



## REVIEW ARTICLE

## Cushing's disease in 2012<sup>☆</sup>

Gabriel Obiols Alfonso<sup>a,\*</sup>, Betina Biagetti Biagetti<sup>a</sup>, Ana Chacón Oreja<sup>b</sup>,  
Javier Salvador Rodríguez<sup>b</sup>

<sup>a</sup> Servicio de Endocrinología y Nutrición, Hospital Vall d'Hebron, Barcelona, Spain

<sup>b</sup> Servicio de Endocrinología y Nutrición, Clínica Universidad de Navarra, Pamplona, Spain

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**Abstract** The aim of this study was to review the literature published and the most important papers presented to meetings on Cushing's disease from October 2011 to September 2012. The selection has been performed according to the authors' criteria. Articles have been classified into five groups: quality of life and perception of the disease, clinical features and pathophysiology, comorbidity conditions, diagnosis, and treatment. The results and conclusions of each publication are discussed.

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### La enfermedad de Cushing en 2012

**Resumen** El objetivo de este trabajo es una revisión de los trabajos publicados y las principales comunicaciones a congresos sobre la enfermedad de Cushing desde octubre de 2011 a septiembre de 2012. La selección de los trabajos se ha realizado bajo el criterio de los autores y han sido agrupados en los apartados siguientes: percepción de la enfermedad y calidad de vida, clínica y fisiopatología, comorbilidades, diagnóstico y tratamiento. Se comentan los resultados y las conclusiones de cada trabajo.

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## Introduction

Cushing's disease (CD) is derived from an ACTH-secreting pituitary tumor that causes increased cortisol secretion. CD is a severe disease, potentially fatal due to significant comorbidities, which greatly impairs quality of life. The diagnosis of both hypercortisolism and the origin of excess ACTH in CD is very difficult. Although surgical treatment is required, pituitary surgery is not successful in all cases and other treatment options should be used.

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\* Corresponding author.

E-mail address: [gobiols@vhebron.net](mailto:gobiols@vhebron.net) (G. Obiols Alfonso).

This review focuses on a selection of advances published and reported from the end of 2011 to September 2012.

## Perception of the disease and quality of life

Health-related quality of life has been recognized as a significant aspect in CD management. The first quality of life questionnaire specific for CD, CushingQoL,<sup>1</sup> has been available since 2008. Tiemensma et al.<sup>2,3</sup> published two articles about the perception of the disease. The first article reported a close correlation between quality of life and disease perception, with much poorer indices than those recorded for other conditions characterized by chronic pain. In a second study, the authors used the patients' perception of their body image, as reflected in drawings, to compare differences before and after treatment. Two significant conclusions were drawn from these studies, applying to quality of life impairment itself, and to the prolongation of such impairment after disease remission has been achieved.

The most relevant publication related to quality of life consists of the development of a new methodology specifically for CD, the Tuebingen-CD25.<sup>4,5</sup> The final version consists of 25 items grouped into six subcategories accounting for 72% of the variance of quality of life in CD: depression, sexual activity, interaction with ones surroundings, eating behavior, body disability, and mental capacity.

This impairment follows a bimodal distribution, so that it is greater in people aged under 31 years and between 51 and 60 years, while it is lower in people aged 31–50 years and in those over 60 years of age.

Quality of life is not related to cortisol or ACTH levels, except for urinary free cortisol (UFC) for the subcategories of eating behavior, mental agility, and concentration capacity.

In a second study, the authors established reference values using an age- and sex-matched adult population. Moderate and severe quality of life impairment was found in 25% and 41% respectively. As regards the reference population, moderate or severe impairment was found in approximately two thirds of cases in each subcategory. Women showed greater impairment than men.

The authors emphasize that the women had higher scores than men, reflecting a lower quality of life, which in turn affected their social surroundings, sexual activity, physical capacity, and eating behavior. Women showed impairment in all subcategories, while only physical capacity and concentration ability or mental agility were impaired in men. As a limitation of this conclusion, it should be noted that the male population only consisted of 11 patients with CD.

