UNIVERSITAT DE BARCELONA

Estudio neuropsicológico, neurorradiológico y clínico en el hipercortisolismo endógeno

(Neuropsychological, neuroradiological and clinical study in endogenous hypercortisolism)

Alicia Santos Vives

ESTUDIO NEUROPSICOLÓGICO, NEURORRADIOLÓGICO Y CLÍNICO EN EL HIPERCORTISOLISMO ENDÓGENO

Alicia Santos Vives

UNIVERSITAT DE BARCELONA

Endocrinology department, Unit CIBERER 747

Estudio neuropsicológico, neurorradiológico y clínico en el hipercortisolismo endógeno

(Neuropsychological, neuroradiological and clinical study in endogenous hypercortisolism)

This thesis is presented by

Alicia Santos Vives

To obtain the degree of Doctor by the University of Barcelona

Supervised by

Prof**. Susan M Webb Youdale**. Department of Medicine UAB

Prof. **Maria Mataró Serrat**. Department of Psychiatry and Clinical Psychology UB

Doctoral programme in Biomedicine

A todos los participantes que

desinteresadamente han realizado

las pruebas de este estudio,

y de los que tanto he aprendido

ACKNOWLEDGEMENTS

Esta tesis es el resultado de un largo periodo de trabajo en un interesante y laborioso proyecto. Creo que los resultados han valido el esfuerzo, y me siento satisfecha de poder estar hoy escribiendo estas páginas. Por supuesto, esto no habría sido posible sin todas las personas que han contribuido a ello.

Primero de todo querría darle las gracias a Susan, por darme la oportunidad de introducirme en el interesante mundo de la investigación. Por su esfuerzo constante para que nuestro pequeño grupo saliera adelante y por ser un ejemplo de vitalidad y energía.

A Maria, por acompañarme también en este largo camino que sería la tesis, por su ayuda, consejos e ideas.

A Eu, mi compañera desde los inicios y co-redactora del proyecto inicial que daría lugar a esta tesis. Por estar siempre ahí durante todo este periodo dándome apoyo incondicional.

A Iris, que con su llegada hizo posible que la montaña de trabajo se pudiese finalmente ir reduciendo.

A Mª Antonia, por su esfuerzo, y por ser un ejemplo de que las metas son alcanzables con trabajo constante.

A Patri, mi primera compañera-psicóloga, por su apoyo en esos momentos iniciales cuando aún se veía muy lejos el final.

A Elena, por su ayuda, y por ser un ejemplo de cómo tomarse las cosas de otro modo.

A Yolanda, por su eficacia y rapidez solucionando cualquier duda o problema.

A Beatriz y Esther por hacer posible la segunda publicación.

A Ignasi, por ayudarme una y otra vez con todos los temas estadísticos.

A Caroline, por las correcciones lingüísticas.

Al personal del banco de sangre, por permitirnos dejar allí nuestra “caja de reclutamiento”.

A todos los que nos han ayudado en la programación de pruebas, Bea, Saiko, Jordi... Y al innumerable personal técnico que ha contribuido al estudio realizando las resonancias magnéticas y analíticas.

A aquellos compañeros del hospital y profesores con los que he colaborado, que en algún momento me han ayudado o aconsejado, o que simplemente me han llevado a aprender algo nuevo durante este periodo, como Carlos, Maria, Albert, Juan Carlos, Quim, Matilde, Maria y tantos otros.

A Anna, por las sesiones de coaching en la última etapa que me ayudaron a visualizar esta tesis acabada.

A todos aquellos que aunque no han estado vinculados directamente con la tesis o el ámbito laboral han estado cerca de mí durante este periodo. A mi familia, mis amigos, a Abel... ¡Muchas gracias a todos!

Y para acabar, también querría expresar mi agradecimiento a un grupo de personas que ha contribuido a este trabajo de forma esencial: los pacientes y controles. A todos ellos, les doy las gracias por su participación desinteresada, por lo que me han ayudado a aprender y por incitarme a través de sus historias a interesarme por otros ámbitos como la psicoterapia. Desearía que este estudio no acabara con los resultados aquí presentados, y que fuera la base para nuevos proyectos para mejorar la calidad de vida de los pacientes con síndrome de Cushing. Porque en este ámbito aún queda muchísimo camino por recorrer...

Prof. Susan M Webb Youdale, professor of the Universitat Autònoma de Barcelona, teaching unit at the Hospital de la Santa Creu i Sant Pau (CIBERER, Unit 747) and Prof. Maria Mataró Serrat, professor agregat at Universitat de Barcelona,

CERTIFY that they have supervised and guided the PhD Thesis entitled "Estudio neuropsicológico, neurorradiológico y clínico en el hipercortisolismo endógeno" presented by Alicia Santos Vives. They hereby assert that this thesis fulfills the requirements to be defended to obtain the degree of Doctor.

Signature,

Prof. Susan M Webb Youdale
Universitat Autònoma de Barcelona
Hospital de la Santa Creu i Sant Pau
(CIBERER 747)

Prof. Maria Mataró Serrat
Universitat de Barcelona

Barcelona, 30th October 2015

The work reported in this thesis was carried out in the CIBERER 747 group at the Hospital de la Santa Creu i Sant Pau, Barcelona.

The present work and the two studies included have been supported by the following grants and institutions: the Instituto de Salud Carlos III, the Spanish Ministry of Science and Innovation (MICINN, FIS080302), the European Commission (ERCUSYN PHP800200) and AGAUR (2014 SGR 355).

The human brain has 100 billion neurons,

each neuron connected to 10 thousand other neurons.

Sitting on your shoulders is the most

complicated object in the known universe.

Michio Kaku

CONTENTS

FOREWORD

This thesis is the result of the work on a project conducted in the CIBERER 747 group at the Hospital de la Santa Creu i Sant Pau, Barcelona.

Both studies included in the thesis are presented as articles. They have been published in an international journal with an impact factor of 4.069 (according to the Journal Citation Reports from ISI Web of Knowledge, inferred for 2014).

Study I:

Santos A, Resmini E, Crespo I, Pires I, Vives-Gilabert Y, Granell E, Valassi E, Gómez-Ansón B, Martínez-Momblán MA, Mataró M, Webb SM. **Small cerebellar cortex volume in patients with active Cushing's syndrome.** *Eur J Endocrinol*, 2014 Oct; 171(4):461-9.

Study II:

Santos A, Resmini E, Gómez-Ansón B, Crespo I, Granell E, Valassi E, Pires I, Vives-Gilabert Y, Martínez-Momblán MA, de Juan M, Mataró M, Webb SM. **Cardiovascular risk and white matter lesions after endocrine control of Cushing's syndrome.** *Eur J Endocrinol* 2015 Dec; 173(6):765-75.

GLOSSARY OF ABBREVIATIONS

ACTH: Adrenocorticotropic hormone

ATPIII: Adult Treatment Panel III

BIPSS: Bilateral inferior petrosal sinus sampling

BMI: Body mass index

CD: Cushing's disease

CRH: Corticotropin releasing hormone

CS: Cushing's syndrome

CT: Computed tomography

DTI: Diffusion-tensor-imaging

GH: Growth hormone

HDL: High density lipoprotein

HPA: Hypothalamic-pituitary-adrenal

NCEP: National Cholesterol Education Program

MRI: Magnetic resonance imaging

MS: Metabolic syndrome

QoL: Quality of life

SMR: Standard mortality rate

VLDL: Very-low-density lipoprotein

WML: White matter lesions

LIST OF FIGURES

LIST OF TABLES

1. INTRODUCTION

1.0. Cortisol and the hypothalamic-pituitary-adrenal axis

Cushing's syndrome (CS) is a disease due to an excess of circulating cortisol. Cortisol is a steroid hormone produced by the adrenal glands which is released in response to challenging situations (which may include illness or stress). Cortisol has a circadian rhythm; higher levels are detected in the morning and lower levels in the evening. It is implicated in several processes including metabolism, cardiovascular tone, fluid volume, response to hemorrhage, immunity, inflammation, neural function, behaviour and reproduction (Sapolsky *et al.* 2000).

Cortisol release depends on the hypothalamic - pituitary - adrenal (HPA) axis. This axis includes the hypothalamus, the pituitary gland and the adrenal cortex. In a challenging situation the hypothalamus releases corticotropin releasing hormone (CRH). This stimulates the production of adrenocorticotropic hormone (ACTH) in the anterior pituitary, which will stimulate the production of cortisol in the adrenal cortex (*Figure 1*). The cortisol release will act as a

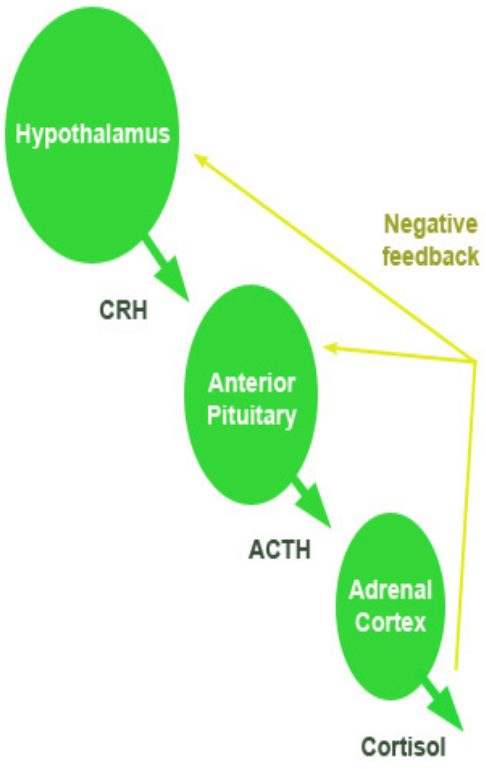


Figure 1: The HPA Axis

negative feedback for the anterior pituitary and the hypothalamus, which will stop releasing hormones.

1.1. Cushing's syndrome: prevalence and aetiology

This thesis will deal with a particular form of CS: endogenous CS. Endogenous CS is a very rare disease, in contrast with exogenous CS, which can ocurr after the chronic exposure to supraphysiological amounts of glucocorticoid drugs prescribed for conditions as autoimmune and inflammatory diseases.

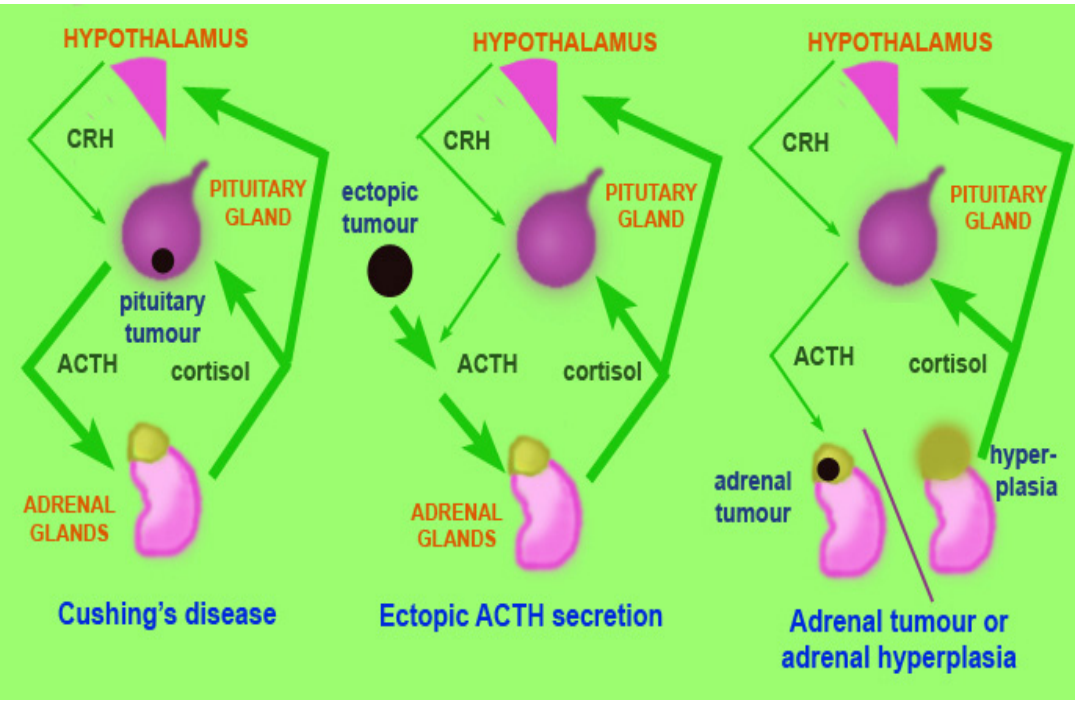
CS is more frequent in women than in men, and the female/male ratio ranges from 3 to 5 (Newell-Price *et al.* 2006). The established incidence of endogenous CS ranges from 0.7 to 2.4 cases per million inhabitants per year (Newell-Price *et al.* 2000, Ambrosi *et al.* 1991, Etxabe *et al.* 1994, Lindholm *et al.* 2001). However, it is important to highlight that these estimations may not be completely accurate, as epidemiological data available in the literature are scarce and not very recent.

In Spain, the estimated prevalence of Cushing's disease (CD), which is the most common cause of CS, is 39.1 patients per million inhabitants (Etxabe *et al.* 1994). According to the Spanish National Institute of Statistics, the total Spanish population in January 2015 was 46.439.864 inhabitants. This would mean an estimation of 1815 patients with CD in the Spanish population in 2015, although further and more recent studies on the prevalence of the disease would be needed to confirm it.

When endogenous, the most common cause of CS is a pituitary ACTH-producing adenoma. This form of the disease is also called CD and involves 70% of the patients. Arround twenty per cent of the cases are ACTH-independent

and involve a cortisol excess due to adrenocortical tumours (10% of the cases), carcinomas (8% of the cases) or bilateral adrenal hyperplasia. Finally, 12% of the cases are due to an ectopic source of ACTH secretion or a CRH production by a neuroendocrine tumour (Boscaro *et al.* 2001). *Figure 2* summarizes the different causes of CS.

Figure 2: The different causes of endogenous Cushing's syndrome



1.2. Cushing's syndrome: clinical manifestations

Patients can present multiple symptoms, including facial plethora (due to the fat deposition in the cheeks and temporal fossae), central obesity, body composition alterations (including increased fat mass, reduced bone mass leading to osteoporosis and decreased lean mass), skin alterations (purple striae, easy bruising, ulceration, poor wound healing), hirsutism, amenorrhea, muscle weakness (or myopathy), hypogonadism, hypertension, insulin resitance

and/or diabetes mellitus, dyslipidemia, prothrombotic state, vascular disease, atherosclerosis, depression, anxiety, cognitive decline and impaired quality of life (QoL) (Arnaldi *et al.* 2003, Nieman *et al.* 2008, Bourdeau *et al.* 2005, Starkman *et al.* 1981, Starkman *et al.* 2001, León-Carrión *et al.* 2009, Dorn *et al.* 2000).

Figure 3: Photographs of a patient, before clinical manifestations (left) and one year later, with active Cushing's syndrome (right)

Footnote: These images were provided by the patient for this thesis and have been included with her consent.

Many of these signs and symptoms of CS may appear in the normal population and can differ from one patient to another, making the diagnosis of the disease difficult. According to data in a big cohort of 481 European patients,

the most common symptoms are weight gain (81%), hypertension (78%), skin alterations (73%) and myopathy (67%) (Valassi *et al.* 2011). When clinical presentation is severe the diagnosis can be made promptly, although unfortunately in most of the cases patients are diagnosed a long time after the initial presentation of the first symptoms. In fact, the median delay to diagnosis is 2 years (Valassi *et al.* 2011). This can have relevant implications, leading to increased comorbidities and low QoL, even after biochemical cure (Lindslay *et al.* 2006, Van Aken *et al.* 2005, Heald *et al.* 2004).

1.3. Cushing's syndrome: diagnosis

When CS is suspected, first steps include an exploration of drug history in order to exclude excessive exogenous glucocorticoid exposure. If this is excluded biochemical testing should be conducted. Guidelines recommend the following first-screening tests (Arnaldi *et al.* 2003, Nieman *et al.* 2008):

- 24-hour urinary free cortisol (at least two measurements)
- 1 mg dexamethasone supression test (or alternatively 2 mg/day for 48 h)
- Late-night salivary cortisol (two measurements)

If at least one test result is abnormal, further endocrinological evaluation would be needed to confirm the diagnosis.

Once the syndromic diagnosis has been established, further tests are needed to determine the differential diagnosis (etiological diagnosis). If plasma ACTH is not supressed, ACTH-dependent causes (pituitary or ectopic origin) should be investigated. Pituitary magnetic resonance imaging (MRI) can evidence an adenoma in approximately 60% of the patients. If no lesion is identified, bilateral inferior petrosal sinus sampling (BIPSS) can be helpful to confirm or exclude a pituitary origin. When the results of the BIPSS test are negative an

ectopic cause can be suspected. It is recommended to perform a computer tomography (CT) and/or MRI of the neck, thorax or abdomen, or a scintigraphy with labelled octreotide (octreoscan), to identify the ectopic source. On the other hand, if ACTH is supressed adrenal CT or MRI is recommended to identify the kind of adrenal lesions causing CS (Arnaldi *et al.* 2003).

1.4. Cushing's syndrome: treatment options

Several treatment options for CS are available nowadays, that may differ depending on the cause of CS. They include surgery (pituitary adenomectomy, adrenalectomy or excision of the ectopic source of ACTH), irradiation or cortisol-lowering drugs (Arnaldi *et al.* 2009, Biller *et al.* 2008).

Very recently the Endocrine Society has established new guidelines to update the recommendations on management and treatment of CS (Nieman *et al.* 2015). For patients with clearly diagnosed and established CS they mainly recommend to normalize cortisol levels or action at its receptors to normalise the signs and symptoms of CS, and also to treat the comorbidities associated with CS. It is not recommended to reduce cortisol levels or its action if the diagnosis of CS is not well established, or if there is only a borderline abnormality of the HPA axis, without specific signs of CS. They also establish several recommendations as first and second-line treatment options.

1.4.1. First-line treatment options: recommendations

According to the guidelines of the Endocrine Society (Nieman *et al.* 2015), the following first-line recommendations are established to treat CS:

-It is highly recommended to perform an initial resection of the primary lesion (or various lesions) underlying CS, including CD, ectopic and adrenal

(cancer, adenoma, and bilateral disease) etiologies. This should be done unless surgery is not possible or if it is unlikely to significantly reduce glucocorticoid excess. Specific recommendations for different kinds of surgery are:

- Adrenal origin: it is recommended to perform unilateral resection (by an experienced adrenal surgeon) for all cases of benign unilateral disease.
- Ectopic origin: it is recommended to localize and resect ectopic ACTH-secreting tumours, with node dissection as appropriate.
- Pituitary origin: it is recommended to perform transsphenoidal selective adenomectomy by an experienced pituitary surgeon as the optimal treatment for CD (in both pediatric and adult patients).
- After transsphenoidal adenomectomy it is recommended to:
  - Measure serum sodium several times during the first 5-14 days.
  - Assess free T_4 and prolactin within 1-2 weeks of surgery in order to evaluate for hypopituitarism.
  - Obtain a postoperative pituitary MRI scan within 1-3 months.
- Surgical resection of bilateral adrenal disorders is recommended, and medical therapy is suggested to block aberrant hormone receptors for bilateral macronodular adrenal hyperplasia.

1.4.2. Second-line treatment options

According to the guidelines of the Endocrine Society, the following second-line recommendations are established to treat CS:

- In patients with ACTH-dependent CS who underwent a non curative surgery or for whom surgery was not possible, a shared decision-making approach is suggested, as there are several available second-line therapies (including repeating transsphenoidal surgery, pituitary radiotherapy, medical therapy, and bilateral adrenalectomy).
    - Bilateral adrenalectomy is suggested for occult or metastatic ectopic ACTH secretion, or as a life-preserving emergency treatment, in patients with very severe ACTH-dependent disease and who can not be promptly controlled by medical therapy.
    - It is recommended to regularly evaluate for corticotrope tumour progression using pituitary MRIs and ACTH levels in:
        - Patients with known CD who undergo bilateral adrenalectomy.
        - Patients who undergo bilateral adrenalectomy for presumed occult ectopic ACTH secretion.
- It is suggested to repeat transsphenoidal surgery particularly in patients with evidence of incomplete resection, or in patients with a pituitary lesion on imaging.
- Before administering radiation therapy or radiosurgery it is recommended to confirm that medical therapy is effective in normalizing cortisol (as this will be needed while awaiting the effect of radiation).
- Further recommendations on radiation therapy or radiosurgery are:
    - It is suggested to perform radiation therapy or radiosurgery in patients who have failed transsphenoidal surgery or have recurrent CD.

- It is recommended to use radiation therapy where there are concerns about the mass effects or invasion associated with corticotroph adenomas.
- It is recommended to measure serum cortisol or urinary free cortisol off-medication periodically (6-12 month intervals) to assess the effect of radiation therapy, and also if patients develop new adrenal insufficiency symptoms while on stable medical therapy.

- Regarding medical treatment, steroidogenesis inhibitors are recommended under the following conditions:
  - 1) As second-line treatment after transsphenoidal surgery in patients with CD (either with or without radiation therapy or radiosurgery).
  - 2) As primary treatment of ectopic ACTH secretions (in patients with occult or metastatic ectopic ACTH secretions).
  - 3) As adjunctive treatment to reduce cortisol levels in adrenocortical carcinoma.
- Other recommendations on medical treatment include:
  - Pituitary-direct medical treatments are suggested in patients with CD who are not surgical candidates or who have persistent disease after transsphenoidal surgery.
  - It is suggested to administer glucocorticoid antagonists (mifepristone) in patients with diabetes or glucose intolerance who are not surgical candidates or who have persistent disease after transsphenoidal surgery. However, this drug is difficult to use, since no biomarker is available for dose adjustment.