## Clinical signs and symptoms and pathophysiology

Mention should first be made of the Mathioudakis et al. study,<sup>6</sup> which compared hormone activity to tumor size and found no relation between them. The clinical signs and symptoms may be even less marked in big tumors, and it may therefore be difficult to suspect hypercortisolism. In fact, only plasma cortisol levels and cortisol/ACTH ratio are significantly lower in macroadenomas as compared to microadenomas. Less skin fragility and muscle weakness are

also seen in bigger tumors. Only headache is more common in macroadenomas.

Pecori Giraldi et al.<sup>7</sup> analyzed this variability in secretory capacity in response to stimulation and suppression tests. They compared 72 ACTH-secreting tumors incubated with CRH or dexamethasone (DXM). Cultures of rat pituitary cells were used as controls. The great variability in the response obtained suggests the existence of multiple phenotypes in patients with CD. Controls did not show this variability.

The authors considered as positive any change greater than 20% as compared to unstimulated samples and found that ACTH increased in 70% of cases at four hours and in 54% of cases at 24 h. ACTH levels increased by more than 50% in only half the patients.

DXM suppression was only greater than 75% in half of the patients, while no change was seen in 30%, and a paradoxal increase was found in 20%.

These results reflect the variability of in vivo responses to CRH and DXM shown by patients with CD.

Desmopressin (DDAVP) is able to stimulate tumoral corticotropic cells, and therefore differentiates CD from a pseudo-Cushing's state. Wang et al.<sup>8</sup> studied responses to DDAVP and CRH before surgery and immunohistochemistry (IHC) to analyze the expression of the CHR receptor (CRHR) and the three subtypes of vasopressin receptor (V1R, V2R, and V3R).

These authors found that V1R, V3R, and CRHR were widely expressed in all patients. By contrast, V2R expression was highly variable and lower in macroadenomas. In vivo ACTH responses to DDAVP correlated to tumor size and expression of V2R, but not V1R or V3R. ACTH response to CRH did not correlate to CRHR expression. The authors suggested that changes in response to DDAVP in CD are due to V2R expression by these tumors.

As regards prognostic and aggressiveness markers in CD, Evang et al.<sup>9</sup> studied cadherin E levels and the expression of its gene. The decreased expression of this membrane protein of epithelial cells was reported in several types of cancer and related to its invasive capacity and the development of metastasis.<sup>10</sup> In somatotropinomas, this low expression has been related to resistance to somatostatin analogues.<sup>11</sup> For IHC, two antibodies recognizing both intracellular and extracellular epitopes have been used. The intracellular domain of cadherin E shows membrane and nucleus staining. This antibody makes it possible to study the fraction of cadherin E bound to the membrane and the fraction internalized to the nucleus. Extracellular domain staining only tests the fraction expressed in the membrane.

Cases are divided into microadenomas, macroadenomas, and Nelson's syndrome, as a reflection of the aggressiveness of the different tumors. A significant difference is seen in nuclear expression, which becomes more intense as aggressiveness increases.

The expression of cadherin E and proopiomelanocortin (POMC) genes has been related to the distribution of staining with the intracellular antibody. The strongest cadherin E expression is found in adenomas predominantly expressed in protein membrane. There are no differences regarding POMC expression.

The authors concluded that CD aggressiveness is characterized by a gradual change in cadherin E expression, which

becomes less strong in the membrane and stronger in the nucleus.

## Comorbidities

Coagulation changes in CD promote a hypercoagulability state. The Vera Popovic team<sup>12</sup> found increases in thrombotic and atheromatous events leading to a five-fold greater mortality as compared to the general population due to endothelial dysfunction, hypercoagulability, and vascular stasis.

In another study, Van der Pas et al.<sup>13</sup> concluded that this trend to thrombosis resulted from a prothrombotic state characterized by a decrease in partial thromboplastin time and increases in fibrinogen, factor VIII, and protein S and, second, from decreased fibrinolysis, characterized by a longer clot lysis time caused by increased levels of plasminogen activation inhibitor, thrombin-activated fibrinolysis inhibitor, and alpha-2-antiplasmin. All 17 patients studied entered a sequential medical treatment protocol with pasireotide, cabergoline, and ketoconazole until UFC normalized. UFC normalization was finally achieved in 15 patients. However, this normalization of UFC was only associated with modest decreases in antithrombin and thrombin-activated fibrinolysis inhibitor. The authors concluded that a hypercoagulability state exists in CD, and that this is not corrected 80 days after the normalization of UFC.

Stuijver et al.<sup>14</sup> analyzed the likelihood of deep venous thrombosis, venous thromboembolism, and pulmonary thromboembolism occurring and found an increased incidence as compared to the general population which did not decrease after transsphenoidal surgery.

In addition, these changes also persist after hypercortisolism is resolved. The need for long-term anticoagulation in CD was suggested one decade ago by Boscaro et al.<sup>15</sup> The Stuijver study concluded that prophylaxis for thromboembolism after surgery for CD should be prolonged similarly as in other conditions with a high risk of deep venous thrombosis.

With regard to the cardiovascular system, Toja et al.<sup>16</sup> studied myocardial structural and functional changes in 44 patients with CD followed up for 46 months after remission following surgery. As compared to a control group, they are found left ventricular and posterior hypertrophy and decreased ejection fraction, which were not corrected upon the normalization of cortisol secretion.

As regards high blood pressure and mortality, a study is available on 80 patients who underwent surgery and were followed up for 4.6 years. The patients were divided into three groups depending on whether they were cured or had persistent or recurrent CD.<sup>17</sup> Mortality was higher in CD, particularly in the group with persistent or recurrent disease, but even cured patients had a higher mortality than expected. This occurred despite significant improvements in their body mass index (BMI) and blood pressure.

With regard to myocardial involvement, a study by the Pereira group<sup>18</sup> found that these patients had myocardial fibrosis, unlike controls and also hypertensive cardiomyopathy. This resolved upon the normalization of cortisol secretion. This fibrosis correlated to systolic and diastolic dysfunction and, according to the authors, was a direct effect of hypercortisolism, independent of blood pressure and ventricular hypertrophy.

Finally, Geer et al.<sup>19</sup> related body composition and cardiovascular risk before and after surgery. They found increases in visceral fat, the BMI, and the visceral/total fat ratio, all of which subsequently decreased after treatment. Improvements were seen in total cholesterol, LDL cholesterol, insulin resistance, and leptin, but not in triglycerides, HDL cholesterol, or the total/HDL cholesterol HDL and LDL/HDL cholesterol ratios. The authors therefore concluded that vascular risk continues to increase, in agreement with the results of previous studies.

## Diagnosis

Three articles related to the measurement of prolactin (PRL) for locating the cause of ACTH-dependent CD, specifically in inferior petrosal sinus sampling (CSPI), deserve special mention.

As is known, IPSS is performed with CRH stimulation. A gradient between basal central ACTH (inferior petrosal sinus [IPS]) and peripheral ACTH higher than 2 or a stimulated gradient higher than 3 localizes the source of excess ACTH. Early studies reported 100% sensitivity, but a false negative rate up to 10% was subsequently seen.<sup>20</sup> This was related to technical problems associated with the hypoplastic petrosal sinuses, inexperience, or other problems, but also to the existence of abnormal venous drainage. Concomitant PRL measurement provided the possibility of increasing method sensitivity, which is very important because IPSS is not free from potential complications. A ratio higher than 1.8 between PRL in IPS and the periphery suggested adequate catheter placement.