1.5. Cushing's syndrome: mortality, cardiovascular risk and cardiovascular disease

Cushing's syndrome was first described in 1912. In the 50's, at a time when successful treatments for the disease were not available, reports described a 50% death rate five years after the diagnosis of the disease (Plotz *et al.* 1952). The therapeutic options available nowadays have led to an improvement in the prognosis, although in some cases it may not be optimal. There is some controversy regarding mortality rates in CS due to increased cardiovascular risk, as some studies found a mortality above that of the normal population (Etxabe *et al.* 1994, Lindholm *et al.* 2001, Bolland *et al.* 2011, Clayton *et al.* 2011, Hassan-Smith *et al.* 2012, Ntali *et al.* 2013, Yaneva *et al.* 2013), while others did not (Swearingen *et al.* 1999, Pikkarainen *et al.* 1999, Hammer *et al.* 2004). However, most studies in patients with persistent moderate hypercortisolism show that the standard mortality rate (SMR) is increased in comparison to normal population (Etxabe *et al.* 1994, Lindholm *et al.* 2001, Hammer *et al.* 2004, Clayton *et al.* 2011, Ntali *et al.* 2013). Disease persistence, older age at diagnosis and presence of hypertension or diabetes have been established as the main determinants of mortality (Clayton *et al.* 2011). In a large series (n=418), 71.4% of deaths in CD were related to cardiovascular causes or infection/sepsis (Ntali *et al.* 2013). Very recently a meta-analysis has established that the mortality risk is increased in both uncured CD (SMR 4.6) and cured CD (SMR 2.5), although the risk is significantly higher in uncured patients (van Haalen *et al.* 2015).

What it is well established is that CS patients have a higher cardiovascular risk related to the cortisol excess, which very often persists after remission of

the disease. This is the result of the combination of different risk factors, which can be metabolic (central obesity, hypercholesterolemia, hypertriglyceridemia and diabetes), vascular (including hypertension and artherosclerosis), cardiac (related to heart functionality and rhythm), or thrombotic (prothrombotic state) (de Leo *et al.* 2010, Colao *et al.* 1999).

1.5.1. Metabolic risk factors and the metabolic syndrome

According to the National Cholesterol Education Program (NCEP)-Adult Treatment Panel III (ATPIII) (Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults, 2001) and the posterior modification from the American Heart Association/National Heart, Lung and Blood Institute (Grundy *et al.* 2005), the metabolic syndrome (MS) is diagnosed when 3 or more of the following factors coexist:

1) Central obesity (waist circumference ≥ 102 cm in males; ≥ 88 cm in females).

2) Fasting hypertriglyceridemia (triglycerides ≥ 1.7mmol/L or 150 mg/dl).

3) Fasting low high density lipoprotein (HDL) cholesterol (HDL-C<40 mg/dL in males, <50 mg/dL in females).

4) Blood pressure ≥ 130/85 mmHg or current use of antihypertensive medications.

5) Fasting plasma glucose ≥5.6 mmol/L or 100 mg/dL, a diagnosis of diabetes or current use of glucose-lowering medication.

In patients suffering CS, approximately 66% of the patients fulfill the criteria for the MS (Chanson *et al.* 2010), which is higher than in normal population, where approximately 20% is affected (Cameron *et al.* 2004). Even if

not all patients have the MS (as described above), most of them have alterations in one or more of the criteria (Chanson *et al.* 2010).

Central obesity is one of the main characteristics of the MS. In CS, obesity or weight gain can be found in approximately 95% of the patients (Newell-Price *et al.* 2006). Possible mechanisms involved could be glucocorticoid inhibition of 5' adenosine monophosphate-activated protein-kinase in the adipose tissue, the increase of inflammatory factors (as tumour necrosis factor α and interleukin-6) or the direct effect of cortisol on omental adipose tissue, which hyperactivates 11-ß-hydroxysteroid dehydrogenase type 1, which stimulates preadypocite differentiation (Barahona *et al.* 2009, Kern *et al.* 2001, Kola *et al.* 2008, Bujalska *et al.* 1997, Stewart *et al.* 2003).

Patients with CS have a higher body mass index (BMI) and waist circumference than normal controls, which persist long time after biochemical cure (Colao *et al.* 1999, Faggiano *et al.* 2003, Giordano *et al.* 2011). Even when compared to BMI-matched controls, patients in remission have a higher waist-hip ratio, indicating central obesity (Colao *et al.* 1999). In fact, the percentage of patients with central obesity, in comparison to BMI-matched controls, is greater in CS, both in the active phase (71%) or after cure (51%) (Barahona *et al.* 2009). Follow-up in patients after cure shows a significant reduction in the percentage of patients with obesity and central obesity (initial 50 and 60% vs final 39 and 45%), although still significantly higher than normal controls (11% with obesity and 13% with central obesity). This reduction was not found in active patients with persistent disease (Terzolo *et al.* 2014). A whole body MRI in patients before and after surgery has revealed that the fat distribution is modified and improved after remission, with a reduction in fat depots. However, most patients still present obesity or are overweight (Geer *et al.* 2012). In general,

despite partial improvement after cure, patients in remission present greater total and abdominal fat mass than healthy population (Barahona *et al.* 2009, Ragnarsson *et al.* 2015).

Dyslipidemia is a common feature in CS characterized by an increase of plasma cholesterol, triglycerides or both (Arnaldi *et al.* 2003). According to some authors, around 7-35% of CS patients have elevated blood triglyceride concentrations and 25-52% have hypercholesterolemia, while 14-36% of the patients have low HDL cholesterol levels (Faggiano *et al.* 2003, Mancini et al. 2004, Greenman 2010). The pathogenic mechanisms that lead to impaired lipid metabolism in CS are multifactorial, and include both direct and indirect action of cortisol on lipolysis, free fatty acid production and turnover, very-low-density lipoprotein (VLDL) synthesis and fatty accumulation in liver (Arnaldi *et al.* 2010). Even if some patients can experiment normalization of cholesterol and triglyceride levels after remission of the disease, some studies report general long-term persistence of these comorbidities (Colao *et al.* 1999, Barahona *et al.* 2010, Terzolo *et al.* 2014). This includes an adverse lipid profile with a higher HDL/cholesterol ratio, which can persist in approximately 30% of the patients (Giordano *et al.* 2011). Taking into account the general increased cardiovascular risk, treatment of dyslipidemia in the patients would be essential.

Both glucose intolerance and diabetes mellitus are frequent characteristics in CS (Arnaldi *et al.* 2003). Glucose intolerance is a pre-diabetic state of hyperglycemia characterised by insulin resistance (a condition where the cells fail to respond to the normal action of insulin), while diabetes mellitus is a more severe disorder characterized by chronic hyperglycemia as a result of impaired insulin secretion, insulin action or both (American Diabetes Association 2010). Diabetes mellitus seems to be more common in ectopic CS than in other

aetiologies (as CD or adrenal source) (Valassi *et al.* 2011). The estimated prevalence of diabetes mellitus in CS is 20-50%, while the estimated prevalence for impaired glucose tolerance is 30-60% (Arnaldi *et al.* 2003, Faggiano *et al.* 2003, Biering *et al.* 2000). However, these numbers may have been underestimated, as glucose tolerance tests are not always performed in the active phase (Arnaldi *et al.* 2003). Mechanisms involved in diabetes in CS include cortisol gluconeogenesis stimulation, disruption of insulin signalling, altered insulin secretion in the pancreas and inhibition of insulin sensitivity in the liver and skeletal muscles (Pivonello *et al.* 2010, Ferrau *et al.* 2015). The presence of diabetes is higher in patients with active disease that in patients in remission. After cure, diabetes rates can decrease up to 40%, although patients still can present altered fasting glucose levels (Faggiano *et al.* 2003, Colao *et al.* 1999). Hyperglycemia can lead to an increased cardiovascular risk, and accelerate atheroesclerosis (mainly when concomitant dyslipidemia is present) (Chait *et al.* 2009). Therefore, adequate diagnosis, hyperglycemic control and treatment (both for hyperglycemia and dyslipidemia) are highly recommended in CS patients.

1.5.2. Vascular risk factors

Hypertension may be the first sign of CS. It is a common symptom, affecting between 55% and 85% of the patients (Arnaldi *et al.* 2003, Faggiano *et al.* 2003, Feelders *et al.* 2012). Several pathophysiological mechanisms are involved, including mechanisms for plasmatic volume regulation, peripheral vascular resistance and cardiac output (Cicala *et al.* 2010). Hypertension has also been correlated with hypercortisolism duration (Cicala *et al.* 2010). In patients of pituitary origin (CD) the physiological nocturnal blood pressure reduction does

not occur, with concomitant cardiac rhythm alterations, presenting only partial improvement after long-term remission of the disease. These factors lead to an even higher cardiovascular risk for patients with CD (Pecori Giraldi *et al.* 2007). These observations may differ for different disease origins. In patients with adrenal adenomas nocturnal blood pressure decline is lower than in patients with essential hypertension in the active phase, although there is an improvement after disease remission (not present in patients of pituitary origin). Severity of hypertension and duration of the disease may also have a role on nocturnal blood pressure decline (Zacharieva *et al.* 2004).

In approximately 44-75% of the patients, hypertension will resolve after cure (Feelders *et al.* 2012). However, hypertension can also persist after remission and patients may require long-term medical treatment to normalize blood pressure (Barahona *et al.* 2009). This hypertension persistance may be more usual in patients presenting older age and with longer duration of hypercortisolism (Feelders *et al.* 2012). Hypertension is associated to high cardiovascular risk, so even if it is usually asymptomatic, it is important to detect it and control it prompty to guarantee adequate health related QoL in these patients.

Patients with CS are at high risk of developing atherosclerosis, as some of the typical complications of hypercortisolism (dyslipidemia, diabetes, obesity, hypertension and the MS) are risk factors for the disease (Crowther *et al.* 2005, Grundy *et al.* 2005). Atherosclerosis is a chronic inflammatory process where artery walls thicken due to the accumulation of plaque. Atherosclerotic plaques have been found in both active CS (14.2-31.2% of the patients) and in patients in remission (26.7% of the patients) (Faggiano *et al.* 2003, Albiger *et al.* 2006, Colao *et al.* 1999). This percentage was significantly higher in comparison to sex

and age matched controls (0%), BMI matched controls (3.3-6.2%), patients with essential hypertension (16.6%) and patients matched for cardiovascular risk (7.1%; matching including smoking habits, BMI, blood pressure and glucose and lipid metabolism) (Fagianno *et al.* 2003, Colao *et al.* 1999, Petramala *et al.* 2015, Albiger *et al.* 2006).

Endothelial dysfunction is thought to be one of the main events linked to atherosclerosis development, and an early marker of the disease. The endothelium (the inner part of blood vessels) has a homeostatic role, keeping the balance between vasodilatation and vasoconstructiction, among other mechanisms. In case of imbalance endothelial dysfunction occurs, and the artery wall is damaged (Davignon *et al.* 2004). Patients with CS have a higher endothelial dysfunction in comparison to healthy controls (Akaza *et al.* 2010, Baykan *et al.* 2007, Kirilov *et al.* 2003). However, this may be reversible after CS cure (Akaza *et al.* 2010, Kirilov *et al.* 2003). Different markers of endothelial dysfunction and/or inflammation have been studied. For instance, high homocysteine levels can be another risk factor for atherosclerosis (Guthikonda *et al.* 2006). They are elevated in active CS, while in patients in remission levels are similar to those in normal controls (Terzolo *et al.* 2004). Elevated endothelin 1 and osteoprotegerin have also been associated with atherosclerosis (Fan *et al.* 2000, Mogelvang *et al.* 2012). They are increased in comparison to controls in CS, and osteoprotegerin is also associated to coronary risk. After cure endothelin levels seem to reverse, but not osteoprotegerin levels (Kirilov *et al.* 2003, Dovio *et al.* 2007, Camozzi *et al.* 2010).

Adipokines are pro-inflammatory peptides that can also contribute to the pathogenesis of atherosclerosis (Valassi *et al.* 2012). There is some controversy in the literature when establishing if their levels significantly differ between CS

patients and controls. Leptin is the adipokine most consistently found to be increased in active CS (Leal *et al.* 1996, Masuzaki *et al.* 1997, Ciza *et al.* 1997, Grottoli *et al.* 2003). Leptin is secreted by adipocytes in proportion to subcutaneous fat and has a role in regulating energy balance (Valassi *et al.* 2012). In female patients with active CS it has been correlated to BMI (Weise *et al.* 1999). Leptin levels seem to decrease after hypercortisolism remission (Widjaja *et al.* 1998, Kresk *et al.* 2004, Ueland *et al.* 2003). In general, plenty of factors seem to contribute to increased risk of atherosclerosis in CS. Some of them may improve after cure, while for others alterations may persist.

1.5.3. Cardiac risk factors

Several studies evidence that CS is associated to cardiac alterations (both functional and structural). Cardiac left ventricular hypertrophy is the feature that has most consistently been described in active CS. Other findings include impaired left ventricular diastolic function, subclinical biventricular systolic dysfunction and subclinical left artrial systolic dysfunction (Muiesan *et al.* 2003, Pereira *et al.* 2010, Kamenicky *et al.* 2014). All these alterations may be reversible after cure (Pereira *et al.* 2010, Kamenicky *et al.* 2014).

Other authors found abnormal left ventricular mass parameters in 70% of the patients, including concentric hypertropathy (42%) and concentric remodelling (23%), while cortisol excess and hypertension contribute to cardiac alterations. There was an improvement after remission, but abnormal values still were more marked than in healthy controls (Toja *et al.* 2012). QT dispersion, an index of spatial dispersion of ventricular recovery times, is increased in patients with CD in comparison to normal controls, indicating repolarization alterations (Alexandraki *et al.* 2011).

A sympathovagal imbalance has also been found in CS, characterized by an increased parasympathetic activity (Fallo *et al.* 2009, Chandran *et al.* 2013). Additionally, CS patients also present a reduced heart rate variability, which has been related to disease duration, age, basal cortisol levels and systolic blood pressure (Chandran *et al.* 2013)

Recently coronary calcifications have been studied in CS. Both calcified plaques and non-calcified coronary plaques were increased in comparison to controls (Neary *et al.* 2013). These alterations seem to persist after remission and show a tendency to higher values in comparison to controls. Coronary calcifications have been found in 31% of CS patients after long-term cure, while non-calcified plaques were found in 20% of the patients (Barahona *et al.* 2013).

1.5.4. Thrombotic risk factors

CS has been related to thrombotic disorders as hypercortisolism can induce hypercoagulability (Coelho *et al.* 2015). This higher predisposition for blood clots production determines a high risk of venous thromboembolism. The risk is approximately 1.9-2.5% when not provoked by surgery, although after surgery rates range from 0 to 5.6%, resulting fatal in 0-1.9% of the patients. Only in one study post surgery risk rates were of 20%, although the sample size was small (10 patients), and it was performed in the 80's. (Van Zaane *et al.* 2009, Rees *et al.* 2002, Zografos *et al.* 2006, Small *et al.* 1983).

Hypercortisolism leads to prothrombotic alterations. On the one hand it determines an increase in coagulation factors (including fibrinogen, factor VIII, IX, XI, XII and von Willebrand factor) (Fatti *et al.* 2000, Casonato *et al.* 1999, Erem *et al.* 2009 , Manetti *et al.* 2010, Patrassi *et al.* 1985). On the other hand, it impairs fibrinolysis reducing its activity (increasing total plasminogen activity

inhibitor and decreasing tissue factor pathway inhibitors) (Erem *et al.* 2009, Manetti *et al.* 2010). Not all these alterations are seen at the same time and in the same patients, although most of the patients have an increase in von Willebrand factor and factor VIII and a reduction in activated partial thromboplastin time (Daidone *et al.* 2011, Isidori *et al.* 2015). The rise of platelets, endothelial dysfunction and hyperhomocysteinemia, present in CS, also have an important role in the prothrombotic state pathogenesis and predispose to a higher cardiovascular risk (Davignon *et al.* 2004, Sato *et al.* 1984, Terzolo *et al.* 2004).

Patients with CS have an increased risk of venous thromboembolism (hazard ratio 2.6), which seems to be already present 3 years before CS is diagnosed. The higher risk corresponds to the first year after diagnosis (hazard ratio 20.6), although it still remains elevated during long-term follow-up (Dekkers *et al.* 2013). However, some thrombotic factors as von Willebrand factor and both VIII and IX factors seem to normalize after successful treatment of CS (van Zaane *et al.* 2009).

1.6. Cardiovascular risk and white matter lesions

As established in the previous section, CS has been associated to a higher cardiovascular risk which usually persists after cure (Colao *et al.* 1999, Barahona *et al.* 2009, de Leo *et al.* 2010, Mancini *et al.* 2004). High cardiovascular risk has been linked to the presence of white matter lesions (WML) in the brain (Gorelick *et al.* 2011, Jeerakathil *et al.* 2004). These kind of lesions have also been referred in the literature as leukoaraiosis or white matter hyperintensities (see *Figure 4*). It is believed that their origin is ischaemic, caused by the obstruction of small cerebral vessels, leading to ischaemia (either

acute or chronic) (Mataró *et al.* 2014). They can be evidenced on brain MRI as hyperintensities on T2 or FLAIR images, usually corresponding to mild gliosis and myelin loss in brain areas (Debette *et al.* 2010). According to immunohistochemistry studies (performed in both aging population and dementia), oligodendroglia apoptosis, activated microglia, upregulated markers of hypoxia and activated microglia are found when analyzing WML (Black *et al.* 2009).

WML are usually found in elderly populations and tend to increase with age. However, they are also very common in populations with a high cardiovascular risk, as hypercholesterolemia, hypertension or diabetes (Verdelho *et al.* 2007). Due to their inherent cardiovascular risk, CS would be very likely to have a high degree of WML. However, to date no studies have been conducted on WML in patients with CS.

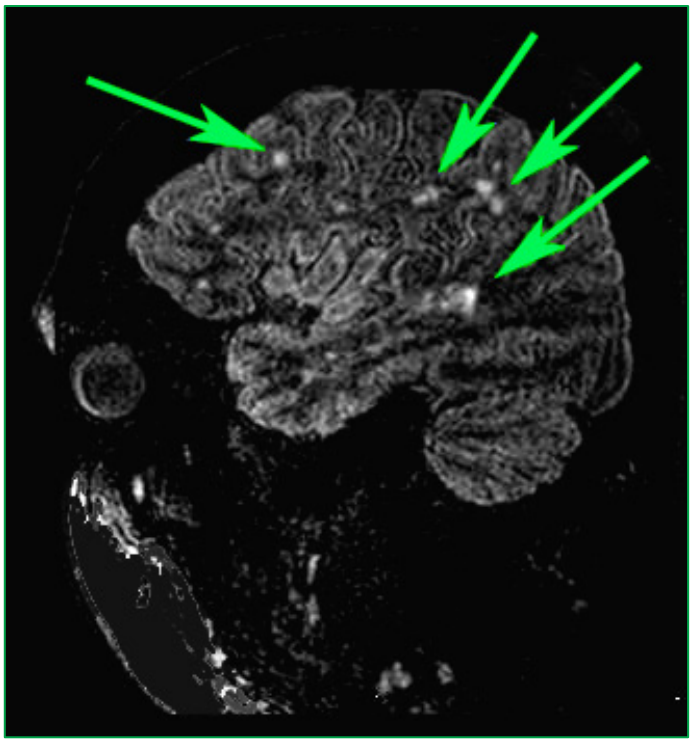


Figure 4: White matter lesions

This would deserve investigation in CS, as WML are not innocuous and can have important clinical implications. They have been related to impaired cognition, mainly executive functioning, but also information processing speed, memory, attention and psychomotor speed (Debette *et al.* 2010, Mataró *et al.* 2014). In addition, they have also been associated with an increased risk of dementia,

stroke and even death (Gorelick 2011). Lesions may worsen over time and increase in number and volume. The most consistent predictor of progression appears to be baseline severity, although other factors include female sex and high blood pressure. When WML progression occurs it is usually associated with cognitive decline (Mataró *et al.* 2014, Xiong *et al.* 2011).

1.7. Cushing's syndrome: brain and cognition

Most of the knowledge we have nowadays on the effects of hypercortisolism on brain and cognition in CS comes from studies developed during the last decades. Nevertheless, initial studies on this topic were conducted a long time ago. The first brain observations (conducted during autopsy) date back to the 1950's, while first neuropsychological evaluations date back to the 80's.

1.7.1. Cushing's syndrome: brain

First autopsy reports described an enlargement of ventricles in patients that had suffered CS (Trethowan *et al.* 1952). This enlargement has usually been related to brain atrophy. Several studies would also report ventricle enlargement, brain atrophy or smaller brain volumes in patients with CS, in comparison to controls, although there is still some controversy on possible recovery after cure (Momose *et al.* 1971, Bourdeau *et al.* 2002, Simmons *et al.* 2000, Resmini *et al.* 2012, Gnjidic *et al.* 2008). In 1971, a pneumoencephalography study revealed that cerebellar atrophy was also present in active CS (Momose *et al.* 1971). The technique used by the authors was not as precise as the ones available nowadays, and no specific measurements on white and grey matter were established. No neuropsychological evaluation was performed either. At that time the

cerebellum was only considered a structure related to coordination, motor control and muscle tone adjustment. However, more recently it has been related to emotional control and cognition, specifically to frontal/executive functions, visual memory, verbal working memory, declarative and procedural memory visuospacial skills, information processing speed, language, fluency and emotional processing (Tirapu-Ustarroz *et al.* 2011, Eckert *et al.* 2010, Schmahmann *et al.* 1998). Surprisingly, no studies had dealt with cerebellar volume and its neuropsychological correlates until now in CS. Only one study conducted in patients in remission found greater volumes of the left posterior lobe of the cerebellum in comparison to controls. However, these findings were the result of a whole brain analysis, where regions with remarkable differences between patients and controls were highlighted, but no analysis was performed on the whole cerebellar volume alone as a possible region of interest (Andela *et al.* 2013).

Several studies have focused on the hippocampus, which is the main brain structure related to memory. Apparently in the active phase its volume is reduced in comparison to normal controls and correlates with plasma cortisol levels and learning and memory deficits, although not with urinary free cortisol levels (Starkman *et al.* 1992). There is some controversy on the possible recovery after cure. According to some studies, successful treatment can only lead to partial recovery (Starkman *et al.* 1999, Starkman *et al.* 2003). Studies performed in patients in remission did not find differences in hippocampal volumes (Resmini *et al.* 2012, Andela *et al.* 2013), although impaired functioning was evidenced with metabolite abnormalities measured by spectroscopy (lower N-Acetyl-aspartate and N-Acetyl-aspartate + N-Acetyl-aspartyl-Glutamate, markers of neuronal dysfunction or loss; as well as higher

Glutamate + Glutamine, indicating glial proliferation as a repair mechanism) (Resmini *et al.* 2013). Only patients with severe memory impairments did have smaller hippocampal volumes in comparison to their matched controls. This group was older, with less years of education and had a trend towards longer duration of hypercortisolism in comparison to the remaining patients (Resmini *et al.* 2012).

Other brain areas also seem to be reduced in CS. In children with active CS, a reduction has been observed in the amygdala in comparison to normal controls, which does not seem to reverse after cure (in contrast to total cerebral volume) (Merke *et al.* 2005). However, studies performed in adult patients in remission did not find any differences in grey matter amygdala volumes between patients and controls (Andela *et al.* 2013). In the same study, differences were found for anterior cingulate cortex volumes, which were smaller in patients with CS than in controls (Andela *et al.* 2013).

The frontal lobe can also be affected by hypercortisolism. Changes in cerebral metabolites have been found in the frontal lobe of patients with active CS, as well as in thalamic areas. They were evidenced as decreased choline/creatine ratios (Khiat *et al.* 1999). Choline levels significantly recovered after successful cure (Khiat *et al.* 2000). However, decreased frontal cortical thickness has been found in eucortisolemic patients in comparison to normal controls. This was evidenced in the left superior frontal cortex, left precentral cortex, left insular cortex, right caudal middle frontal cortex and left and right rostral anterior cingulate cortex (Crespo *et al.* 2014).

Diffusion-tensor-imaging (DTI) have also demonstrated alterations in white matter integrity in both active CS patients and CS patients in remission, evidenced by fractional anisotropy reductions and increased values for mean

diffusivity, axial diffusivity and radial diffusivity (van der Werff *et al.* 2014, Pires *et al.* 2014). These findings suggest a loss of white matter integrity and brain demyelization.

Regarding functional studies, altered activation in brain structures relevant to emotion perception, processing and regulation has been found in patients with CS. More specifically, patients had less activation in the left anterior superior temporal gyrus and higher activation in frontal, medial, and subcortical regions, in comparison to controls (Langenecker *et al.* 2012). Adolescents with CS show increased activation in the amygdala and hippocampus during face encoding (Maheu *et al.* 2008). Patients in remission show less activation in the medial prefrontal-orbitofrontal cortex than controls when processing emotional faces, while no differences have been found for amygdala activation (Bas-Hoogendam *et al.* 2015)

1.7.2. Cushing's syndrome: cognition

The first study dealing with neuropsychological performance in CS was performed in 1980. Two thirds of the patients showed some kind of impairment. The degree of deficit was variable and corresponded to a wide range of neuropsychological functions (Whelan *et al.* 1980). Many studies emerged later to establish the neuropsychological profile in patients with CS.

Memory can be considered the most studied cognitive function in CS. Patients usually complain of poor memory performance, and studies have consistently demonstrated impairment in both visual and verbal memory (Whelan *et al.* 1980, Forget *et al.* 2000, León-Carrión *et al.* 2009, Mauri *et al.* 1993, Starkman *et al.* 2001, Michaud *et al.* 2009). Memory performace shows a trend for a negative correlation with urinary free cortisol levels (Starkman *et al.*

2001). The reversability of these alterations is still a matter of debate. Most of the studies have found some degree of improvement after disease remission (Mauri *et al.* 1993, Martignoni *et al.* 1992, Starkman *et al.* 2003, Hook *et al.* 2007), associated with a decrease in serum cortisol and urinary free cortisol decrease (Starkman *et al.* 2003, Hook *et al.* 2007), while only one study did not find any improvement (Forget *et al.* 2002). Nevertheless, more recent data on patients after long-term remission suggests that some degree of memory impairment may still be present after endocrine cure (Resmini *et al.* 2012, Tiemensma *et al.* 2010). Memory performance in patients in remission is negatively associated with urinary free cortisol levels (Resmini *et al.* 2012) and positively associated with duration of remission (Tiemensma *et al.* 2010).

For other cognitive functions, there is still some controversy. Even if data are consistent regarding memory, where alterations during the active disease have repeatedly been reported, results differ for other neuropsychological funtions. Impairment in executive functions, attention, visuoconstructive functions, language, information processing speed and motor functions are also present in patients with CS, although less consistently (Forget *et al.* 2000, Michaud 2009, Starkman 1992, Crespo *et al.* 2014, Whelan 1980). *Table 1*, on page 45, summarizes findings in different studies.

Despite controversy, several studies have found some degree of impairment in attention or executive functions. The latter involve multiple capacities that help to regulate behaviour and skills, as the ability to focus attention and select relevant information, planning, abstract thinking, inhibition, rule acquisition or flexibility. When a pattern of high number of intrusions or repetitions in neuropsychological tests is found, it is usually an indicator of executive dysfunction. This has been reported in some studies, involving both active CS

patients and CS patients in remission (León-Carrión *et al.* 2009, Michaud *et al.* 2009, Tiemensma *et al.* 2010).

Working memory, measured by Digit Span Backwards, seems to be impaired in active CS disease, although it may improve after cure (Forget *et al.* 2000, Mauri *et al.* 1993, Tiemensma *et al.* 2010, Ragnarsson *et al.* 2012). On the contrary, studies reporting performance on verbal fluency (both letter and category) and inhibition (measured by the Stroop Test) are controversial, as some studies report a poor performance in comparison to controls and others do not (Forget *et al.* 2000, León-Carrión *et al.* 2009, Mauri *et al.* 1993, Tiemensma *et al.* 2010, Ragnarsson *et al.* 2012). Impairment may still be present after cure, although there is controversy for verbal fluency (Tiemensma *et al.* 2010, Ragnarsson *et al.* 2012). Planning, as well as decision making, has only been evaluated once. Planning was measured using the Tower of Hanoi, where patients performed similarly to controls (León-Carrión *et al.* 2009). Decision making, measured with the Iowa Gambling Task, showed a poor performance in eucortisolemic CS patients (Crespo *et al.* 2014).

Regarding attention, studies are also controversial regarding selective attention (measured with the Digit Span Forward and Visual Target Detection), both when reporting performance in active CS patients or improvement after cure (Forget *et al.* 2000, León-Carrión *et al.* 2009, Mauri *et al.* 1993, Hook *et al.* 2007, Michaud *et al.* 2009, Forget *et al.* 2002). Nevertheless impairment seems to be present in patients after long-term remission, both in selective and sustained attention (Tiemensma *et al.* 2010, Ragnarsson *et al.* 2012).

Table 1: Summary of findings related to cognition in different studies dealing with Cushing's syndrome:

Cognitive functions			Impairment or poorer performance than controls found?		Improvement after cure?	
			YES	*NO*	*YES*	*NO*
Memory	***Verbal***		142, 190, 68, 106, 119, 160, 169		119, 116, 161, 84	69
	Visual		142, 190, 68, 119, 121, 169	160		69
Executive functions and attention	***Working memory***		68, 119, 139	169		
	Verbal fluency		68, 139	119, 169	84	
	Inhibition		68	106, 169		
	Planning			106		
	Decision making		42			
	Attention	***Selective***	68, 121, 169	106, 119	119	84, 69
		Sustained	139			
Other functions	***Visuoconstructive skills***		68, 119, 160, 121, 69, 53	106	69, 53	
	Language		68, 119, 160	121	161	
	Motor functions		190, 68	119, 160, 169, 139		
	Information processing speed		68, 119, 53, 169, 139	160	53	

Footnote: *Turquoise colour: The study involved a sample that included both active CS patients and CS patients in remission together.* ***Violet colour****: Results in patients in remission.* ***Black colour:*** *Results in patients with active CS.*

Most studies dealing with CS have found some sort of impairment in other cognitive functions, although not all agree on the exact functions impaired. Regarding visuoconstructive functions, most studies have found poorer performance than controls in different tasks (including Object Assembly, Block Design and Visual Reproduction) (Forget *et al.* 2000, León-Carrión *et al.* 1999, Mauri *et al.* 2003, Michaud *et al.* 2009,), negatively correlated with urinary free cortisol levels (Starkman *et al.* 2001). Performance may improve after endocrine cure (Mauri *et al.* 2003, Hook *et al.* 2007, Forget *et al.* 2002). No data are available for CS patients in remission.

Information processing speed, measured by performance on the Symbol Dygit Substitution Test also seems to be impaired in CS patients, both active and in remission, although it may improve after cure (Forget *et al.* 2000, Mauri *et al.* 1993, Starkman *et al.* 2001, Michaud *et al.* 2009, Forget *et al.* 2002, Ragnarsson *et al.* 2012, Dorn *et al.* 2000). Scores are negatively correlated with urinary free cortisol in active CS patients (Starkman *et al.* 2001).

Regarding language, part of the studies found poorer performance than controls in Vocabulary and Comprehension (Forget *et al.* 2000, Mauri *et al.* 1993, Starkman *et al.* 2001) while other did not (Michaud *et al.* 2009). One study suggested that 25% of the CS patients improved their performance on Vocabulary after disease remission (Starkman *et al.* 2003).

For motor functions, results are also controversial. In one study, impaired performance was found in 40% of the patients for the Purdue test, while another study found no differences in comparison to normal controls (Whelan *et al.* 1980, Starkman *et al.* 2001). Scores in Trail Making Test A showed poorer performance than controls in one study, while not in another (Forget *et al.*

2000, Mauri *et al.* 1993), although performance in patients in remission of CS seemed to be normal (Tiemensma *et al.* 2010, Ragnarsson *et al.* 2012).

1.8. Cushing's syndrome: psychological comorbidities and quality of life

Cushing's syndrome has repeatedly been linked to psychopathology. Most of the studies describe depression and anxiety as usual comorbidities in CS, which may persist after cure (Starkman *et al.* 1981, Whelan *et al.* 1980, Dorn *et al.* 2000, Kelly 1996, Sonino *et al.* 1998, Tiemensma *et al.* 2010, Ragnarsson *et al.* 2012, Andela *et al.* 2013). Depression may be considered the most usual comorbidity, ocurring in approximately 54-57% of the patients (Sonino *et al.* 1998, Kelly 1996). In a minority of patients suicidal thoughts may also be present (Starkman *et al.* 1981, Dorn *et al.* 1997). Depression has been associated to female sex, older age, higher basal urinary cortisol, more severe clinical condition and absence of detectable pituitary adenoma (Sonino *et al.* 1998).

Emotional lability is also very common during the active phase of CS, and sometimes can take the form of irritability (Starkman *et al.* 1981). It may persist after cure. In fact, some authors report that in patients in remission 36% of the patients had clinically significant scores in the irritability scale, and 44% of the patients in the apathy scale. When comparing scores on both scales with matched controls, even if they were higher in CS, significant differences were only found for apathy, while a tendency was found for irritability. Increased social fobia (measured with the Phobia Subscale of the Fear Questionnaire) was also found in patients in remission of CS in comparison to matched controls (Andela *et al.* 2013).

In a minority of patients expansive euphoric affect with episodes of hyperactivity, and also paranoid trends have been described, which tend to disappear after disease progression (Starkman *et al.* 1981). Other alterations including insomnia and reduced libido or impotence, have also been described in patients with CS (Starkman *et al.* 1981, Martínez-Momblán *et al.* 2015, Gotch 1994).

Even if some degree of psychopathology may persist after cure, successful treatment seems to improve symptomatology. Improvement seems to be parallel to the recovery of the hypothalamic-pituitary-axis (Dorn *et al.* 1997). Some studies have detected improvements after treatment in several subscales of the Symptom Checklist 90, including Anxiety, Depression, Somatization, Obsessive- Compulsive and Paranoid Ideation (Starkman *et al.* 2007). Nevertheless, the percentage of patients that do not improve, or even worsen after cure should not be neglected. Interestingly, the recently published Endocrine Society Guidelines recommend that patients receive monitoring and treatment of psychiatric disorders, as well as providing education to patients and their families (Nieman *et al.* 2015).

Few studies have assessed personality in CS. In patients in remission no differences were found in any personality dimension of the Tridimensional personality questionnaire (Sonino *et al.* 2006). However, a study performed in a larger sample found an increased prevalence of maladaptative personality traits. Patients had worse performance than non-functioning pituitary adenomas in 11 of 18 personality scales of the Dimensional Assessment of Personality Pathology short-form. When compared to healthy controls they had worse performance in 10 scales, although when depression and anxiety were included as covariates

only affective lability and anxiousness remained significant (Tiemensma *et al.* 2010).

QoL is also impaired in CS. Patients have poorer QoL than healthy controls and patients with other pituitary adenomas (Lindslay *et al.* 2006, Lindholm *et al.* 2001, Hawn *et al.* 2002, Johnson *et al.* 2003, van der Klaauw *et al.* 2008). Active CS patients have even worse QoL than patients in remission (Lindslay *et al.* 2006, Lindholm *et al.* 2001, Webb *et al.* 2008, Santos *et al.* 2012). However, some degree of impairment seems to persist after cure (van Aken *et al.* 2002, Heald *et al.* 2004, Lindslay *et al.* 2006). Both physical and psychological factors may influence QoL. Depression seems to be the main predictor of QoL, when assessed with the disease-specific CushingQoL questionnaire (Valassi *et al.* 2011). Female gender has also been related to poorer QoL (Webb *et al.* 2008). On the contrary, QoL seems to be independent from the kind of treatment received (pituitary surgery, adrenal surgery or irradiation) (Wahenmakers *et al.* 2012).

CS patients in remission seem to have worse illness perceptions (measured with the Illness Perception Questionnaire) than reference data from patients with vestibular Schwannoma and acute and chronic pain. Illness perceptions negatively correlate with QoL (measured with the EuroQoL-VAS and CushingQoL), while perceived treatment control shows positive correlations with QoL (Tiemensma *et al.* 2011). It is also important to remember that recurrence rates after 10 years reach 25% (Arnaldi *et al.* 2009), and many patients can feel the fear of having a possible recurrence in the future (Andela *et al.* 2015).

When patients have been asked through a questionnaire about their perception of their disease, 71% reported that CS affected their life “greatly”, and

20% “a lot”; 80% of the patients referred that this affected their family life, due to interpersonal conflicts arising form their irritable mood states and feelings of being left out due their fatigue and weakness. Fifty-six % reported that it affected their work/school performance. Some of them had to quit studies or jobs, or even were fired. Interestingly, 56% of the patients found patient education to be helpful for coping with CS (Gotch 1994). Patient education is a key issue that has recently begun to receive more attention (Nieman *et al.* 2015, Martinez-Momblán *et al.* 2015). Future studies and clinical practice should take it into consideration in the future, as a tool to improve patients QoL.

2. AIMS

The general aims of this thesis were to investigate cerebellar volumes and brain white matter lesions in patients with Cushing's syndrome, and their relationship with neuropsychological performance, clinical and hormonal status. The specific aims were:

I. Analyze cerebellar volume in patients with CS (separating white and grey matter) **(Study I).**

II. Study the relationship between cerebellar volume and neuropsychological performance, cortisol levels and other clinical parameters **(Study I).**

III. Investigate the presence of brain white matter lesions in patients with CS **(Study II).**

IV. Investigate the relationship between cardiovascular risk, white matter lesions, neuropsychological performance and brain volume in CS patients **(Study II).**

3. STUDIES

Methods and results can be found in the two papers that comprise this thesis. Details on the studies are included below; a copy of each paper can be found on the following pages.

Study I:

Santos A, Resmini E, Crespo I, Pires I, Vives-Gilabert Y, Granell E, Valassi E, Gómez-Ansón B, Martínez-Momblán MA, Mataró M, Webb SM. **Small cerebellar cortex volume in patients with active Cushing's syndrome.** *Eur J Endocrinol*, 2014 Oct; 171(4):461-9.

Study II:

Santos A, Resmini E, Gómez-Ansón B, Crespo I, Granell E, Valassi E, Pires I, Vives-Gilabert Y, Martínez-Momblán MA, de Juan M, Mataró M, Webb SM. **Cardiovascular risk and white matter lesions after endocrine control of Cushing's syndrome.** *Eur J Endocrinol* 2015 Dec; 173(6):765-75.

Small cerebellar cortex volume in patients with active Cushing's syndrome

Alicia Santos[1,†], Eugenia Resmini[1], Iris Crespo[1], Patricia Pires[2], Yolanda Vives-Gilabert[2], Esther Granell[3], Elena Valassi[1], Beatriz Gómez-Anson[3], M Antonia Martínez-Momblán[1,4], Maria Mataró[5] and **Susan M Webb[1]**

[1]Endocrinology/Medicine Departments, Hospital Sant Pau, Centro de Investigación Biomédica en Red de Enfermedades Raras (CIBER-ER, Unidad 747), ISCIII, IIB-Sant Pau, Universitat Autònoma de Barcelona (UAB), 08193 Bellaterra (Cerdanyola del Vallès), Spain, [2]INNDACYT, Avenida Europa 20, Hospitalet de Llobregat, Barcelona, Spain, [3]Neuroradiology Unit, Hospital Sant Pau, and IIB-Sant Pau, Universitat Autònoma de Barcelona (UAB), Barcelona, Spain, [4]Escola Universitària d'Infermeria, Hospital Sant Pau. Universitat Autònoma de Barcelona (UAB), Barcelona, Spain and [5]Department of Psychiatry and Clinical Psychobiology, Psychology Faculty, Institute for Brain, Cognition and Behaviour (IR3C), Universitat de Barcelona (UB), Barcelona, Spain
[†]A Santos is now at Department of Endocrinology, Hospital de la Santa Creu i Sant Pau, C/Sant Antoni Maria Claret 167, 08025 Barcelona, Spain

Correspondence should be addressed to A Santos
Email
asantos@santpau.cat

European Journal of Endocrinology

Abstract

Objective: Cushing's syndrome (CS) is associated with neuropsychological deficits. As the cerebellum plays a key role in neuropsychological functions it may be affected in CS. The aim of this study was to investigate whether patients with CS have a smaller cerebellar volume than healthy controls, and to analyse whether cerebellar volume is associated with neuropsychological performance and clinical parameters.
Design: A cross-sectional study was performed.
Methods: Thirty-six CS patients (15 with active CS and 21 with CS in remission) and 36 controls matched for age, sex, and education underwent neuropsychological testing, quality of life assessment, clinical evaluation, and magnetic resonance imaging brain scan. Cerebellar volumes (white matter and cortex, bilateral) were calculated using FreeSurfer Software.
Results: Patients with active CS showed smaller bilateral cerebellar cortex volumes than controls (left, $P=0.035$ and right, $P=0.034$), as well as a trend toward smaller right cerebellar cortex volumes than patients in remission CS ($P=0.051$). No differences were observed in the volume of cerebellar white matter between the three groups. Both right and left cerebellar cortex volumes correlated negatively with triglyceride levels (right: $r=-0.358$, $P=0.002$ and left: $r=-0.317$, $P=0.005$) and age at diagnosis (right: $r=-0.433$, $P=0.008$ and left: $r=-0.457$, $P=0.005$). Left cerebellar cortex volume also correlated positively with visual memory performance ($r=0.245$, $P=0.038$). Right cerebellar cortex volume positively correlated with quality-of-life scores ($r=0.468$, $P=0.004$).
Conclusions: The cerebellar cortex volume is smaller in active CS patients than in controls. This finding is associated with poor visual memory and quality of life and is mostly pronounced in patients with higher triglyceride levels and older age at diagnosis.

European Journal of Endocrinology (2014) **171**, 461–469

Introduction

Cushing's syndrome (CS) is a rare disease caused by chronic glucocorticoid excess. It is characterized by central obesity, moon face, muscle weakness, red or purple striae, easy bruising, bone loss, hypertension, fatigue, lack of libido, emotional lability, and depression (1, 2, 3). It has also been associated with cognitive impairment, particularly affecting memory and frontal functions (4, 5, 6, 7, 8, 9).

www.eje-online.org
DOI: 10.1530/EJE-14-0371

Published by Bioscientifica Ltd.

Chronic exposure to excess glucocorticoids can exert a neurotoxic effect (10, 11). In CS, this has been specifically associated with aging-like effects on the brain (12). Aging is associated with annual decreases in the volume of whole brain, the hippocampus, and the cerebellum (13, 14, 15). Whole brain and hippocampal atrophy and neuropsychological dysfunction have been described in CS, but consensus is lacking about the reversibility of the effects of cortisol on the brain after biochemical control (6, 7, 8, 9, 16, 17, 18, 19). Furthermore, information regarding the cerebellum in CS is scarce. A 1971 study suggested that cerebellar atrophy was involved in CS, but no neuropsychological evaluation was performed and imaging techniques were not as precise as they are today (20).

The cerebellum has classically been associated with coordination, motor control, and muscle tone adjustments during movement to keep balance. However, it has long been suspected that the cerebellum has other roles, not only because it has multiple connections to cortical and subcortical brain regions, but also because more than half of the brain neurons are located in the cerebellum (21). Recently, the cerebellum has been related to emotional control and cognition, and linked to frontal/executive functions, visuospacial skills, visual memory, verbal working memory, declarative and procedural memory, information processing speed, language, fluency, and emotional processing (21, 22, 23). Cerebellar atrophy has been found in depression and in posttraumatic stress disorder, conditions in which hypercortisolism seems to have an important role (24). A reduction in cerebellar volumes has also been observed in patients with rheumatoid arthritis, a population that requires long-term glucocorticoid treatment (25). Taken together, these data suggest that hypercortisolism is involved in cerebellar volume reduction.

Our aim was to investigate whether patients with CS have a smaller cerebellar volume than healthy controls, and to analyse whether cerebellar volume is associated with neuropsychological performance, clinical parameters, and cortisol levels.