When a false negative result is suspected due to the abovementioned reasons, the Findling et al.<sup>20</sup> correction formula allows the gradient to be corrected so that values higher than 0.8 suggest CD, while values less than 0.6 suggest ectopic ACTH.

In the first study conducted by Grant et al.<sup>21</sup> using this methodology, 83 patients with CS, 72 of them with CD, were accurately diagnosed, which increased sensitivity to 100%. A false positive result was found, and specificity was therefore 91%, markedly higher than for the results not taking PRL into account.

In another series of 28 CD patients reported by Mulligan et al.,<sup>22</sup> six patients were correctly lateralized using correction by PRL, because the ACTH gradient provided an incorrect adenoma location.

In a third study by Sharma et al.,<sup>23</sup> discordant results were found in two of the eight patients between both petrosal sinuses using the corrected gradient, and the same occurred in two CD patients with positive values on both sides and in two additional patients with an occult origin. The authors therefore stated that PRL measurement should be reserved for patients with a negative corrected gradient, and that currently established values should be changed, increasing to 1.3 the cut-off point for considering the gradient positive, even at the expense of having some patients with an indeterminate gradient. At the end of the article, the authors proposed a flow chart for the management of ACTH-dependent CS taking into account agreement with standard tests, petrosal sinus venography and, finally, the gradient corrected with the new values.

Finally, Tirabassi et al.<sup>24</sup> analyzed the DDAVP and CRH tests in 30 CD patients, 18 with pseudo-Cushing, and 12 controls. Agreement was found between them, with 96% sensitivity and 100% specificity for both. When both tests are negative, CD may be ruled out. They have a greater value than any other combination.

## Treatment

Surgical results in large series have not been reported, but several studies on the dynamics of adrenal axis changes after surgery aimed at assessing cure criteria are available.

A first study reported a rapid decrease in adrenal response to ACTH after complete resection of corticotrophic adenoma.<sup>25</sup> This decrease occurred after stimulation of 45 CD patients with 1  $\mu\text{g}$  of ACTH six days after surgery. Mean follow-up time was 56 months. In 24 of 28 patients in remission, cortisol levels after ACTH did not exceed 28  $\mu\text{g}/\text{dL}$ . Two patients in whom remission occurred later did not achieve this level either. By contrast, levels higher than 28  $\mu\text{g}/\text{dL}$  were seen in 14/15 surgery failures, thus giving values of 93% sensitivity and 87% specificity. The authors attributed this behavior to down-regulation of the adrenal ACTH receptor after adenoma resection.

An additional study<sup>26</sup> characterized the timing of hormonal changes in 21 patients with recurrent CD, defined as changes in nighttime cortisol (in serum or saliva) and UFC levels. If only one of these changes was found, recurrence was considered to be mild. When each of the tests became positive, the authors found that DDAVP and CRH tests showed an earlier change in nighttime cortisol and UFC in most patients.

Adrenal axis recovery has also been reported in children after adenoma resection. In 57 patients younger than 18 years with CD<sup>27</sup> ACTH stimulation was performed every six months for up to three years. Axis integrity was defined as cortisol levels higher than 18  $\mu\text{g}/\text{dL}$ . Recovery was found in 29 patients at 12 months, and in 14 patients at 18 months. Cortisol peaks and time to adrenal axis recovery were analyzed, as well as Kaplan–Meier curves detailing the recovery time for patients overall and by age group and Tanner stage. The results led them to conclude that a cortisol peak of 11  $\mu\text{g}/\text{dL}$  at six months predicts for axis recovery with 70–80% sensitivity and 64–73% specificity. The authors proposed an action algorithm for hydrocortisone dose and its reduction. Starting with a hydrocortisone dose of 8–12  $\text{mg}/\text{m}^2/\text{day}$ , in two divided doses as usual, dosage is reduced by 2.5 mg every 4–6 weeks until a dose of 5 mg daily is reached. The first ACTH stimulation is performed in the sixth month. If a cortisol peak of 18  $\mu\text{g}/\text{dL}$  is reached, hydrocortisone is discontinued. Otherwise, the test is repeated six months later.