Patients and methods

Patients

CS patients who were routinely followed at Hospital de la Santa Creu i Sant Pau were recruited during their routine endocrinology visits. Healthy controls were donors from the blood donor service and individuals who had participated in previous studies at the center. All patients and controls gave signed informed consent to participate in the study, which was approved by the Hospital Ethics Committee.

Thirty-six CS patients (15 patients with active CS and 21 with CS in remission) and 36 matched controls were included in the study (Table 1). Each patient was matched to a control participant of the same sex, age (±3 years), and years of education (±3 years) to prevent the influence of age, sex, and education level. All patients and controls were right handed as the study involved brain magnetic resonance imaging (MRI; Edinburgh Handness Inventory >80) (26). Exclusion criteria for both CS patients and controls were as follows: age >65 years, growth hormone (GH) deficiency, history of drug or alcohol abuse, brain damage, and severe psychiatric or neurological illness. Diabetes mellitus was also considered as an exclusion criterion as it has been associated with a reduction in cerebellar volume (27). For controls, exclusion criteria additionally included history of endocrine disease or glucocorticoid exposure.

CS patients were considered in remission after surgery if they presented adrenal insufficiency or if morning cortisol suppression (<50 nmol/l) was observed after 1 mg dexamethasone overnight (28), and if repeated

Table 1 Clinical characteristics of CS patients and controls.

	CS in remission ($n=21$)	**Active CS** ($n=15$)	**Controls** ($n=36$)	***P***
Age (years)	41.9±10.4	44.2±9.3	42.7±9.9	NS
Sex (female/male)	17/4	13/2	30/6	NS
Years of education	13.1±3.4	13.7±2.7	13.4±3.5	NS
Origin of CS	18 pituitary and three adrenal	Ten pituitary, three adrenal, one AIMAH, and one ectopic	–	NS
Duration of hypercortisolism (months)	61.8±32.2	62.2±59.1	–	NS
Delay to diagnosis (months)	42.4±32.9	47.4±51.6	–	NS
Age at diagnosis	34.9±9.3	40.2±9.6	–	NS

CS, Cushing's syndrome; AIMAH, ACTH-independent macronodular adrenal hyperplasia.

24-h urinary free cortisol measures were within the normal range (<280 nmol/24 h). Patients who did not fulfill these criteria were considered active.

Clinical interview, neuropsychological assessment, and biochemistry

Patients and controls underwent a complete clinical interview that included demographic and clinical data, family and medical history, current medical treatment and blood pressure, height, weight, and waist circumference assessment. CS patients were also asked about the history of their disease. Information was completed with data collection from their clinical files.

Duration of hypercortisolism was estimated as the time from symptom onset (in months) to the date of hypercortisolism remission (or current date in active patients), as previously described (9). The time from symptom onset was estimated through a detailed patient interview and from review of clinical notes and photographs. Delay to diagnosis was considered as the time from symptom onset (in months) to the date of diagnosis of CS.

Both patients and controls performed a battery of neuropsychological tests related to cerebellar functions. Only total scores were included in the analysis, except for the Wisconsin Card Sorting Test (WCST), where mean time and perseverative errors were recorded. We specifically used the Grooved Pegboard (dominant and non-dominant hand) and the Trail Making Test A to evaluate motor functions, the Object Assembly and Block Design from WAIS-III for visuoconstructive functions, the Boston Naming Test and Vocabulary from WAIS-III for language, the Rey-Osterrieth Complex Figure for visual memory, the Symbol Digit Modality Test and WCST (mean time) for information processing speed, and Animals, FAS, WCST (perseverative errors), Digit Span Backwards and Trail Making Test B for executive functions. Table 2 provides further information about each test. Participants also performed two complementary questionnaires to assess depression (BDI-II) and anxiety (STAI), both the actual state (STAI State) and the personality trait (STAI Trait).

CS patients also completed a disease-specific quality-of-life questionnaire, the Cushing QoL (29). All neuropsychological assessments were performed by the same neuropsychologist (A Santos) to avoid inter-examiner variability.

The participants underwent blood and urine tests. We used a 24-h collection to assess urinary free cortisol using

Table 2 Description of the neuropsychological tests performed (in alphabetical order).

	Test's name	Description
1	Animals	A test that measures semantic fluency. Patients have to enumerate all the animals they can recall in 1 min
2	Block Design (from Wechsler Adult Intelligence Scale (WAIS-III))	This task evaluates visuospacial skills. Patients have to build figures following a model in a picture card. They have to use cubes with different colored sides (white, red or half white, and half red)
3	Boston Naming Test (abbreviated version)	A language test that measures denomination. Patients are asked to name the pictures they are shown
4	Digit Span Backwards (from WAIS-III)	This test measures working memory. The patients have to repeat a series of numbers backwards (i.e. '719' would be '917')
5	FAS	This test assesses phonetic fluency. Patients have to enumerate all the words they can in 1 min. Words must begin with a specific letter (i.e. 'F')
6	Grooved Pegboard	A test that measures fine motor skills (coordination and motor speed). Patients have to insert some pegs in a pegboard, as quick as possible. They will do it with both the dominant (DH) and non-dominant hand (NDH)
7	Object Assembly (from WAIS-III)	This test measures visuospatial skills. It consists in assembling a series of puzzles, as quickly as possible
8	Rey-Osterrieth Complex Figure (ROCF)	A test that assesses visual memory. Patients have to copy a figure looking to a model and then draw it again without the model. They will also have to draw it again after 20 min
9	Symbol Digit Modality Test (SDMT)	A test that measures information processing speed, divided attention, visual scanning, and tracking. The patients see a model were symbols correspond to numbers. A sequence of symbols is shown. Using the model, patients will have to say the numbers that correspond to symbols as quickly as possible
10	Trail Making Test B (TMTB)	This test measures divided attention. The patients have to connect numbers and letters in ascending order (i.e. 1-a–2-b–3-c)
11	Vocabulary (from WAIS-III)	A test that assesses language. The patient has to say the meaning of different words
12	Wisconsin Card Sorting Test (WCST)	A computerized test that measures cognitive flexibility. Four model cards are shown to patients. They have to match their cards with one of the models, trying to find the correct criteria (i.e. color). Criteria change over time

a commercial RIA. Standard assay methods were used to assess cholesterol, triglyceride, and glucose levels from blood samples.

MRI and cerebellar volumes

MRI was obtained using a 3-Tesla Philips Achieva scanner (software version 2.1.3.2) and a specific acquisition protocol: 3DMPRAGE whole-brain sequence (repetition time = 6.7 ms; echo time = 3.1 ms, 170 slices; and voxel size = 0.889 × 8.889 × 1.2).

All images were postprocessed by the Port d'Informació Científica (PIC) at the Universitat Autònoma de Barcelona using the FreeSurfer Software (http://surfer.nmr.mgh.harvard.edu/). The volumes of right and left white matter and right and left cortex were obtained. The total cerebellar volume was calculated by adding up all these volumes.

Volumetric segmentation was performed automatically using FreeSurfer version 4.3.1 Image Analysis Software (http://surfer.nmr.mgh.harvard.edu/). This software is composed of 170 HP blades with two quad-cores CPU (Hewlett Packard), each one with 16 GB of RAM, running over Scientific Linux version 5 (https://www.scientificlinux.org/). FreeSurfer processing includes motion correction, removal of non-brain tissue using a hybrid watershed/surface deformation procedure (30), automated Talairach transformation, and segmentation of the subcortical white matter and deep grey matter volumetric structures (31, 32). Postprocessing was launched using the PICNIC tool (https://neuroweb.pic.es). Both the automated image processing and the visual check were done by a single, blinded investigator.

All volumetric scores where normalized to the estimated intracranial volume of each individual, as previously described (9).

Statistical analysis

Statistical analysis was performed using IBM SPSS 21 Software (SPSS, Inc.). Normal distribution was analyzed using the Kolmogorov–Smirnov test. Comparisons between groups were performed using ANOVA followed by a Bonferroni's correction. Differences were considered significant when $P<0.05$. The χ^2 test was used to compare categorical variables, and correlations were assessed using Pearson's coefficient. Data are reported as mean ± S.D.

Direct scores of the tests were transformed into *Z*-scores to obtain a general score for each cognitive function. The general scores for each cognitive function were obtained by calculating the mean between the *Z*-scores of the tests included for each cognitive function. Tests in which higher scores were not related to a better performance were inverted.

Results

No differences were found between groups for age, sex, or education level. Table 1 summarizes clinical and demographic characteristics of CS patients and controls.

Patients in remission included 21 CS patients (17 females) who achieved cure of their hypercortisolism after treatment: 18 were of pituitary origin and three of adrenal origin. At the time of the study, six were on hydrocortisone therapy for adrenal insufficiency after surgery, five were taking antidepressants, and five had had previous radiotherapy.

Active patients included 15 CS patients (13 females) with active hypercortisolism: ten of pituitary origin, three of adrenal origin, one ectopic adrenocorticotropic hormone (ACTH) secretion of unknown origin, and one ACTH-independent macronodular adrenal hyperplasia (AIMAH). Fourteen of the 15 active patients were taking medication: 11 were on ketoconazole or metyrapone treatment, one was taking cabergoline, one was taking losartan treatment (a patient with AIMAH who responded to angiotensin receptor antagonists), and one was taking antidepressants.

Total cerebellar volume was calculated. Both grey matter (cortex) and white matter were analyzed separately

Table 3 Mean cerebellar volumes in patients and controls.

	CS in remission ($n=21$)	**Active CS** ($n=15$)	**Controls** ($n=36$)
Left cerebellar cortex (mm^3)	43 170 ± 3626	40 853 ± 3757[a]	44 110 ± 4444
Right cerebellar cortex (mm^3)	43 742 ± 3403	40 634 ± 3178[a]	43 646 ± 4146
Left cerebellar white matter (mm^3)	15 501 ± 1959	14 721 ± 2838	15 065 ± 1577
Right cerebellar white matter (mm^3)	15 747 ± 1871	14 971 ± 2740	15 276 ± 1605
Total cerebellar volume (mm^3)	118 160 ± 9924	111 179 ± 11 417	118 097 ± 10 577

[a]Significant differences between active CS patients and controls ($P<0.05$).

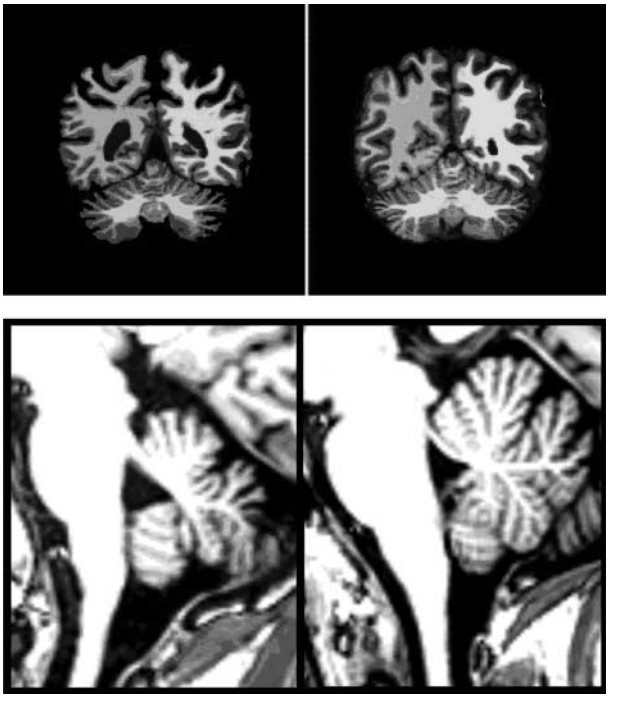


Figure 1
Images obtained from FreeSurfer and MRI (T1), corresponding to an active CS patient (left) and its matched healthy control (right). Reduction in cerebellar volume can be observed in the CS patient.

for each cerebellar hemisphere. Table 3 shows mean cerebellar volumes.

No differences were found between groups for total cerebellar volumes. However, in patients with active CS, cerebellar cortex volumes were smaller than those in controls (left, $P=0.035$ and right, $P=0.034$). The results did not change when the patient taking antidepressants and the corresponding healthy control were excluded from the analysis (left, $P=0.046$ and right, $P=0.032$). In contrast, cerebellar cortex volumes in CS patients in remission did not differ from those in controls. When the two CS patient groups were compared, active patients also tended to have a smaller right cerebellar cortex volume than patients in remission ($P=0.051$). No differences were found in cerebellar white matter volumes between the three groups. Figure 1 shows an example of the MRI of a patient and her matched control.

Regarding neuropsychological functions, patients with active CS had a poorer performance in visual memory than controls ($P=0.006$), and a tendency for poorer performance when compared with patients in remission ($P=0.066$). Visual memory correlated with left cerebellar cortex volumes ($r=0.245$, $P=0.038$). No difference between groups was found for other functions (fine motor skills, visuoconstructive function, language, information processing speed, or executive function; Fig. 2). Table 4 gives mean and S.D.s of each neuropsychological test. Furthermore, both patient groups had more depression (both $P<0.001$) and anxiety scores than controls (STAI State, $P=0.014$ for active CS and $P=0.024$ for patients in remission; STAI Trait, $P<0.001$ for both patient groups), although no difference was found between patients with active disease or those in remission. No correlation was found between these scores and cerebellar volumes.

Regarding clinical parameters, active CS patients had higher urinary free cortisol ($P<0.001$), triglycerides ($P=0.010$), waist circumference ($P=0.001$), systolic blood pressure ($P=0.006$), and diastolic blood pressure ($P<0.001$) than controls. CS patients in remission showed higher waist circumference ($P=0.031$), systolic blood pressure ($P=0.002$), and diastolic blood pressure ($P<0.001$) than controls. They also showed a tendency to higher triglycerides ($P=0.062$). When the whole sample was analyzed, only triglycerides correlated negatively with right and left cerebellar cortex volumes (right: $r=-0.358$, $P=0.002$ and left: $r=-0.317$, $P=0.005$). Both right and

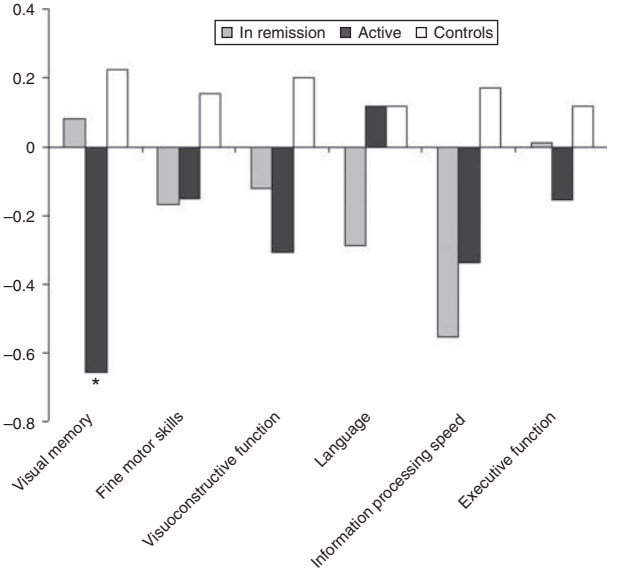


Figure 2
Z-scores of each cognitive function in CS patients (active and in remission) and controls, obtained from neuropsychological testing. *Significant differences between active CS patients and controls ($P=0.006$).

Table 4 Mean and SDS of neuropsychological tests.

				P value		
	CS in remission	Active CS	Controls	CS in remission vs controls	Active CS vs controls	CS in remission vs active
Visual memory						
ROCF immediate	21.3±6.5	16.4±5.7	21.7±6.7	1.000	**0.028**	0.086
ROCF delayed	20.8±6.4	15.8±4.8	22.3±6.7	1.000	**0.004**	0.062
Fine motor skills						
Grooved DH[a]	71.0±11.4	69.5±11.4	64.4±8.2	0.057	0.285	1.000
Grooved NDH[a]	72.8±10.5	73.9±14.2	72.6±11.8	1.000	1.000	1.000
Visuoconstructive						
Object Assembly	28.8±9.1	26.2±6.9	32.8±12.9	0.649	0.191	1.000
Block Design	40.2±21.1	38.5±11.2	44.1±13.2	0.808	0.449	1.000
Language						
Vocabulary	39.7±9.2	43.7±6.8	43.1±8.8	0.429	1.000	0.490
Boston Naming Test	12.8±1.3	13.3±1.0	13.4±1.5	0.446	1.000	0.987
Information processing speed						
Symbol Digit	62.6±10.8	57.9±10.2	61.9±10.9	1.000	0.685	0.604
WCST (mean time)[a]	2281.4±927.1	2371.2±675.4	1869.9±556.3	0.102	0.064	1.000
Executive						
WCST (perseverative errors)[a]	10.4±9.9	11.4±10.5	10.8±11.0	1.000	1.000	1.000
TMTB[a]	58.6±17.5	72.1±27.5	58.8±19.2	0.126	1.000	0.175
Digits Backwards	5.5±1.5	6.0±1.9	5.9±1.7	1.000	1.000	1.000
FAS	42.9±9.4	39.5±10.0	45.8±14.5	0.314	1.000	1.000
Animals	26.2±4.9	25.60±4.5	25.3±5.5	1.000	1.000	1.000

ROCF, Rey-Osterrieth Complex Figure; DH, dominant hand; NDH, non-dominant hand; WCST, Wisconsin Card Sorting Test; TMTB, Trail Making Test B. Total scores are given except when indicated in brackets.
[a]For these tests higher scores indicate poorer performance (Grooved DH, Grooved NDH, WCST mean time, WCST perseverative errors, and TMTB).

left cerebellar cortex volumes were negatively correlated to age at diagnosis (right: $r=-0.433$, $P=0.008$ and left: $r=-0.457$, $P=0.005$). Current age did not correlate with cerebellar volumes in active CS or patients in remission, but a correlation was found in the control group (right: $r=-0.426$, $P=0.010$ and left: $r=-0.559$, $P=0.000$).

The volume of the right cerebellar cortex correlated positively with the Cushing QoL quality-of-life scores ($r=0.468$, $P=0.004$). No correlations were found for cerebellar volumes (white matter and cortex), levels of cholesterol, glucose and urinary free cortisol, duration of hypercortisolism, or delay to diagnosis.

Discussion

CS is associated with a decrease in whole brain and hippocampal volumes, although its reversibility after biochemical control of hypercortisolism is still a matter of debate (9, 16, 18, 19, 33). In this study, we found that the cerebellar cortex (grey matter) was smaller in patients with active hypercortisolism than in control individuals. Glucocorticoids have been linked to cell death in brain regions such as the cerebellum and hippocampus (34). They are also known to impair neurogenesis, present in both these structures (35). Glucocorticoids and stress can also modify dendritic structure, reducing synapsis and dendritic atrophy (dendritic simplification and retraction) (36, 37). As dendrites are part of the cortex, our results could also be explained by a reduction in dentrites induced by glucocorticoids.

In contrast with these findings in active patients, the cerebellar cortex was not smaller in CS patients in remission. Based on these data, we can speculate that hypercortisolemia leads to cerebellar shrinkage, which may be partially reversible after cure. Several studies have found that hippocampal volume increases after cure, so it is feasible that other brain structures may also improve after remission (16, 19). Our results are in line with a recent study in CS patients in remission that did not find the volume of cerebellar grey matter was smaller. In fact, the authors reported that the left posterior lobe of the cerebellum was larger (33). They suggested that neuronal reorganization after chronic stress could lead to dendrite atrophy in parts of the brain or to dendritic hypertrophy in others. This could explain the larger cerebellar volume that they found in contrast with smaller volumes in

other structures. Following this hypothesis, even if dendritic atrophy occurred during the active phase of the disease, it could be compensated by a dendritic hypertrophy after biochemical cure. Longitudinal studies would be necessary to confirm these hypotheses.

In this study, smaller cerebellar cortex volumes were associated with several clinical variables. First, age at diagnosis correlated negatively with cerebellar volume, meaning that the volume of cerebellar cortex was smaller in patients who were diagnosed at an older age. Current age did not correlate with cerebellar volumes in active patients or in patients in remission, so the correlation with age at diagnosis may be related to other factors, such as exposure to hypercortisolism. The capacity for neurogenesis decreases significantly throughout life (38). Exposure to glucocorticoids at a later age may therefore be related to an increased risk of cerebellar reduction, as the capacity of neurons to regenerate is reduced with aging.

Second, we found a negative correlation between triglyceride levels and the volume of the cerebellar cortex, meaning that elevated triglycerides were related to a smaller cerebellar cortex volume. This is consistent with a study in which rats fed a medium-chain triglyceride supplemented diet had an aging-like effect in the cerebellar cortex, leading to a lower number of synapsis and synaptic mitochondria (39). The authors claimed that this diet could have aging or anti-aging effects, depending on the neuronal vulnerability of the cells, and the cerebellar cortex is particularly vulnerable to age. Therefore, high triglyceride levels, together with cortisol exposure, could have an aging-like effect in the cerebellar cortex in CS patients, leading to a volumetric reduction. Control of triglyceride levels may be helpful to avoid cerebellar impairment. However, further studies on dyslipidemia and cerebellar function are needed.

Third, quality of life, evaluated with the disease-specific Cushing QoL questionnaire, correlated directly with right cerebellar cortex volume. This relation between quality of life and cerebellar cortex has not been reported previously, but since the cerebellum plays a role in the modulation of emotional responses (23), it may also be involved in self-perceived quality of life. In patients with right cerebellar insults, lesion size has been correlated with the severity of depression (40). Indeed, the cerebellum has been associated with emotional processing. Although we did not find any correlation between depression and cerebellar cortex, it is feasible that there may be a certain degree of lateralization for emotional processing, mood, and depression in the cerebellum.

Regarding neuropsychological results, visual memory performance correlated with left cerebellar cortex volume. Even if memory has classically been related to the hippocampus, the cerebellum also seems to play a role in visual memory (21, 23). Specifically, visuospatial memory has been associated with the left-superior-posterior lobe of the cerebellum in both children with early deprivation and normal children (41). Our data show that patients with smaller left cerebellar volumes have poorer visual memory skills. This could lead to problems such as difficulties in remembering information from maps, or losing one's way in unknown places. These findings may have implications during the diagnostic process because neuropsychological impairment may be present. Apart from a neuropsychological evaluation, a detailed neurological examination may be helpful to identify further impairment.

It is also of note that patients in remission showed poorer neuropsychological performance than active patients in language and information processing speed, although this difference was not statistically significant. CS predisposes to chronic inflammation and cerebrovascular disease, even after cure (42). Long-term inflammation has been associated with impaired information processing speed (43), while cerebrovascular disease can lead to vascular dementia. Interestingly, in patients with vascular dementia, language function progressively declines (44). Patients in remission have been exposed to inflammation and cerebrovascular risk for a longer time than active patients, which may explain their worse performance. As language tends to be more preserved than information processing speed with normal aging (45), it is conceivable that newly diagnosed patients with active hypercortisolism still maintain normal language function.

A smaller cerebellar volume may have other clinical implications. As this portion of the brain is related to motor control and gait, a smaller volume might account for poor postural control and balance deficits in these patients (46, 47). These deficits may be even more severe in patients with obesity, a symptom also related to impaired balance and postural control (48) and to more falls than in normal-weight subjects (49). The risk of falls should be kept in mind in patients with CS as they have a higher incidence of osteoporosis and therefore a higher risk of fracture. The risk of falls should be specially considered in older patients, as it has been associated with higher morbidity, greater use of health care resources, and even a higher mortality (50). It would be interesting in the future to prospectively study the relationship between cerebellar volume and the number of falls.

This study has several limitations. The first one is the small sample size and the heterogeneity of the sample,

challenges inherent to a rare disease such as CS. The number of patients included in the study was further reduced by the strict exclusion criteria. However, this restriction avoided the influence of factors which could affect cerebellar volume, such as diabetes mellitus, GH deficiency, older age, brain damage, history of drug or alcohol abuse, and severe psychiatric or neurological illnesses. The second limitation is that FreeSurfer is an automatic technique and it may not be as accurate as manual segmentation. It is feasible that measurement noise may have masked clearer correlations with clinical and neuropsychological parameters. A further limitation is that we did not perform a standard neurological evaluation, and such a study might have identified subtle cerebellar dysfunction in these CS patients. A final potential weakness is the cross-sectional design of the study. Longitudinal studies could also confirm whether the cerebellar cortex volume in active patients increases after cure, and if so, how long this takes to occur. Studies analysing the possible benefits of neuropsychological rehabilitation could also lead to improvements in QoL and neuropsychological functions.

In conclusion, we found that patients with active CS have a smaller cerebellar cortex, associated with poorer visual memory, decreased QoL, higher blood triglyceride levels, and older age at diagnosis. The absence of this volumetric reduction in patients in remission is encouraging, suggesting that it may be reversible, at least in part, after cortisol normalization.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

Funding

This work was supported by the Instituto de Salud Carlos III, the Spanish Ministry of Science and Innovation (MICINN, FIS080302), the European Commission (ERCUSYN PHP800200), and AGAUR (2014 SGR 355).

Acknowledgements

The authors thank all the patients and controls who participated in the study.

References

1 Arnaldi G, Angeli A, Atkinson AB, Bertagna X, Cavagnini F, Chrousos GP, Fava GA, Findling JW, Gaillard RC, Grossman AB *et al*. Diagnosis and complications of Cushing's syndrome: a consensus statement. *Journal of Clinical Endocrinology and Metabolism* 2003 **88** 5593–5602. (doi:10.1210/jc.2003-030871)

2 Nieman LK, Biller BM, Findling JW, Newell-Price J, Savage MO, Stewart PM & Montori VM. The diagnosis of Cushing's syndrome: an Endocrine Society clinical practice guideline. *Journal of Clinical Endocrinology and Metabolism* 2008 **93** 1526–1540. (doi:10.1210/jc.2008-0125)

3 Starkman MN, Schteingart DE & Schork MA. Depressed mood and other psychiatric manifestations of Cushing's syndrome: relationship to hormone levels. *Psychosomatic Medicine* 1981 **43** 3–18. (doi:10.1097/00006842-198102000-00002)

4 Starkman MN, Giordani B, Berent S, Schork MA & Schteingart DE. Elevated cortisol levels in Cushing's disease are associated with cognitive decrements. *Psychosomatic Medicine* 2001 **63** 985–993. (doi:10.1097/00006842-200111000-00018)

5 León-Carrión J, Atutxa AM, Mangas MA, Soto-Moreno A, Pumar A, Leon-Justel A, Martín-Rodriguez JF, Venegas E, Domínguez-Morales MR & Leal-Cerro A. A clinical profile of memory impairment in humans due to endogenous glucocorticoid excess. *Clinical Endocrinology* 2009 **70** 192–200. (doi:10.1111/j.1365-2265.2008.03355.x)

6 Dorn LD & Cerrone P. Cognitive function in patients with Cushing's syndrome: a longitudinal perspective. *Clinical Nursing Research* 2000 **9** 420–440. (doi:10.1177/10547730022158672)

7 Forget H, Lacroix A & Cohen H. Persistent cognitive impairment following surgical treatment of Cushing's syndrome. *Psychoneuroendocrinology* 2002 **27** 367–383. (doi:10.1016/S0306-4530(01)00059-2)

8 Tiemensma J, Kokshoorn NE, Biermasz NR, Keijser BJ, Wassenaar MJ, Middelkoop HA, Pereira AM & Romijn JA. Subtle cognitive impairments in patients with long-term cure of Cushing's disease. *Journal of Clinical Endocrinology and Metabolism* 2010 **95** 2699–2714. (doi:10.1210/jc.2009-2032)

9 Resmini E, Santos A, Gómez-Anson B, Vives Y, Pires P, Crespo I, Portella MJ, de Juan-Delago M, Barahona MJ & Webb SM. Verbal and visual memory performance and hippocampal volumes, measured by 3-Tesla magnetic resonance imaging, in patients with Cushing's syndrome. *Journal of Clinical Endocrinology and Metabolism* 2012 **97** 663–671. (doi:10.1210/jc.2011-2231)

10 Joëls M, Karst H, Krugers HJ & Lucassen PJ. Chronic stress: implications for neural morphology, function and neurogenesis. *Frontiers in Neuroendocrinology* 2007 **28** 72–96. (doi:10.1016/j.yfrne.2007.04.001)

11 McEwen BS. Physiology and neurobiology of stress and adaptation: central role of the brain. *Physiological Reviews* 2007 **87** 873–904. (doi:10.1152/physrev.00041.2006)

12 Michaud K, Forget H & Cohen H. Chronic glucocorticoid hypersecretion in Cushing's syndrome exacerbates cognitive aging. *Brain and Cognition* 2009 **71** 1–8. (doi:10.1016/j.bandc.2009.02.013)

13 Enzinger C, Fazekas F, Matthews PM, Ropele S, Schmidt H, Smith S & Schmidt R. Risk factors for progression of brain atrophy in aging: six-year follow-up of normal subjects. *Neurology* 2005 **64** 1704–1711. (doi:10.1212/01.WNL.0000161871.83614.BB)

14 Scahill RI, Frost C, Jenkins R, Whitwell JL, Rossor MN & Fox NC. A longitudinal study of brain volume changes in normal aging using serial registered magnetic resonance imaging. *Archives of Neurology* 2003 **60** 989–994. (doi:10.1001/archneur.60.7.989)

15 Raz N, Lindenberger U, Rodrigue KM, Kennedy KM, Head D, Williamson A, Dahle C, Gerstorf D & Acker JD. Regional brain changes in aging healthy adults: general trends, individual differences and modifiers. *Cerebral Cortex* 2005 **15** 1676–1689. (doi:10.1093/cercor/bhi044)

16 Bourdeau I, Bard C, Noël B, Leclerc I, Cordeau MP, Bélair M, Lesage J, Lafontaine L & Lacroix A. Loss of brain volume in endogenous Cushing's syndrome and its reversibility after correction of hypercortisolism. *Journal of Clinical Endocrinology and Metabolism* 2002 **87** 1949–1954. (doi:10.1210/jcem.87.5.8493)

17 Simmons NE, Do HM, Lipper MH & Laws ER. Cerebral atrophy in Cushing's disease. *Surgical Neurology* 2000 **53** 72–76. (doi:10.1016/S0090-3019(99)00197-4)

18 Gnjidić Z, Sajko T, Kudelić N, Malenica M, Vizner B, Vrkljan M, Hat J & Rumboldt Z. Reversible "brain atrophy" in patients with Cushing's disease. *Collegium Antropologicum* 2008 **32** 1165–1170.

19 Starkman MN, Giordani B, Gebarski SS, Berent S, Schork MA & Schteingart DE. Decrease in cortisol reverses human hippocampal atrophy following treatment of Cushing's disease. *Biological Psychiatry* 1999 **46** 1595–1602. (doi:10.1016/S0006-3223(99)00203-6)

20 Momose KJ, Kjellberg RN & Kliman B. High incidence of cortical atrophy of the cerebral and cerebellar hemispheres in Cushing's disease. *Radiology* 1971 **99** 341–348. (doi:10.1148/99.2.341)

21 Tirapu-Ustarroz J, Luna-Lario P, Iglesias-Fernandez MD & Hernaez-Goni P. Cerebellar contribution to cognitive process: current advances. *Revue Neurologique* 2011 **53** 301–315.

22 Eckert MA, Keren NI, Roberts DR, Calhoun VS & Harris KC. Age-related changes in processing speed: unique contributions of cerebellar and prefrontal cortex. *Frontiers in Human Neuroscience* 2010 **4** 10. (doi:10.3389/neuro.09.010.2010)

23 Schmahmann JD & Sherman JC. The cerebellar cognitive affective syndrome. *Brain* 1998 **121** 561–579. (doi:10.1093/brain/121.4.561)

24 Baldaçara L, Borgio JG, Lacerda AL & Jackowski AP. Cerebellum and psychiatric disorders. *Revista Brasileira de Psiquiatria* 2008 **30** 281–289. (doi:10.1590/S1516-44462008000300016)

25 Bekkelund SI, Pierre-Jerome C, Husby G & Mellgren SI. Quantitative cerebral MR in rheumatoid arthritis. *AJNR. American Journal of Neuroradiology* 1995 **16** 767–772.

26 Oldfield RC. The assessment and analysis of handedness: the Edinburgh inventory. *Neuropsychologia* 1971 **9** 97–113. (doi:10.1016/0028-3932(71)90067-4)

27 Manor B, Newton E, Abduljalil A & Novak V. The relationship between brain volume and walking outcomes in older adults with and without diabetic peripheral neuropathy. *Diabetes Care* 2012 **35** 1907–1912. (doi:10.2337/dc11-2463)

28 Barahona MJ, Sucunza N, Resmini E, Fernández-Real JM, Ricart W, Moreno-Navarrete JM, Puig T, Farrerons J & Webb SM. Persistent body fat mass and inflammatory marker increases after long-term cure of Cushing's syndrome. *Journal of Clinical Endocrinology and Metabolism* 2009 **94** 3365–3371. (doi:10.1210/jc.2009-0766)

29 Webb SM, Badia X, Barahona MJ, Colao A, Strasburger CJ, Tabarin A, van Aken MO, Pivonello R, Stalla G, Lamberts SW *et al*. Evaluation of health-related quality of life in patients with Cushing's syndrome with a new questionnaire. *European Journal of Endocrinology* 2008 **158** 623–630. (doi:10.1530/EJE-07-0762)

30 Ségonne F, Dale AM, Busa E, Glessner M, Salat D, Hahn HK & Fischl B. A hybrid approach to the skull stripping problem in MRI. *NeuroImage* 2004 **22** 1060–1075. (doi:10.1016/j.neuroimage.2004.03.032)

31 Fichl B, Salat DH, Busa E, Albert M, Dieterich M, Haselgrove C, van der Kouwe A, Killiany R, Kennedy D, Klaveness S *et al*. Whole brain segmentation: automated labeling of neuroanatomical structures in the human brain. *Neuron* 2002 **33** 341–355. (doi:10.1016/S0896-6273(02)00569-X)

32 Fichl B, Salat DH, van der Kouwe AJ, Makris N, Ségonne F, Quinn BT & Dale AM. Sequence-independent segmentation of magnetic resonance images. *NeuroImage* 2004 **23** (Suppl 1) S69–S84. (doi:10.1016/j.neuroimage.2004.07.016)

33 Andela CD, van der Werff SJ, Pannekoek JN, van den Berg SM, Meijer OC, van Buchem MA, Rombousts SA, van der Mast RC, Romijn JA, Tiemensma J *et al*. Smaller grey matter volumes in the anterior cingulate cortex and greater cerebellar volumes in patients with long-term remission of Cushing's disease: a case–control study. *European Journal of Endocrinology* 2013 **169** 811–819. (doi:10.1530/EJE-13-0471)

34 Bhatt AJ, Feng Y, Wang J, Famuyide M & Hersey K. Dexamethasone induces apoptosis of progenitor cells in the subventricular zone and dentate gyrus of developing rat brain. *Journal of Neuroscience Research* 2013 **91** 1191–1202. (doi:10.1002/jnr.23232)

35 Wang J, Barak LS, Mook RA Jr & Chen W. Glucocorticoid hedgehog agonists in neurogenesis. *Vitamins and Hormones* 2011 **87** 207–215. (doi:10.1016/B978-0-12-386015-6.00030-5)

36 Tata DA, Marciano VA & Anderson BJ. Synapse loss from chronically elevated glucocorticoids: relationship to neuropil volume and cell number in hippocampal area CA3. *Journal of Comparative Neurology* 2006 **498** 363–374. (doi:10.1002/cne.21071)

37 Kleen JK, Sitomer MT, Killeen PR & Conrad CD. Chronic stress impairs spatial memory and motivation for reward without disrupting motor ability and motivation to explore. *Behavioral Neuroscience* 2006 **120** 842–851. (doi:10.1037/0735-7044.120.4.842)

38 Galvan V & Jin K. Neurogenesis in the aging brain. *Clinical Interventions in Aging* 2007 **2** 605–610.

39 Balietti M, Fattoretti P, Giorgetti B, Casoli T, Di Stefano G, Platano D, Aicardi G, Lattanzio F & Bertoni-Freddari C. Effect of two medium chain triglycerides-supplemented diets on synaptic morphology in the cerebellar cortex of late-adult rats. *Microscopy Research and Technique* 2009 **72** 933–938. (doi:10.1002/jemt.20737)

40 Lauterbach EC, Harris JB & Bina WF III. Mood and neurobehavioral correlates of cerebellar lesions. *Cognitive and Behavioral Neurology* 2010 **23** 63–73. (doi:10.1097/WNN.0b013e3181cfb541)

41 Bauer PM, Hanson JL, Pierson RK, Davidson RJ & Pollak SD. Cerebellar volume and cognitive functioning in children who experienced early deprivation. *Biological Psychiatry* 2009 **66** 1100–1106. (doi:10.1016/j.biopsych.2009.06.014)

42 Resmini E. Persistent comorbidities in Cushing's syndrome after endocrine cure. *Advances in Endocrinology* 2014. (doi:10.1155/2014/231432)

43 Heringa SM, van den Berg E, Reijmer YD, Nijpels G, Stehouwer CG, Teerlink T, Scheffer PG, van den Hurk K, Kappelle LJ, Dekker JM *et al*. Markers of low-grade inflammation and endothelial dysfunction are related to reduced information processing speed and executive functioning in an older population – the Hoorn Study. *Psychoneuroendocrinology* 2014 **40** 108–118. (doi:10.1016/j.psyneuen.2013.11.011)

44 Gustand J, Brickman AM, Paul RH, Browndyke J, Moser DJ, Ott BR, Gordon N, Haque O & Cohen RA. Progressive morphometric and cognitive changes in vascular dementia. *Archives of Clinical Neuropsychology* 2005 **20** 229–241. (doi:10.1016/j.acn.2004.07.001)

45 Scherder E. Functional circuits in cognitive aging and mild cognitive decline. In *Aging and Dementia: Neuropsychology, Motor Skills, and Pain*, ch 2, p 49. Amsterdam: VU University Press, 2011.

46 Dichgans J & Mauritz KH. Patterns and mechanisms of postural instability in patients with cerebellar lesions. *Advances in Neurology* 1983 **39** 633–643.

47 Horak FB & Diener HC. Cerebellar control of postural scaling and central set in stance. *Journal of Neurophysiology* 1994 **72** 479–493.

48 Singh D, Park W, Lewy MS & Jung ES. The effects of obesity and standing time on postural sway during prolonged quiet standing. *Ergonomics* 2009 **52** 977–986. (doi:10.1080/00140130902777636)

49 Fjeldstad C, Fjeldstad AS, Acree LS, Nickel KJ & Gardner AW. The influence of obesity on falls and quality of life. *Dynamic Medicine* 2008 **7** 4. (doi:10.1186/1476-5918-7-4)

50 Rubenstein LZ. Falls in older people: epidemiology, risk factors and strategies for prevention. *Age and Ageing* 2006 **35** (Suppl 2) ii37–ii41. (doi:10.1093/ageing/afl084)

Received 9 May 2014
Revised version received 30 June 2014
Accepted 8 July 2014

Cardiovascular risk and white matter lesions after endocrine control of Cushing's syndrome

Alicia Santos[1], Eugenia Resmini[1], Beatriz Gómez-Ansón[2], Iris Crespo[1], Esther Granell[2], Elena Valassi[1], Patricia Pires[3], Yolanda Vives-Gilabert[3], M Antonia Martínez-Momblán[1,4], Manuel de Juan[2], Maria Mataró[5] and **Susan M Webb[1]**

[1]Endocrinology/Medicine Departments, Hospital Sant Pau, Centro de Investigación Biomédica en Red de Enfermedades Raras (CIBERER, Unidad 747), IIB-Sant Pau, ISCIII and Universitat Autònoma de Barcelona (UAB), Sant Antoni Maria Claret n. 167, 08025 Barcelona, Spain, [2]Neuroradiology Unit, Hospital de Sant Pau, and IIB-Sant Pau, UAB, Barcelona, Spain, [3]INNDACYT, Avda. Europa, 20, planta baja puerta D 08907, Hospitalet de Llobregat, Spain, [4]Escola Universitària d'Infermeria, Hospital de Sant Pau. Universitat Autònoma de Barcelona (UAB), Barcelona, Spain and [5]Department of Psychiatry and Clinical Psychobiology, Psychology Faculty, Institute for Brain, Cognition and Behaviour (IR3C), Universitat de Barcelona (UB), Barcelona, Spain

Correspondence should be addressed to A Santos
Email
asantos@santpau.cat

Abstract

Objective: Cushing's syndrome (CS) is associated with high cardiovascular risk. White matter lesions (WML) are common on brain magnetic resonance imaging (MRI) in patients with increased cardiovascular risk.

Aim: To investigate the relationship between cardiovascular risk, WML, neuropsychological performance and brain volume in CS.

Design/methods: Thirty-eight patients with CS (23 in remission, 15 active) and 38 controls sex-, age- and education-level matched underwent a neuropsychological and clinical evaluation, blood and urine tests and 3Tesla brain MRI. WML were analysed with the Scheltens scale. Ten-year cardiovascular risk (10CVR) and vascular age (VA) were calculated according to an algorithm based on the Framingham heart study.

Results: Patients in remission had a higher degree of WML than controls and active patients ($P<0.001$ and $P=0.008$ respectively), which did not correlate with cognitive performance in any group. WML severity positively correlated with diastolic blood pressure ($r=0.659$, $P=0.001$) and duration of hypertension ($r=0.478$, $P=0.021$) in patients in remission. Both patient groups (active and in remission) had higher 10CVR ($P=0.030$, $P=0.041$) and VA than controls ($P=0.013$, $P=0.039$). Neither the 10CVR nor the VA correlated with WML, although both negatively correlated with cognitive function and brain volume in patients in remission ($P<0.05$). Total brain volume and grey matter volume in both CS patient groups were reduced compared to controls (total volume: active $P=0.006$, in remission $P=0.012$; grey matter: active $P=0.001$, in remission $P=0.003$), with no differences in white matter volume between groups.

Conclusions: Patients in remission of Cushing's syndrome (but not active patients) have more severe white matter lesions than controls, positively correlated with diastolic pressure and duration of hypertension. Ten-year cardiovascular risk and vascular age appear to be negatively correlated with the cognitive function and brain volume in patients in remission of Cushing's syndrome.

European Journal of Endocrinology (2015) **173**, 765–775

European Journal of Endocrinology

Introduction

Cushing's syndrome (CS) is a rare disease due to a chronic glucocorticoid excess. When endogenous, the most common cause is a pituitary adenoma and, less frequently, an adrenal or ectopic tumour (1). CS is associated with severe morbidities and increased mortality, due to systemic complications, that may persist after cure.

Published by Bioscientifica Ltd.

These include central obesity, increased fat mass, reduced bone and lean body mass, excessive fatigue, skin lesions (purple striae, easy bruising, ulceration), hirsutism, hypogonadism, hypertension, insulin resistance and/or diabetes mellitus, dyslipidemia, prothrombotic state, vascular disease, atherosclerosis, depression, anxiety, cognitive decline and impaired health-related quality of life (1, 2, 3, 4, 5, 6). Available treatments include surgery (pituitary adenomectomy, adrenalectomy or excision of the ectopic source of the adrenocorticotrophic hormone (ACTH)), irradiation and cortisol-lowering drugs (7, 8).

Glucocorticoids can be neurotoxic to the brain by modifying both neuronal structure and neurotransmission (3). This can impair adult neurogenesis, lead to region-specific alterations in dendrite and spine morphology (i.e., reducing dendritic length and branching morphology), impair hippocampal long-term potentiation and synaptic plasticity and also imply inappropriate functional responses after a brief stress exposure (9, 10). Reduced brain volume, both general and specifically in the cerebellum or the hippocampus, has been described in CS (11, 12, 13, 14, 15). Patients also present neuropsychological alterations, mainly involving memory. Moreover impairment in executive functions, attention, visuoconstructive functions, language, information processing speed and motor functions are also present in these patients, although less consistently (10, 11, 13, 14, 16, 17). Because some brain and neuropsychological alterations are still present after biochemical cure, it is suggested that the consequences of cortisol excess on the brain are not completely reversible (3, 18, 19).