A third study analyzed changes over time in ACTH levels after transsphenoidal surgery (TSS) for CD.<sup>28</sup> Its purpose was to assess ACTH as an early marker of remission after adenoma resection. For this, ACTH and cortisol were measured every six hours under no corticoid treatment. Remission was defined as cortisol levels <2  $\mu\text{g}/\text{dL}$  or <5  $\text{g}/\text{dL}$  with clinical signs of adrenal insufficiency. Remission was seen in nine of the 12 patients studied. ACTH decreased faster in patients in remission, all of them having ACTH levels <20  $\text{pg}/\text{mL}$

17h after surgery. Two previous studies with the same scheme but using cortisol as a prediction parameter are available.<sup>29,30</sup> In the current study, ACTH decrease preceded cortisol decrease by 3–36h. The authors concluded that postoperative ACTH discriminates cases with disease persistence and remission after TSS surgery for CD.

As regards medical treatment, a very interesting study by Fleseriu et al.<sup>31</sup> analyzed the effects of mifepristone on diabetes mellitus or carbohydrate intolerance and on high blood pressure in endogenous hypercortisolism. Mifepristone is a progesterone receptor antagonist and, in high doses, a glucocorticoid receptor antagonist. It has no mineralocorticoid effect. Authors found at least a 25% decrease in the area under the curve of glucose levels during the oral glucose tolerance test 0–120 min ( $\text{AUC}^{\text{gluc-120}}$ ) in 60% of patients, and the decrease persisted over the 24 weeks of the study.

In 38% of patients, diastolic blood pressure decreased at least 5 mmHg. When the data were combined, 87% of patients experienced some type of significant improvement, resulting in decreases in HbA1c and insulin resistance, as reflected by reductions in plasma insulin levels and the homeostatic model assessment (HOMA) index.

An experimental study by Fukuoka et al. used cultures of dog and human corticotropinomas to study the value of the epidermal growth factor receptor (EGFR), expressed by these tumors, as a therapeutic target in CD.<sup>32</sup> Increased EGFR expression in these tumors causes increased POMC expression. If EGFR is transfected to mouse corticotrope cells, POMC expression and ACTH production increase. EGFR uses the MAPK pathway, so that when EGFR expression is blocked with gefitinib, a tyrosine kinase inhibitor, POMC and ACTH expression levels are decreased. Such reduced levels are associated with decreases in corticosterone level and in the size of explanted tumors. Reductions also occur in hypercortisolemia, elevated glucose levels, and visceral adipose tissue. The authors concluded that EGFR may be a therapeutic target in CD.

Finally, oral communications on the effect of LC1699, an 11-beta hydroxylase inhibitor, in 11 patients with CD were presented at the meetings of both the European Society of Endocrinology<sup>33</sup> and the Endocrine Society.<sup>34</sup> The dosage was increased from 4 to 100 mg over 10 weeks until UFC levels normalized. The primary endpoint was UFC at study end and cortisol precursors. Twelve patients completed the study. UFC levels normalized in 11 patients, and decreased more than 50% in all others. UFC levels increased at 12 weeks. ACTH, 11DOCs, testosterone, and DOCA levels increased, while renin and aldosterone levels decreased. The drug was well tolerated. Moderate signs of adrenal insufficiency such as fatigue, nausea, and abdominal pain were detected, as well as hypokalemia. LC1699 was recommended as a good treatment alternative in CD.