It is well known that suffering or having suffered CS determines a higher cardiovascular risk (20, 21). Cardiovascular risk has consistently been linked to cognitive impairments and brain white matter lesions (WML) (22). WML (also known as leukoaraiosis) are evidenced on brain MRI as hyperintensities on T2 or FLAIR images and usually correspond to myelin loss and mild gliosis in brain areas (23). In both aging and dementia, immunohistochemistry on WML has revealed activated microglia, clasmatodendritic astrocytosis, oligodendroglia apoptosis and upregulated markers of hypoxia (24). They are supposed to have an ischemic origin, due to the obstruction of small cerebral vessels, which lead to acute or chronic ischaemia (25). These WML are a common finding in patients with high cardiovascular risk, like in hypertension, hypercholesterolemia or diabetes, although they are also common in the elderly, increasing with age (26). WML have important clinical implications, as they have been related to poor cognition, mainly in tests associated to executive functioning but also with information processing speed, memory, attention and psychomotor speed (23, 25). Furthermore, they have also been related to an increased risk of dementia, stroke and death (22). These lesions are not static and may worsen over time, increasing in number and volume. Baseline severity is perhaps the most consistent predictor of progression, although other reported factors are female sex and high blood pressure. WML progression has also been associated with cognitive decline (25, 27).

Patients with CS have increased cardiovascular risk and therefore may be prone to develop WML. Reduced integrity of brain white matter has been reported in CS, indicating axon impairment (28, 29). This has been established using diffusion tension imaging, a non-invasive neuroimaging technique that assesses the motion of water molecules along and across neuronal axons (28). A specific software is needed to perform this kind of analysis. In contrast, WML are characterized by macroscopic lesions, which can be seen and evaluated directly on MRI by physicians in their clinical practice. Therefore, the aim of this study was to investigate the relationship between cardiovascular risk and WML, neuropsychological performance and brain volume in CS. Our hypothesis was that cardiovascular risk would have a role on WML and that patients with CS (both active and in remission) would have more WML than controls. Furthermore, both cardiovascular risk and WML would be related to cognitive deficits and decreased brain volume.

Subjects and methods

Patients

The study included 38 patients with CS (23 in remission, 15 active) routinely followed in our hospital and 38 controls matched for sex, age and years of education ($\pm$3 years). Initially, 59 patients were approached; five were not right-handed and were excluded. From the 54 remaining patients, five declined to participate (citing lack of time or interest), nine had claustrophobia and could not undergo an MRI and two had medical incompatibilities for the MRI. Controls were recruited from the blood donor's center of the hospital and also from other current studies. A matched control was identified and recruited for each patient. Initially, 63 controls were invited to participate in the study; seven were not right-handed and were excluded. From the remaining controls, eight declined to participate (citing lack of time or interest), nine were excluded as they could

not undergo an MRI due to claustrophobia and one was excluded due to exogenous chronic glucocorticoid exposure. The study was approved by the hospital's ethics committee, and all patients and controls signed an informed consent before they were enrolled in the study.

Exclusion criteria for patients with CS were age above 65 years, growth hormone (GH) deficiency, known prior cerebrovascular disease, severe neurological or psychiatric illness and history of drug or alcohol abuse. For controls, endocrine disease and glucocorticoid exposure were additional exclusion criteria. Only right-handed patients with CS and controls were included (Edinburgh Handedness Inventory >80) (30).

Patients were considered biochemically cured of CS after surgery if adrenal insufficiency was demonstrated or if morning cortisol suppression (<50 nmol/l) was observed after 1 mg dexamethasone overnight (16) and if repeated 24-h urinary-free cortisol measures were normal (<280 nmol/l). Patients who did not fulfil these criteria were considered to have active CS.

Clinical interview, neuropsychological assessment and biochemistry

Both patients and controls underwent a complete clinical interview, which included demographic data, clinical history, family history, any current treatments and an assessment of blood pressure, height, weight and waist circumference. The clinical interview for patients with CS also included details on disease history, with a review of clinical files.

Patients and controls also underwent a neuropsychological battery of tests. Tests evaluating functions that have been related to WML in the literature (23, 25) were selected, including Memory: Rey-Osterrieth Complex Figure (31) and Rey Auditory Verbal-Learning Test (32); Attention: Digit Span Forward (33) and Continuous Performance Test II (34); Executive function: Trail Making Test B (35), FAS (36), Animals (37), Wisconsin Card Sorting Test (38) and Digit Span Backwards (33); Information processing speed: Symbol Digit Modalities Test (39), Wisconsin Card Sorting Test (mean time) (38) and Continuous Performance Test – II (hit reaction time); Motor functions: Grooved Pegboard (40) and Trail Making Test A (35). A further description of the tests can be found in Supplementary Table 1, see section on supplementary data given at the end of this article. Individual test scores were converted into Z-scores. In addition, Z-scores where higher scores were related to a poorer performance were inverted to guarantee that all scores were equivalent. A Z-score of each cognitive domain was calculated, as the mean of all Z-scores of the tests selected for a particular cognitive function. Specifically, the cognitive domains included the following scores: Memory: Rey-Osterrieth Complex Figure (immediate and delayed recall) and Rey Auditory Verbal-Learning Test (Rey 5 and Recognition A); Attention: Digit Span Forward (total score) and Continuous Performance Test II (omissions); Executive function: Trail Making Test B (total time), FAS (total score), Animals (total score), Wisconsin Card Sorting Test (perseverative errors) and Digit Span Backwards (total score); Information processing speed: Symbol Digit Modalities Test (total score), Wisconsin Card Sorting Test (mean time) and Continuous Performance Test II (hit reaction time); Motor functions: Grooved Pegboard (total time for both dominant and non-dominant hand) and Trail Making Test A (total time) (41, 42).

Two questionnaires to assess depression (Beck Depression Inventory II (BDI-II)) and anxiety (State Trait Anxiety Inventory (STAI), which assesses both state and trait anxiety) were also included in the study protocol. To rule out an intra-examiner effect, all assessments were performed by one neuropsychologist (A Santos).

Participants also had blood and urine tests. Urinary free cortisol was determined from a 24-h collection using a commercial radioimmunoassay. To assess cholesterol, triglycerides and glucose from blood samples, standard assay determinations were performed.

Magnetic resonance imaging

All participants underwent 3-Tesla magnetic resonance imaging (MRI) of the whole brain. MRI was obtained using a 3-Tesla Philips Achieva facility (Software version 2.1.3.2) and a dedicated acquisition protocol: 3DMPRAGE whole brain sequence (repetition time$=$6.7 ms; echo time$=$3.1 ms, 170 slices; voxel size$=1\times1\times1.2$; field of view$=256\times256\times204$) and FLAIR (repetition time$=$8000 ms; echo time$=$332 ms; voxel size$=1.1\times1.1\times0.6$; field of view$=250\times250\times250$). The complete acquisition protocol lasted approximately 45 min. FLAIR images were analysed by two blinded neuroradiologists, who scored the degree of WML according to the semiquantitative rating scale described by Scheltens (Scheltens scale) (43).

The Scheltens scale can range from 0 to 84 in which a higher score indicates a higher degree of WML. The score is the sum of four subscores rated in a semiquantitave way. It includes white matter hyperintensities in the periventricular area (score: 0–6), the cerebral lobes (score: 0–24), the basal ganglia (score 0–30) and the infratentorial area

(score 0–30). Scores depend on both the presence of lesions in different areas and the size of the lesion.

Furthermore, the routine MRI reports prepared by the radiologists before using the Scheltens scale were collected from the clinical files of patients and controls to analyse in how many the presence of WML had already been registered. This information was categorized in a dichotomic way (detecting WML: yes/no).

Additionally, structural T1 MRIs were processed at the Port d'Informació Científica (PIC) of the Universitat Autonoma de Barcelona. Whole brain, grey matter and white matter volumes were measured using FreeSurfer v5.3 Software (http://surfer.nmr.mgh.harvard.edu/). These volumes were normalised to the estimated intracranial volume (eTIV) of each individual, as previously described (14).

Cardiovascular risk evaluation

Ten-year cardiovascular risk and vascular age were calculated with a sex-specific multivariable risk factor algorithm, which includes sex, age, systolic blood pressure, smoking, diabetes, HDL and total cholesterol, based on the Framingham heart study data (44). This algorithm was developed using the longitudinal data of 8491 patients. The algorithm provides two scores: a percent risk of suffering a cardiovascular event over the next 10 years (10-year cardiovascular risk) and a further quantification of this risk in the form of vascular age/heart age (vascular age).

Statistical analysis

Statistical analysis was performed using IBM SPSS 21 software (SPSS, Inc.). Normal distribution was analysed using the Kolmogorov-Smirnov test. ANOVA followed by a *post hoc* analysis using Bonferroni was used for comparisons between the three groups (controls, active patients and patients in remission). For non-normal data (10-year cardiovascular risk, Scheltens score, Beck depression score and triglyceride level), the Kruskal-Wallis test was used, and the *post hoc* analysis was performed with a Mann Whitney *U* test. Student's *t*-test or Mann Whitney *U* test for non-parametric variables were used when comparing two groups. χ^2 was used to compare categorical variables. Correlations were assessed using the Pearson coefficient or the Sperman's ρ for non-parametric variables. Differences were considered significant when $P<0.05$.

Results

Demographic and clinical characteristics of patients and controls are shown in Table 1. There were no differences between groups for age, sex and educational level, as expected due to prior matching.

Cardiovascular risk

The first analysis was devoted to cardiovascular risk. Regarding the 10-year cardiovascular risk, both CS patient groups (active and in remission) had a higher risk of suffering a cardiovascular event during the next 10 years

Table 1 Clinical and demographic characteristics of patients (CS, active and in remission) and controls.

	CS		
	Active ($n=15$)	In remission ($n=23$)	**Controls** ($n=38$)
Age	44.3±9.3	42.9±10.6	42.7±10.3
Sex (female/male)	13/2	19/4	32/6
Years of education	13.7±2.7	13.2±3.3	13.7±3.3
Origin of CS (pituitary/adrenal/ectopic/AIMAH†)	10/3/1/1	20/3/0/0	–
Surgery (transsphenoidal/adrenal)	1/0	20/3	–
Radiotherapy	–	5	–
Cortisol lowering medication (metyrapone/ketokonazole/ cabergoline/losartan)	4/7/1/1	–	–
Hydrocortisone replacement	–	8	–
Waist circumference (cm)	**98.0±11.7***	**93.1±14.8***	82.2±11.3
Total cholesterol (mmol/l)	5.2±0.7	5.2±1.0	5.1±0.9
Triglycerides (mmol/l)	**1.0 (0.6–2.2)***	**1.2 (0.6–3.9)***	0.7 (0.5–2.4)
Systolic blood pressure (mm/Hg)	**132.0±21.7***	**131.2±21.3***	112.8±15.9
Diastolic blood pressure (mm/Hg)	**84.0±15.6***	**82.3±10.7***	69.9±11.2
Glucose (mmol/l)	4.9±0.54	4.9±0.51	4.8±0.54

*Significant differences between patients and controls ($P<0.05$). All values are expressed as mean±S.D., except for triglycerides, expressed as median and range.
†ACTH-independent macronodular adrenal hyperplasia.

Table 2 Scheltens scale scores in patients (CS, active and in remission) and normal controls. The number of patients and controls who showed each score is indicated.

	Scheltens scale scores[a]																
	0*	1	2	3	4	5	6	7	8	9	10	11	12	13	14	>15	Median and range
Active CS ($n=15$)	5	4	2	2	1	0	0	0	1	0	0	0	0	0	0	0	1; 0–8
In remission CS ($n=23$)	4	4	1	3	1	1	2	1	1	3	0	0	0	1	1	0	3; 0–14
Controls ($n=38$)	17	10	4	2	2	2	1	0	0	0	0	0	0	0	0	0	1; 0–6

[a]Higher scores indicate a higher degree of WML.
*No lesions.

than controls ($P=0.037$ and $P=0.044$ respectively). More specifically, active patients had a 8.1% risk, followed by patients in remission (7.4% risk) and controls (3.9% risk).

Regarding vascular age, both patient groups had a higher vascular age than controls (56.1±16.8 years in active patients with CS, $P=0.013$; 52.5±19.8 years in patients in remission, $P=0.039$; vs 42.1±11.9 years in controls). This was remarkable, as the sample was initially matched for age (mean between 42.7 and 44.3 years). In fact, both patient groups (but not controls) had an increased mean vascular age compared to their current age (active CS 11.8 years more, $P=0.005$; CS in remission 9.6 years more, $P=0.002$).

Regarding specific parameters associated with cardiovascular risk, patients with CS (both in remission and active) had a greater waist circumference ($P=0.005$, $P<0.001$), triglyceride level ($P=0.001$, $P=0.002$), diastolic blood pressure ($P=0.001$, $P<0.001$) and systolic blood pressure ($P=0.001$, $P=0.004$) than controls (Table 1). No differences were found for glucose level, cholesterol level or smoking habits between groups. Patients in remission had a longer hypertension duration (measured in months since diagnosis) than active patients ($P=0.048$).

No differences were found for 10-year cardiovascular risk or vascular age when comparing patients of pituitary or adrenal origin, either active or in remission.

White matter lesions

A second analysis was devoted to WML, which mainly presented as small periventricular foci in patients and controls. Patients with CS in remission had a higher degree of WML than controls and active CS, measured with the Scheltens scale ($P=0.001$ and $P=0.035$ respectively) (Table 2 and Fig. 1). Active patients did not differ in WML compared to controls. There were also significant differences in the number of patients whose routine MRI report described the presence of WML (in remission 73.9%, active 46.7%, controls 28.9%; $P=0.001$).

No differences were found in the Scheltens scale when comparing pituitary and adrenal origin in active patients and patients in remission. However, patients in remission who were on hydrocortisone replacement had a higher degree of WML than patients in remission who were not taking hydrocortisone ($P=0.023$).

Neuropsychological evaluation

A third analysis was devoted to a neuropsychological evaluation (Table 3). Patients with active CS had worse neuropsychological performance in the memory domain

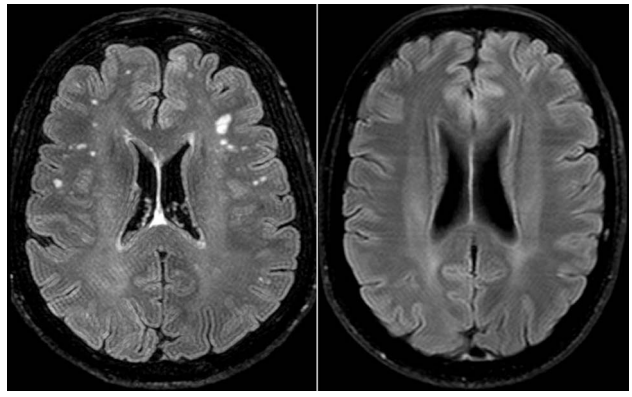


Figure 1
White matter lesions (the white spots seen across the MRI) in a patient with CS in remission (left) in comparison to her matched control (right).

Table 3 *Z*-scores of neuropsychological test results and normalized brain volumes of patients (CS active and in remission) and normal controls.

	CS		
	Active ($n=15$)	In remission ($n=23$)	**Controls** ($n=38$)
Memory (*Z*-scores)[a]	**−0.49±0.46***	−0.05±0.76	0.22±0.66
Attention (*Z*-scores)[b]	0.05±0.76	0.01±0.55	−0.03±0.83
Executive function (*Z*-scores)[c]	−0.14±0.46	0.03±0.39	0.04±0.53
Information processing speed (*Z*-scores)[d]	−0.35±0.65	−0.21±0.86	0.15±0.72
Motor functions (*Z*-scores)[e]	−0.14±1.09	−0.28±1.04	0.22±0.75
Depression (total score)[f]	**9 (3–19)***	**12 (0–23)***	2.5 (0–16)
Anxiety (total scores)[g]			
State	**20.27±10.37***	**20.27±11.15***	11.36±7.56
Trait	**24.93±9.86***	**24.17±9.96***	13.58±7.97
Brain white matter (mm^3)	382 267±64 888	386 751±40 564	408 458±31 648
Brain grey matter (mm^3)	**357 470±33 918***	**365 632±36 581***	397 766±34 190
Total brain volume (mm^3)	**739 737±91 350***	**752 383±73 460***	806 223±53 847

*Significant differences between patients and controls ($P<0.05$). All values are expressed as mean±S.D., except for depression, expressed as median and range.
[a]Includes Rey-Osterrieth Complex figure (immediate and delayed recall) and Rey Auditory Verbal-Learning Test (Rey 5 and Recognition A).
[b]Includes Digit Span Forward (total score) and Continuous Performance Test II (omissions).
[c]Includes Trail Making Test B (total time), FAS (total score), Animals (total score), Wisconsin Card Sorting Test (perseverative errors) and Digit Span Backwards (total score).
[d]Includes Symbol Digit Modalities Test (total score), Wisconsin Card Sorting Test (mean time) and Continuous Performance Test II (Hit Reaction Time).
[e]Includes Grooved Pegboard (total time for both dominant and non-dominant hand) and Trail Making Test A (total time).
[f]Beck Depression Inventory-II.
[g]State Trait Anxiety Inventory.

European Journal of Endocrinology

than controls ($P=0.002$). No differences were found between patients in remission and controls. Both CS patient groups had more anxiety and depression than controls (in remission: STAI-State $P=0.017$, STAI-Trait $P<0.001$, BDI-II $P<0.001$; active CS: STAI-State $P=0.008$, STAI-Trait $P<0.001$, BDI-II $P<0.001$).

No differences were found in cognitive function when comparing patients of pituitary or adrenal origin (active or in remission). In patients in remission, no cognitive differences were found when comparing patients requiring hydrocortisone or not or who had undergone radiotherapy or not. Beck depression scores did not correlate with cognitive function in any of the patient groups.

Brain volumes

A final analysis was devoted to brain volumes (Table 3). Total brain volume and grey matter volume in both CS patient groups were reduced compared to controls (total volume: active CS $P=0.006$, in remission CS $P=0.012$; grey matter: active CS $P=0.001$, in remission CS $P=0.003$). There were no differences in white matter volume between groups. No differences were found in brain volumes when comparing pituitary and adrenal origin in active patients and patients in remission.

Correlations

Neither the 10-year cardiovascular risk nor the vascular age correlated with the Scheltens scale score, although both negatively correlated with cognitive function and brain volume in patients in remission and controls (Table 4). Ten-year cardiovascular risk and vascular age did not correlate with anxiety or depression scores in any group.

The Scheltens scale did not correlate with total brain volume, grey matter, white matter, cognitive domains or depression and anxiety scores, either in the CS patient groups or in the control group (data not shown). WML severity positively correlated with diastolic blood pressure ($r=0.617$, $P=0.002$) and hypertension duration ($r=0.543$, $P=0.007$) in patients in remission. In active patients these clinical parameters did not correlate with the Scheltens scale. The Scheltens scale did not correlate with age, years of education, waist circumference, total cholesterol, glucose, triglycerides, urinary free cortisol, duration of hypercortisolism or delay to diagnosis in any group.

Discussion

Our study demonstrates that 10-year cardiovascular risk and vascular age (as described by the Framingham heart study (44)) are negatively associated with brain volume

Table 4 Correlations of 10-year cardiovascular risk and vascular age with both brain volumes (white matter, grey matter and total brain volume) and cognitive function in CS patients and controls.

	Active CS patients ($n=15$)		**CS patients in remission** ($n=23$)		**Controls** ($n=38$)	
	10-year CV risk (Framingham)*	Vascular age (Framingham)*	10-year CV risk (Framingham)*	Vascular age (Framingham)*	10-year CV risk (Framingham)*	Vascular age (Framingham)*
Brain volume						
White matter volume	None	None	None	None	None	None
Grey matter volume	None	None	**$r=-0.515$, $P=0.012$**	**$r=-0.544$, $P=0.007$**	None	**$r=-0.481$, $P=0.002$**
Total brain volume	None	None	**$r=-0.465$, $P=0.025$**	**$r=-0.486$, $P=0.019$**	None	None
Cognitive function						
Memory	None	None	**$r=-0.442$, $P=0.035$**	**$r=-0.425$, $P=0.043$**	**$r=-0.380$, $P=0.019$**	**$r=-0.467$, $P=0.006$**
Attention	None	None	**$r=-0.508$, $P=0.013$**	**$r=-0.575$, $P=0.004$**	None	None
Executive function	None	None	**$r=-0.759$, $P<0.001$**	**$r=-0.769$, $P<0.001$**	None	**$r=-0.365$, $P=0.024$**
Information processing speed	None	None	**$r=-0.588$, $P=0.003$**	**$r=-0.591$, $P=0.003$**	**$r=-0.465$, $P=0.003$**	**$r=-0.467$, $P=0.003$**
Motor functions	None	None	**$r=-0.560$, $P=0.007$**	**$r=-0.521$, $P=0.013$**	**$r=-0.521$, $P=0.001$**	**$r=-0.484$, $P=0.002$**

*As described in (44). CV, cardiovascular.

and cognitive function in patients in remission of CS. Patients in remission also have a higher degree of WML, measured with the Scheltens scale, than controls and patients with active CS. WML have been related to cardiovascular risk factors in the normal population (26), and our findings also show a relationship between WML and hypertension in patients in remission of CS.

Ten-year cardiovascular risk correlated with both brain volumes and cognitive function in patients in remission of CS. This is in line with previous findings in large samples of normal population (45, 46, 47, 48, 49). Special attention should be paid to high 10-year cardiovascular risk, which may imply decreased cognitive function and reduced brain volumes. Current 10-year cardiovascular risk has also been associated with poorer future executive functions, in line with our results. This association has been found in middle aged but also in young populations (18–30 years), highlighting the importance of controlling cardiovascular risk even at earlier ages (48, 49).

The correlation between cardiovascular risk and both brain volume and cognitive function was not found in active patients with CS. This may be due to the small sample size of the active group, which may have prevented finding significant results. An analysis in a larger sample would be needed to confirm this hypothesis. Additionally, the effects of chronic hypercortisolism, still present in active patients, may affect brain volume and cognitive function more than cardiovascular risk. Hypercortisolism itself causes cognitive dysfunctions and reduces brain volume (10, 11, 12, 13, 14, 15, 16, 17). Possibly in the active population, hypercortisolism plays a more important role than cardiovascular risk on both cognitive function and brain volume. As active patients were affected by current hypercortisolism, it is possible that this situation (and the role of hypercortisolism on cognition and brain volume) may have prevented finding associations between cardiovascular risk and both cognitive function and brain volume.

We have not found any studies correlating vascular age and both brain volumes and cognitive function. The concept of 'vascular age' may be a useful tool in clinical practice for teaching patients, who may understand better that their vascular age is higher than their current age, rather than concepts like 10-year cardiovascular risk. In younger individuals, knowing their 'heart-age' has a high emotional impact, leading to changes in their lifestyle (50). Knowing that vascular age has been linked to poorer cognitive function and reduced brain volumes may also encourage patients to improve their healthy habits. In fact, Canadian guidelines for the diagnosis and treatment of dyslipidemia already recommend communicating vascular age to patients to improve hypertension and lipid control (51).

The fact that WML were increased only in cured patients even if their vascular age was comparable to active patients may be surprising, although it is important to highlight that patients in remission had been exposed to cardiovascular risk for a longer time. In fact, the algorithm for 10-year cardiovascular risk and vascular age only includes current risk factors, not a measure of time exposed to cardiovascular risk. The correlations between WML and length of hypertension exposure are in line with this hypothesis.

According to some authors, the presence of WML has been reported in 11–21% of 64-year-old patients. The presence of WML lesions increases with time, and the percentage of older patients with WML at the age of 82 increases to 94% (23). Taking into account the number of patients whose routine MRI reports described the presence of WML (in remission 73.9%, mean age 44.3±9.3; active 46.7%, mean age 42.9±10.6), it seems that the WML presence in our patient population is higher than expected, maybe in line with the brain-aging effect suggested by some authors in Cushing's syndrome (52).

Our results also showed a relationship between hypertension and WML in patients in remission. Hypertension is a known strong predictor for WML (53, 54). More specifically, the severity of hypertension and bad control in treated hypertensive patients has been associated with the presence of WML (55, 56, 57). These lesions may worsen over time. In uncontrolled hypertension, progression is higher in untreated rather than in treated patients, suggesting that hypertension treatment could reduce WML progression (58).

The duration of hypertension has also been associated with WML in the normal elderly population (57). In fact, apart from current hypertension, hypertension established 5 or 20 years before has also been associated to current WML in the old age (57, 59). This is in line with our results and could explain the differences in WML between active patients and patients in remission. The latter have longer hypertension exposure than active CS, and WML severity positively correlated with hypertension duration in patients in remission. These data indicate that the cause of WML may not be CS itself but rather long-term hypertension exposure caused by CS.

Regarding cognitive function, it is important to highlight that all *Z*-scores were within one s.d. both in active and treated CS, implying no severe impairments in cognition. These data are in line with previous literature in patients in remission in which all *Z*-scores were also within one s.d., even if significant differences in cognition between patients in remission and both normal controls and non-functioning adenomas were found (3). Regarding active patients, according to the literature, greater deficits may be expected (16, 60). Furthermore, significant differences with controls were only found for the memory domain. The fact that most of the active patients were on a cortisol-lowering medication may have prevented finding greater impairment. We had no data on cognitive performance before suffering CS; nevertheless, most of the patients complained about having a poor cognitive performance, mainly memory problems, in comparison to their prior capacities.

WML have been associated with reduced brain volumes and impaired cognitive function (23, 25, 61), although this was not the case in our study. The Scheltens scale scores did not correlate with cognitive function or brain volume in patients with CS. Most of the literature studies on WML have been performed in older populations, where cognitive decline and brain atrophy are more pronounced. It is possible that the younger age of our patients may have prevented finding any significant correlations; however, a higher degree of WML may be related to poorer cognitive performance and reduced brain volumes when they reach an older age, which, given the cross-sectional nature of this study, could not be evaluated.

In fact, even if we did not find correlations between WML and cognitive function, WML are known to predispose to dementia, stroke and cognitive decline in old age (23, 25). This is a key finding with important clinical implications as it implies that this patient cohort may be cognitively compromised in the future. Nevertheless, control of hypertension may prevent progression of WML (56, 57) and reduce the risk of developing dementia and cognitive decline (62, 63). Thus, adequate control of hypertension would appear to be mandatory both in patients in remission, usually exposed to hypertension for a long time and with a higher degree of WML, and in active patients to prevent WML progression at an early stage. Patients should be informed on the risks of inadequate control of hypertension and on healthy habits to control hypertension. Education on drugs that may worsen hypertension such as non-steroidal anti-inflammatory drugs (64, 65) is worth recommending.

Patients in remission taking hydrocortisone had a higher degree of WML than patients who were not on hydrocortisone replacement. This would suggest limiting the use of hydrocortisone as much as possible; in practice, it may be difficult for patients to differentiate symptoms related to adrenal insufficiency from other causes. This

may lead to taking extra doses of hydrocortisone, which may worsen hypertension (66). They should be encouraged to check their blood pressure regularly and to not 'routinely' take extra hydrocortisone doses when suffering from common complaints like tiredness or headache.

This study has several limitations. The small sample size is difficult to avoid in rare diseases, such as CS. It may have led to a low statistical power and possibly prevented the identification of significant findings, mainly in the active patients. Therefore, confirmatory analysis in larger samples of active patients would be needed, for instance, to check if there is a relationship between cardiovascular risk and both cognitive function and brain volume. Another limitation is the heterogeneity of the sample, because different causes of CS have been included, at different stages of the disease. Additionally, for non-parametric data in which differences were identified, no post hoc correction was performed. Longitudinal studies would be necessary to analyse WML progression in active patients and also to investigate the role of hypertension control in both active and in remission CS to prevent WML worsening.

In conclusion, our study describes negative correlations between cardiovascular risk and both brain volume and cognitive function in CS patients in remission. Moreover, it describes a high prevalence of WML in these patients, probably mediated by hypertension. These findings emphasize the importance of controlling cardiovascular risk factors to prevent brain damage in patients who have been diagnosed and treated for CS.

Supplementary data
This is linked to the online version of the paper at http://dx.doi.org/10.1530/EJE-15-0600.

Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

Funding
This work was supported by the Instituto de Salud Carlos III, Spanish Ministry of Science and Innovation (MICINN, FIS080302), the European Commission (ERCUSYN PHP800200) and AGAUR (2014 SGR 355).

Acknowledgements
The authors thank all of the patients and controls that voluntarily participated in the study. We thank Dr Ignasi Gich for the critical review of the statistical analysis.

References

1 Arnaldi G, Angeli A, Atkinson AB, Bertagna X, Cavagnini F, Chrousos GP, Fava GA, Findling JW, Gaillard RC, Grossman AB *et al*. Diagnosis and complications of Cushing's syndrome: a Consensus Statement. *Journal of Clinical Endocrinology and Metabolism* 2003 **88** 5593–5602. (doi:10.1210/jc.2003-030871)

2 Nieman LK, Biller BM, Findling JW, Newell-Price J, Savage MO, Stewart PM & Montori VM. The diagnosis of Cushing's syndrome: an Endocrine Society Clinical Practice Guideline. *Journal of Clinical Endocrinology and Metabolism* 2008 **93** 1526–1540. (doi:10.1210/jc.2008-0125)

3 Tiemensma J, Kokshoorn NE, Biermasz NR, Keijser BJ, Wassenaar MJ, Middelkoop HA, Pereira AM & Romijn JA. Subtle cognitive impairments in patients with long-term cure of Cushing's disease. *Journal of Clinical Endocrinology and Metabolism* 2010 **95** 2699–2714. (doi:10.1210/jc.2009-2032)

4 Tiemensma J, Biermasz NR, Middlekoop HA, van der Masr RC, Romijn JA & Pereira AM. Increased prevalence of psychopathology and maladaptative personality traits after long-term cure of Cushing's disease. *Journal of Clinical Endocrinology and Metabolism* 2010 **95** E129–E141. (doi:10.1210/jc.2010-0512)

5 van Aken MO, Pereira AM, Biermasz NR, van Thiel SW, Hoftijzer HC, Smit JW, Roelfsema F, Lamberts SW & Romijn JA. Quality of life in patients after long-term biochemical cure of Cushing's disease. *Journal of Clinical Endocrinology and Metabolism* 2005 **90** 3279–3286. (doi:10.1210/jc.2004-1375)

6 Lindsay JR, Nansel T, Baid S, Gumowski J & Nieman LK. Long-term impaired quality of life in Cushing's syndrome despite initial improvement after surgical remission. *Journal of Clinical Endocrinology and Metabolism* 2005 **91** 447–453. (doi:10.1210/jc.2005-1058)

7 Arnaldi G & Boscaro M. New treatment guidelines on Cushing's disease. *F1000 Medicine Reports* 2009 **1** 64. (doi:10.3410/M1-64)

8 Biller BM, Grossman AB, Stewart PM, Melmed S, Bertagna X, Bertherat J, Buchfelder M, Colao A, Hermus AR, Hofland LJ *et al*. Treatment of adrenocorticotropin-dependent Cushing's syndrome: a consensus statement. *Journal of Clinical Endocrinology and Metabolism* 2008 **93** 2454–2462. (doi:10.1210/jc.2007-2734)

9 Joëls M, Karst H, Krugers HJ & Lucassen PJ. Chronic stress: implications for neuronal morphology, function and neurogenesis. *Frontiers in Neuroendocrinology* 2007 **28** 72–96. (doi:10.1016/j.yfrne.2007.04.001)

10 Forget H, Lacroix A, Somma M & Cohen H. Cognitive decline in patients with Cushing's syndrome. *Journal of the International Neuropsychological Society* 2000 **6** 20–29. (doi:10.1017/S1355617700611037)

11 Santos A, Resmini E, Crespo I, Pires P, Vives-Gilabert Y, Granell E, Valassi E, Gómez-Ansón B, Martínez Momblán MA, Mataró M *et al*. Small cerebellar cortex volume in patients with active Cushing's syndrome. *European Journal of Endocrinology* 2014 **171** 461–469. (doi:10.1530/EJE-14-0371)

12 Bourdeau I, Bard C, Forget H, Boulanger Y, Cohen H & Lacroix A. Cognitive function and cerebral assessment in patients who have Cushing's syndrome. *Endocrinology and Metabolism Clinics of North America* 2005 **34** 357–369. (doi:10.1016/j.ecl.2005.01.016)

13 Starkman MN, Gebarski SS, Berent S & Schteingart DE. Hippocampal formation volume, memory dysfunction, and cortisol levels in patients with Cushing's syndrome. *Biological Psychiatry* 1992 **32** 756–765. (doi:10.1016/0006-3223(92)90079-F)

14 Resmini E, Santos A, Gómez-Anson B, Vives Y, Pires P, Crespo I, Portella MJ, de Juan-Delago M, Barahona MJ & Webb SM. Verbal and visual memory performance and hippocampal volumes, measured by 3-Tesla magnetic resonance imaging, in patients with Cushing's syndrome. *Journal of Clinical Endocrinology* 2012 **97** 663–671. (doi:10.1210/jc.2011-2231)

15 Andela CD, van Haalen FM, Ragnarsson O, Papakokkinou E, Johannson G, Santos A, Webb SM, Biermasz NR, van der Wee NJ, Pereira AM *et al*. Mechanisms in Endocrinology: Cushing's syndrome causes irreversible effects on the human brain: a systematic review of structural and functional magnetic studies. *European Journal of Endocrinology* 2015 **173** R1–R14. (doi:10.1530/EJE-14-1101)

16 Mauri M, Sinforiani E, Bono G, Vignati F, Berselli ME, Attanasio R & Nappi G. Memory impairment in Cushing's disease. *Acta Neurologica Scandinavica* 1993 **87** 52–55. (doi:10.1111/j.1600-0404.1993.tb04075.x)

17 Crespo I, Granell-Moreno E, Santos A, Valassi E, De Juan-Delago M, Vives-Gilabert Y, Webb SM, Gómez-Ansón B & Resmini E. Impaired decision-making process and thinner prefrontal cortex in patients with Cushing's syndrome. *Clinical Endocrinology* 2014 **81** 826–833. (doi:10.1111/cen.12564)

18 Ragnarsson O, Berglund P, Eder DN & Johannsson G. Long-Term cognitive impairments and attentional deficits in patients with Cushing's disease and cortisol-producing adrenal adenoma in remission. *Journal of Clinical Endocrinology and Metabolism* 2012 **97** 1640–1648. (doi:10.1210/jc.2012-1945)

19 Andela CD, van der Werff SJ, Pannekoek JN, van den Berg SM, Meijer OC, van Buchem MA, Rombousts SA, van der Mast RC, Romijn JA, Tiemensma J *et al*. Smaller grey matter volumes in the anterior cingulate cortex and greater cerebellar volumes in patients with long-term remission of Cushing's disease: a case–control study. *European Journal of Endocrinology* 2013 **169** 811–819. (doi:10.1530/EJE-13-0471)

20 Colao A, Pivonello R, Spiezia S, Faggiano A, Ferone D, Filippella M, Marzullo P, Cerbone G, Siciliani M & Lombardi G. Persistence of increased cardiovascular risk in patients with Cushing's disease after five years of successful cure. *Journal of Clinical Endocrinology and Metabolism* 1999 **84** 2664–2672. (doi:10.1210/jcem.84.8.5896)

21 Barahona MJ, Sucunza N, Resmini E, Fernández-Real JM, Ricart W, Moreno-Navarrete JM, Puig T, Farrerons J & Webb SM. Persistent body fat mass and inflammatory marker increases after long-term cure of Cushing's syndrome. *Journal of Clinical Endocrinology and Metabolism* 2009 **94** 3365–3371. (doi:10.1210/jc.2009-0766)

22 Gorelick PB, Scuteri A, Black SE, Decarli C, Greenberg SM, Iadecola C, Launer LJ, Laurent S, Lopez OL, Nyenhuis D *et al*. Vascular contributions to cognitive impairment and dementia: a statement for healthcare professionals from the American Heart Association/American Stroke Association. *Stroke* 2011 **42** 2672–2713. (doi:10.1161/STR.0b013e3182299496)

23 Debette S & Markus HS. The clinical importance of white matter hyperintesities on brain magnetic resonance imaging: systematic review and meta-analysis. *British Medical Journal* 2010 **331** c3666. (doi:10.1136/bmj.c3666)

24 Black S, Gao F & Bilbao J. Understanding white matter disease: imaging-pathological correlations in vascular cognitive impairment. *Stroke* 2009 **40** S48–S52. (doi:10.1161/STROKEAHA.108.537704)

25 Mataró M, Soriano-Raya JJ, López-Oróliz J, Miralbell J & Dacosta-Aguayo R. Cerebrovascular markers in lowered cognitive function. *British Medical Journal* 2014 **42** (Suppl 4) S383–S391.

26 Verdelho A, Madureira S, Ferro JM, Basile AM, Chabriat H, Erkinjuntti T, Fazekas F, Hennerici M, O'Brien J, Pantoni L *et al*. LADIS study differential impact of cerebral white matter changes, diabetes, hypertension and stroke on cognitive performance among non-disabled elderly. The LADIS study. *Journal of Neurology, Neurosurgery, and Psychiatry* 2007 **78** 1325–1330. (doi:10.1136/jnnp.2006.110361)

27 Xiong YY & Mok V. Age-related white matter changes. *Journal of Aging Research* 2011 **2011** 617927. (doi:10.4061/2011/617927)

28 van der Werff SJ, Andela CD, Nienke Pannekoek J, Meijer OC, van Buchem MA, Rombouts SA, van der Mast RC, Biermasz NR, Pereira AM & van der Wee AJ. Widespread reductions of white matter integrity in patients with long-term remission of Cushing's disease. *NeuroImage. Clinical* 2014 **4** 659–667. (doi:10.1016/j.nicl.2014.01.017)

29 Pires P, Santos A, Vives-Gilabert Y, Webb SM, Sainz-Ruiz A, Resmini E, Crespo I, de Juan-Delago M & Gómez-Anson B. White matter alterations in the brain of patients with active, remitted and cured Cushing's syndrome: A DTI study. *AJNR. American Journal of Neuroradiology* 2015 **36** 1043–1048. (doi:10.3174/ajnr.A4322)

30 Oldfield RC. The assessment and analysis of handedness: the Edinburgh inventory. *Neuropsychologia* 1971 **9** 97–113. (doi:10.1016/0028-3932(71)90067-4)

31 Rey A. *L'examin Clinique en Psychologie*. Paris: Presses Universitaires de France, 1958.

32 Rey A. L'examen psychologique dans les cas d'encephalopathie traumatique. *Archives de Psychologie* 1941 **28** 286–340.

33 Wechsler D. WAIS-III, Wechsler Adult Intelligence Scale-Third Edition. *Administration and Scoring Manual*. San Antonio, TX, USA: The Psychological Corporation, 2001.

34 Conner's CK. Conner's Continuous Performance Test II, Version 5 for Windows (CPT II): Technical Guide and Software Manual. North Tonawanda, NY: Multi-Health Systems, 2008.

35 Reitan R. Trail making test: Manual for administration, scoring, and interpretation. Bloomington: Indiana University, 1956.

36 Benton AL, Hamsher Kd. Multilingual Aphasia Examination. Iowa City: University of Iowa, 1976.

37 Goodglass H, Kaplan E, Barresi B. Boston diagnostic aphasia examination set, 3/E. Philadelphia: Lippincott Williams & Wilkins, 2001.

38 Kongs SK, Thompson LL, Iverson GL, Heaton RK. Wisconsin Card Sorting Test-64 Card Version. Professional Manual. Lutz, FL: Psychological Assessment Resources, 2000.

39 Smith A. Symbol Digit Modalities Test. Los Angeles, California: Western Psychological Services, 1973.

40 Lafayette Instrument Company. Grooved Pegboard: Model 32025; User's Manual. Lafayette: Lafayette Instrument Company, 2003.

41 Lezak MD, Howieson DB, Bigler ED, Tranel D. Neuropsychological assessment. 5th edn, New York: Oxford University Press, 2012.

42 Strauss E, Sherman EM, Spreen O. A compendium of neuropsychological tests, administration, norms and commentary. 3rd edn. NewYork: Oxford University Press, 2006.

43 Scheltens P, Barkhof F, Leys D, Pruvo JP, Naura JJ, Vermersch P, Steinling M & Valk J. A semiquantitative rating scale for the assessment of signal hyperintensities on magnetic resonance imaging. *Journal of the Neurological Sciences* 1993 **114** 7–12. (doi:10.1016/0022-510X(93)90041-V)

44 D'Agostino RB, Vasan RS, Pencina MJ, Wolf PA, Cobain M, Massaro JM & Kannel WB. General cardiovascular risk profile for use in primary care: the Framingham Heart Study. *Circulation* 2008 **117** 743–753. (doi:10.1161/CIRCULATIONAHA.107.699579)

45 Knopman DS, Mosley TH, Catellier DJ, Sharrett AR & Atherosclerosis Risk in Communities (ARIC) Study. Cardiovascular risk factors and cerebral atrophy in a middle-aged cohort. *Neurology* 2005 **65** 876–881. (doi:10.1212/01.wnl.0000176074.09733.a8)

46 Longstreth WT, Arnold AM, Manolio TA, Burke GL, Bryan N, Jungreis CA, O'Leary D, Enright PL & Fried L. Clinical correlates of ventricular and sulcal size on cranial magnetic resonance imaging of 3,301 elderly people. The Cardiovascular Health Study. Collaborative Research Group. *Neuroepidemiology* 2000 **19** 30–42. (doi:10.1159/000026235)

47 Seshadri S, Wolf PA, Beiser A, Elias MF, Au R, Kase CS, D'Agostino RB & DeCarli C. Stroke risk profile, brain volume, and cognitive function: the Framingham Offspring Study. *Neurology* 2004 **63** 1591–1599. (doi:10.1212/01.WNL.0000142968.22691.70)

48 Nishtala A, Preis SR, Beiser A, Devine S, Hankee L, Seshadri S, Wolf PA & Au R. Midlife cardiovascular risk impacts executive function: Framingham offspring study. *Alzheimer Disease and Associated Disorders* 2014 **28** 16–22. (doi:10.1097/WAD.0b013e3182a715bc)

49 Yaffe K, Vittinghoff E, Pletcher MJ, Hoang TD, Launer LJ, Whitmer R, Coker LH & Sidney S. Early adult to midlife cardiovascular risk factors

and cognitive function. *Circulation* 2014 **129** 1560–1567. (doi:10.1161/CIRCULATIONAHA.113.004798)

50 Soureti A, Hurling R, Murray P, van Mechelen W & Cobain M. Evaluation of a cardiovascular disease risk assessment tool for the promotion of healthier lifestyles. *European Journal of Cardiovascular Prevention and Rehabilitation* 2010 **17** 519–523. (doi:10.1097/HJR.0b013e328337ccd3)

51 Anderson TJ, Grégoire J, Hegele RA, Couture P, Mancini GB, McPherson R, Francis GA, Poirier P, Lau DC, Grover S *et al*. 2012 Update of the Canadian Cardiovascular Society guidelines for the diagnosis and treatment of dyslipidemia for the prevention of cardiovascular disease in the adult. *Canadian Journal of Cardiology* 2013 **29** 151–167. (doi:10.1016/j.cjca.2012.11.032)

52 Michaud K, Forget H & Cohen H. Chronic glucocorticoid hypersecretion in Cushing's syndrome exacerbates cognitive aging. *Brain and Cognition* 2009 **71** 1–8. (doi:10.1016/j.bandc.2009.02.013)

53 Longstreth WT Jr, Manolio TA, Arnold A, Burke GL, Bryan N, Jungreis CA, Enright PL, O'Leary D & Fried L. Clinical correlates of white matter findings on cranial magnetic imaging of 3301 elderly people: The Cardiovascular Health Study. *Stroke* 1996 **27** 1274–1282. (doi:10.1161/01.STR.27.8.1274)