The predominance of somatostatin receptor type 5 (SSTR5) over the type 2 receptor (SSTR2) in ACTH-secreting pituitary adenomas and the development of pasireotide, a somatostatin (SS) analog acting on four SS receptors but with a predominant affinity for SSTR5, has provided a new and promising therapeutic window in the treatment of CD.<sup>35</sup> By 2012, early results had confirmed its activity both as a monotherapy and when combined with cabergoline and ketoconazole. It may potentially achieve biochemical control in CD in 90% of patients.<sup>36,37</sup>

A phase III multicenter, randomized, double-blind study conducted on patients with de novo, persistent, or recurrent CD treated for one year with pasireotide 1200 or 1800  $\mu\text{g}$  daily, administered subcutaneously, was reported in 2012.<sup>38</sup> A significant UFC reduction was seen with both doses. At three months of treatment, UFC normalization was achieved in 16% and 29% of patients treated with 1200 and 1800  $\mu\text{g}$  daily of pasireotide respectively, and this persisted after one year of treatment. Overall mean reductions in UFC levels at 12 months as compared to baseline were 67.6% and 62.4% with the doses of 1200 and 1800  $\mu\text{g}$  daily respectively. On the other hand, and no less significantly, pasireotide was more effective in patients with moderate baseline UFC elevations as compared to those with baseline UFC levels more than five-fold higher than normal. In addition, the biochemical response achieved in the first 2–3 months was highly predictive of the long-term effect.<sup>38</sup> Using these data, those patients who will benefit the most from the long-term administration of pasireotide can be targeted. As expected, mean basal ACTH levels gradually decreased over the treatment period, with a 16.9% reduction at 12 months as compared to baseline values.

The data provided suggest that 900  $\mu\text{g}$  twice daily decreases corticotropinoma size by 9% and 43% at six and three months respectively. This is highly significant, because it suggests that a pharmacological approach able to exert an effect on tumor volume may be available. Although the final mechanisms mediating this action have not yet been fully established, the phenomenon probably results from the antiproliferative effects mediated by the activation of different SSTRs.<sup>39</sup> This represents a different approach as compared to other treatments aimed at controlling high cortisol levels.

The effects on associated comorbidity reflect reductions in body weight, systolic and diastolic blood pressure, triglycerides, and LDL, as well as quality of life improvement, occurring in parallel to UFC decrease and confirming the benefits of decreased cortisol production on classical cardiovascular risk factors. The side effects of pasireotide are those typical of a somatostatin analog and include nausea, diarrhea, and biliary tract pathology.<sup>38</sup> However, because of its ability to activate SSTR5 to inhibit insulin secretion without significantly affecting glucagon secretion,<sup>40</sup> pasireotide has a greater hyperglycemic effect than octreotide, which explains the development of hyperglycemia in up to 40% of treated patients. This effect is dose-dependent, and more marked in patients who already require antidiabetic treatment at the onset and have HbA1c values > 7%.

Studies conducted on normal subjects to elucidate the pathophysiological mechanisms leading to hyperglycemia suggest incretin system deficiency, and DPP-IV inhibitors or GLP-1 analogs are therefore more helpful in attenuating this effect.<sup>41</sup> However, internationally accepted clinical guidelines, including the combination of oral drugs or even insulin therapy, whether or not associated with drugs with an incretin effect, should be followed.<sup>42</sup>

The data most recently reported suggest that, after two years of treatment, pasireotide achieves an overall percent UFC reduction even greater than at one year (62.7% vs 54.7%) and a higher proportion of patients with normal UFC levels (34.5% vs 25%). Moreover, no additional cases of

hyperglycemia were seen as compared to those identified after one year of treatment.<sup>43</sup>

A new extended release formulation of pasireotide allowing for once monthly intramuscular administration has been shown to have an acceptable tolerability profile in normal subjects,<sup>44</sup> and may provide increased efficacy, tolerability, and convenience in treatment compliance in patients with CD.

The chance of a significant action on SSTR5 expands the spectrum of CD treatment. Both as monotherapy or, probably more frequently, combined with drugs acting through other mechanisms,<sup>45–47</sup> pasireotide opens up new treatment alternatives, thus promoting the consolidation and individualization of treatment options for this devastating disease.

## Conflicts of interest

The authors state that they have no conflicts of interest.

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