54 Basile AM, Pantoni L, Pracucci G, Asplund K, Chabriat H, Erkinjuntti T, Fazekas F, Ferro JM, Hennerici M, O'Brien J *et al*. Age, hypertension and lacunar stroke are the major determinants of the severity of age-related white matter changes. The LADIS (Leukoaraiosis and Disability in the Elderly) Study. *Cerebrovascular Diseases* 2006 **21** 315–322. (doi:10.1159/000091536)

55 Shimada K, Kawamoto A, Matsubayashi K & Ozawa T. Silent cerebrovascular disease in the elderly. Correlation with ambulatory pressure. *Hypertension* 1990 **16** 692–699. (doi:10.1161/01.HYP.16.6.692)

56 Liao D, Cooper L, Cai J, Toole JF, Bryan N, Hutchinson RG & Tyroler HA. Presence and severity of cerebral white matter lesions and hypertension, its treatment and its control. The ARIC Study. *Stroke* 1996 **27** 2262–2270. (doi:10.1161/01.STR.27.12.2262)

57 De Leeuw FE, de Groot JC, Oudkerk M, Witteman JC, Hofman A, van Gijn J & Breteler MM. Hypertension and cerebral white matter lesions in a prospective cohort study. *Brain* 2002 **125** 765–772. (doi:10.1093/brain/125.4.765)

58 Verhaaren BF, Vernooij MW, de Boer R, Hofman A, Niessen WJ, van der Lugt A & Ikram MA. High blood pressure and cerebral white matter lesion progression in the general population. *Hypertension* 2013 **61** 1354–1359. (doi:10.1161/HYPERTENSIONAHA.111.00430)

59 Debette S, Seshadri S, Beiser A, Au R, Himali JJ, Palumbo C, Wolf PA & DeCarli C. Midlife vascular risk factor exposure accelerates structural brain aging and cognitive decline. *Neurology* 2011 **77** 461–468. (doi:10.1212/WNL.0b013e318227b227)

60 Whelan TB, Schteingart DE, Starkman MN & Smith A. Neuropsychological deficits in Cushing's syndrome. *Journal of Nervous and Mental Disease* 1980 **168** 753–757. (doi:10.1097/00005053-198012000-00008)

61 Appelman AP, Exalto LG, van der Graaf Y, Biessels GJ, Mali WP & Geerlings MI. White matter lesions and brain atrophy: more than shared risk factors? A systemiatic review *Cerebrovascular Diseases* 2009 **28** 227–242. (doi:10.1159/000226774)

62 Forette F, Seux ML, Staessen JA, Thijs L, Birkenhäger WH, Babarskiene MR, Babeanu S, Bossini A, Gil-Extremera B, Girerd X *et al*. Prevention of dementia in randomised double-blind placebo-controlled Systolic Hypertension in Europe (Syst-Eur) trial. *Lancet* 1998 **352** 1347–1351. (doi:10.1016/S0140-6736(98)03086-4)

63 Levi Marpillat N, Macquin-Mavier I, Tropeano AI, Bachoud-Levi AC & Maison P. Antihypertensive classes, cognitive decline and incidence of dementia: a network meta-analysis. *Journal of Hypertension* 2013 **31** 1073–1082. (doi:10.1097/HJH.0b013e3283603f53)

64 Johnson AG. NSAIDs and increased blood pressure. What is the clinical significance? *Drug Safety* 1997 **17** 277–289. (doi:10.2165/00002018-199717050-00001)

65 Kalafutova S, Juraskova B & Vlcek J. The impact of combinations of non-steroidal anti-inflammatory drugs and anti-hypertensive agents on blood pressure. *Advances in Clinical and Experimental Medicine* 2014 **23** 993–1000. (doi:10.17219/acem/37357)

66 Sudhir K, Jennings GL, Esler MD, Korner PI, Blombery PA, Lambert GW, Scoggins B & Whitworth JA. Hydrocortisone-induced hypertension in humans: pressor responsiveness and sympathetic function. *Hypertension* 1989 **13** 416–421. (doi:10.1161/01.HYP.13.5.416)

Received 17 June 2015
Revised version received 29 August 2015
Accepted 4 September 2015

4. SUMMARY OF RESULTS AND DISCUSSION

CS has been related to a higher cardiovascular risk, even after successful cure (Colao *et al.* 1999, Mancini *et al.* 2004, Barahona *et al.* 2009, de Leo *et al.* 2010). It has also been associated to cognitive impairments and a decrease in whole brain and hippocampal volumes, although its complete reversibility after biochemical control is still a matter of debate (Momose *et al.* 1971, Bourdeau *et al.* 2002, Simmons *et al.* 2000, Resmini *et al.* 2012, Gnjidic *et al.* 2008, Starkman *et al.* 1999, Starkman *et al.* 2003, Resmini *et al.* 2012, Andela *et al.* 2013). In the present thesis we have analysed if other structures as the cerebellum could be decreased in CS, and have examined the possible relationship between the cerebellum and neuropsychological functions and other clinical parameters. We have also studied if WML are present in the brains of patients with CS and investigated the relationship between cardiovascular risk, white matter lesions, neuropsychological performance and brain volume.

4.1. Cerebellar volumes

Cerebellar atrophy has been described in conditions in which hypercortisolism seems to have an important role, as depression or posttraumatic stress disorder (Baldaçara *et al.* 2008). Smaller cerebellar volumes have also been found in patients with longstanding rheumatoid arthritis, which is a population that requires long-term glucocorticoid treatment

(Bekkelund SI *et al.* 1995). These data suggested that hypercortisolism may have a role in cerebellar volume reduction.

Results from *study I* were in line with this hypothesis. Active CS patients had a smaller cerebellar cortex than controls, indicating less cerebellar grey matter, although no differences were found for cerebellar white matter or total cerebellar volumes. Accordingly, our results indicate that hypercortisolism may lead to a selective grey matter reduction in the cerebellum of active CS patients. Different mechanisms could have led to a volumetric reduction in the cerebellar cortex. Animal studies suggest that glucocorticoid exposure can lead to neurodegeneration and cell death in regions such as the cerebellum, the hippocampus or the prefrontal cortex (Bhatt *et al.* 2013, Hu *et al.* 2015). Interestingly, in the three structures smaller volumes in comparison to normal controls or published norms have been described (Momose *et al.* 1971, Starkman *et al.* 1992, Crespo *et al.* 2014). Another possible mechanism would be related to impaired neurogenesis. Neurogenesis is present in both the cerebellum and the hippocampus, as a mechanism to compensate neural damage. However, glucocorticoids induce neurogenesis reduction, which may also lead to reduced brain volumes (Wang *et al.* 2011). A third mechanism would involve dendritic structure. Both glucocorticoids and stress can modify the dendritic structure, reducing the synapsis number and leading to dendritic atrophy (a simplification of dendrites and retraction) (Tata *et al.* 2006, Kleen *et al.* 2006). Dendrites are part of the brain cortex, so our results could also be explained by a dendritic reduction induced by a chronic glucocorticoid exposure.

In contrast with the findings in active CS, cerebellar cortex volumes in patients in remission of CS were not smaller than those observed in controls.

Putting these data together, we can speculate that hypercortisolemia leads to a cerebellar reduction that may be partially reversible after cortisol normalization. In fact, some studies in CS have found a partial hippocampal volume increase after cure. Accordingly, it would be feasible that other brain structures may also improve after remission (Bourdeau *et al.* 2002, Starkman *et al.* 1999). Some other data in patients in remission of CS are in line with this hypothesis. In a cross-sectional study, even if the authors did not measure the volumes of the whole cerebellum, they reported that its left posterior lobe was larger in comparison to controls. When discussing their results, the authors suggested that neuronal reorganization after chronic stress could lead to dendrite atrophy in some parts of the brain (as in the anterior cingulate cortex, where they found smaller volumes) and to dendritic hypertrophy in others (as the cerebellum) (Andela *et al.* 2013). This would be a plausible explanation for the different results found in active patients and patients in remission. Following this hypothesis, in the cerebellum, even if dendritic atrophy may occur during the active phase of the disease, it may be compensated by dendritic hypertrophy after biochemical cure. This may explain why in our study patients in remission did not have smaller cerebellar cortex volumes. Anyway, longitudinal studies would be necessary to confirm this hypothesis.

It is important to highlight the clinical implications that a smaller cerebellar volume may have. This portion of the brain is related to motor control and gait. A smaller volume might account for poor postural control and balance deficits in these patients (Horak *et al.* 1994, Dichgans *et al.* 1983). In fact, during clinical practice some patients in the active phase of the disease have reported falling, although this issue has not been further investigated. These deficits may be even more severe when obesity is present, also related to impaired balance

and postural control (Singh *et al.* 2009), as well as to more falls than in normal-weight subjects (Fjeldstad *et al.* 2008). The risk of falls should be kept in mind in patients with CS, as they also have an increased risk of osteoporotic fractures. In fact, patients suffer from more low-energy fractures than controls (Vestergaard *et al.* 2002). The risk of falls should be especially considered in older patients, as it has been associated with greater use of health care resources, higher morbidity and even higher mortality (Rubenstein 2006).

4.2. Cerebellum, correlations with neuropsychological performance and clinical parameters

In *study I*, cerebellar cortex volumes were associated with different clinical variables. First of all, age at diagnosis was negatively correlated with bilateral cerebellar volume. This indicated that the cerebellar cortex volume was smaller in patients diagnosed at an older age. Current age did not correlate with cerebellar volumes in active CS patients or in patients in remission; therefore, the correlation with age at diagnosis may be related to other factors, like hypercortisolism exposure. In fact, neurogenesis capacity decreases significantly throughout life (Galvan *et al.* 2007). Consequently, exposure to glucocorticoids at a later age may be a risk factor for higher cerebellar shrinkage, as the capacity of the neurons to regenerate is reduced with aging.

We also found a negative correlation between triglyceride levels and bilateral cerebellar cortex volume. This meant that elevated triglycerides were related to smaller cerebellar cortex volumes. This is consistent with an animal study in which rats fed a medium-chain triglyceride supplemented diet had an aging-like effect in the cerebellar cortex, which led to a reduced number of synapsis and of synaptic mitochondria (Balietti *et al.* 2009). According to the

authors, this diet could have aging or anti-aging effects, depending on neural vulnerability. The cerebellar cortex is particularly vulnerable to age. Therefore, high triglyceride levels, together with hypercortisolemia exposure, could have an aging-like effect in the cerebellar cortex of CS patients, which may lead to a volumetric reduction. Control of triglyceride levels may be helpful in order to avoid cerebellar impairment, although further confirmatory studies on dyslipidemia and cerebellar function would be required.

QoL was evaluated with the disease-specific CushingQoL questionnaire and scores correlated positively with the right cerebellar cortex volume. This relationship between QoL and cerebellar cortex has not been previously reported. Nevertheless, since the cerebellum plays a role modulating emotional responses (Schmahmann *et al.* 1998), it may also be involved in self-perceived QoL. In fact, in patients with right cerebellar insults, lesion size has been correlated with the severity of depression (Lauterbach *et al.* 2010). The cerebellum has also been associated with emotional processing. Although we did not find correlations between depression and cerebellar cortex volumes, it is feasible that there may be some degree of lateralization for emotional processing, mood and depression in the cerebellum.

Regarding neuropsychological results, a positive correlation was found between visual memory performance and left cerebellar cortex volume. Memory has classically been related to the hippocampus, but the cerebellum seems to play a role in visual memory (Tirapu-Ustarroz *et al.* 2011, Schmahmann et al. 1998). More specifically, visuospatial memory has been associated with the left-superior-posterior lobe of the cerebellum in both normal children and children with early deprivation (Bauer *et al.* 2009). According to our data, patients with smaller left cerebellar cortex volumes have poorer visual memory skills. These

findings may have implications during the diagnostic process, as neuropsychological impairment may be present. A neuropsychological evaluation would be important to assess cognitive functions in these patients, as well as a detailed neurological examination may be helpful to identify further impairment.

4.3. Neuropsychological findings and mood

According to *study I*, active CS patients had poorer visual memory than controls. In daily life, this could lead to problems such as difficulties in remembering visual information (as information from maps or charts), or losing one's way in unknown places. However, in *study II*, the memory domain included both visual and verbal memory, and showed that active CS patients also had poorer performance than controls. This would imply that patients may also have difficulties remembering verbal information. In clinical practice this would mean that patients may have difficulties remembering the information provided by doctors, like daily doses of drugs or clinical recommendations. Therefore, it may be helpful to provide extra written information to the patients, to be sure that they will remember the doctor's indications. Furthermore, when explaining to the patients what CS is for the first time, it would also be helpful to provide information brochures that they may be able to read at home, to further understand the disease and its implications. Some of the brochures can be found online, as informative guidelines on the disease (https://pituitarysociety.org/sites/all/pdfs/ Pituitary Society Cushings ES.pdf) or as educational guidelines on the comorbidities of the disease (http://www.lb.de/ercusyn/wMedia/pdf/brochure/Guia educativa para pacientes con sindrome de Cushing .pdf).

When analysing the results in cognitive function in both studies, it is important to highlight that all Z-scores were within one standard deviation of the mean, both in active CS and in treated CS patients. This implied no severe impairments in cognitive performance. Our data are in line with previous literature in patients in remission of CS, where all Z-scores were also within one standard deviation, even if significant differences in cognition were found between patients in remission of CS and both normal controls and non-functioning pituitary adenomas (Tiemensma *et al.* 2010). In our study, in contrast with most studies found in the literature, patients with growth hormone (GH) deficiency were excluded. GH deficiency is a possible complication after pituitary surgery or irradiation, and is related to cognitive deficits (van Nieuwpoort *et al.* 2008). This may explain the lack of differences found in cognitive function, in contrast to other studies (Tiemensma *et al.* 2010, Ragnarsson *et al.* 2012).

Regarding active CS patients, according to the literature greater deficits may have been expected (Mauri *et al.* 1993, Whelan *et al.* 1980). Significant differences in comparison with controls were only found for the memory domain. However, as most of the active CS patients were on cortisol-lowering medication, this may have prevented finding greater impairment. On the other hand it is important to highlight that we had no data on cognitive performance before suffering CS, which would have been the perfect situation to establish if the disease had caused any cognitive impairment. However, most of the patients complained about having a poor cognitive performance, mainly related to memory problems, in comparison to their prior capacities.

Mood was also altered in both active CS patients and patients in remission. Both patient groups presented higher scores than controls for anxiety (both

state and trait) and depression, although scores did not correlate with clinical parameters. These higher scores are in line with the literature in patients with CS (Starkman *et al.* 1981, Whelan *et al.* 1980, Dorn *et al.* 2000, Kelly 1996, Sonino *et al.* 1998, Tiemensma *et al.* 2010, Ragnarsson *et al.* 2012, Andela *et al.* 2013) and confirm that mood alterations may be present long term after cure. As established by the guidelines, it would be important to provide proper monitoring and treatment of psychiatric disturbances for these patients (Nieman *et al.* 2015).

4.4. White matter lesions

When WML were analysed in *study II*, CS patients in remission had a higher degree of WML (measured with the Scheltens scale) in comparison to controls and patients with active disease. Cardiovascular risk factors in normal population have been related to WML (Jeerakathil *et al.* 2004, Gorelick *et al.* 2011). This is in line with our findings, which also evidence a relationship between WML and hypertension in patients in remission of CS, as WML severity is positively correlated to diastolic blood pressure and duration of hypertension.

Regarding WML degree, it may seem surprising that WML were increased only in cured patients. In fact, their vascular age was comparable to that of active CS patients. However, it is important to highlight that CS patients in remission had been exposed to a higher cardiovascular risk for a longer time than active CS patients. The algorithm for 10-year cardiovascular risk and vascular age only includes current risk factors, but is not a measure of total time exposed to cardiovascular risk. Therefore, patients in remission of CS, with a long-time exposure to cardiovascular risk factors may have more risk to present

WML than active CS patients. The positive correlations we found between WML and duration of hypertension exposure are in line with this hypothesis. It is important to highlight that this also implies that active CS patients may also develop a higher degree of WML in the future if cardiovascular risk remains increased.

On the other hand, WML are also related to aging (Xiong *et al.* 2011). According to some studies, in the general population the presence of WML has been reported in 11-21% of 64-year old adults, although it seems to increase with time. In fact, the percentage of older adults with WML at the age of 82 increases to 94% (Debette *et al.* 2010). If we analyse the number of patients whose routine MRI report described the presence of WML (in remission 73.9%, mean age 44.3 ± 9.3; active 46.7%, mean age 42.9 ± 10.6), it seems that the presence of WML in our patient population is higher than expected. This could be in line with the brain-aging effect suggested in CS by some authors (Michaud *et al.* 2009).

The relationship found between hypertension and WML in patients in remission of CS was not surprising. Hypertension is well-known as a strong predictor for WML (Longstreth *et al.* 1996, Basile *et al.* 2006, Xiong et al. 2011). The severity of hypertension and bad control in treated hypertensive patients have been associated with WML presence (Shimada *et al.* 1990, Liao *et al.* 1996, De Leeuw *et al.* 2002). Lesions may worsen over time, and hypertension may play a role on it. In uncontrolled hypertension, progression is higher in untreated than in treated patients, which suggests that hypertension treatment could be a useful tool to reduce WML progression (Verhaaren *et al.* 2013). Hypertension duration has also been associated with WML in a normal elderly population (De Leeuw *et al.* 2002). Apart from current hypertension, hypertension established 5 or 20 years before has also been associated to

current WML in old age (De Leeuw *et al.* 2002, Debette *et al.* 2011). These results are in line with our findings and could explain the differences in WML seen between active CS patients and patients in remission, as the latter have longer hypertension exposure. These data also indicate that the cause of WML may not be CS, but long-term hypertension caused by CS. This is important, as hypertension is a modifiable risk factor.

On the other hand WML have usually been associated with reduced brain volumes and impaired cognitive function (Debette *et al.* 2010, Mataró *et al.* 2014, Appelman *et al.* 2009), but in our study this was not the case. We did not find any correlations between the Scheltens scale scores and cognitive function or brain volume in patients with CS. It is important to highlight that most studies on WML have been performed in older populations, when cognitive decline and brain atrophy are more pronounced. Thus, it is possible that the younger age of our patients may have prevented finding significant correlations. Nevertheless, WML are known to predispose to cognitive decline, dementia and stroke in old age (Debette *et al.* 2010, Mataró *et al.* 2010). Therefore, it is important to highlight that this cohort may be at risk of being cognitively compromised in the future. This would be a pending issue to evaluate, as given the cross-sectional nature of this study it was not possible to assess longitudinal cognitive performance. Anyway, hypertension control may be helpful to prevent progression of WML (Liao *et al.* 1996, De Leeuw *et al.* 2002) and to reduce the risk of developing cognitive decline and dementia (Forette *et al.* 1998, Levi *et al.* 2013). Therefore, adequate control of hypertension would be necessary in patients with CS, both in patients in remission, usually exposed to hypertension for a longer time and with a higher degree of WML, but also in active patients, to prevent WML progression at an early stage. To achieve an optimal control of

hypertension patient education would be essential. Patients should be informed on the risks of inadequate control of hypertension and on healthy habits that may be helpful to control it.

Furthermore, education on drugs which may worsen hypertension like non steroidal anti-inflammatory drugs (Johnson 1997, Kalatufova *et al.* 2014) would be recommended. According to our results, patients in remission that were on hydrocortisone replacement had a higher degree of WML than patients who were not taking hydrocortisone. These findings would suggest trying to limit the use of hydrocortisone as much as possible. In some situations patients may have difficulties to differentiate symptoms related to adrenal insufficiency from other causes. Therefore, they may take extra doses of hydrocortisone, which may worsen hypertension (Sudhir *et al.* 1989). Patients should be encouraged to check their blood pressure regularly to identify possible increases, and avoid taking extra hydrocortisone doses regularly when suffering common complaints as headache or tiredness.

4.5. Cardiovascular risk, brain volumes and neuropsychological performance

Results of *study II* confirm previous findings and show that cardiovascular risk was increased in comparison with the control group in both active CS patients and in patients in remission (Colao *et al.* 1999, Mancini *et al.* 2004, Faggiano *et al.* 2003). These data confirm that cardiovascular risk is one of the comorbidities that can persist after cure. Furthermore, our results also show that both patient groups have smaller total and grey matter brain volumes in comparison to controls, in line with previous studies (Resmini *et al.* 2012, Simmons *et al.* 2000, Momose *et al.* 1971).

Study II has also evidenced that cardiovascular risk, measured as 10-year cardiovascular risk, is inversely correlated with both brain volumes and cognitive function in patients in remission of CS. These associations are in line with previous studies in large samples with more than 1700 participants from the normal population (Knopman *et al.* 2005, Longstreth *et al.* 2000, Seshadri *et al.* 2004, Nitshala *et al.* 2014, Yaffe *et al.* 2014, Dregan *et al.* 2013, Kaffashian *et al.* 2011). According to our results, special attention should be paid to high 10-year cardiovascular risk, as it may imply reduced brain volumes and decreased cognitive function. Furthermore, 10-year cardiovascular risk may also have implications in future cognitive performance, as it has been associated to poorer future neuropsychological function. These associations have been found when studying middle aged cohorts and their cognitive performance 10-15 years later, but also in young populations (18-30 years) followed for 25 years (Nitshala *et al.* 2014, Yaffe *et al.* 2014). This information is very relevant, and highlights the importance of controlling cardiovascular risk even at earlier ages to prevent cognitive decline.

For active CS patients, the correlation between cardiovascular risk and both brain volume and cognitive function was not found. The small sample size of the active CS group may have prevented finding significant associations, so an analysis in a larger sample would be necessary to confirm this hypothesis. Alternatively, it is possible that cardiovascular risk needed some time to affect brain volume and cognitive functions. As active CS patients had not been exposed to a higher cardiovascular risk for a long time, it is possible that this may have prevented finding associations. On the other hand, the effects of chronic hypercortisolism, that was still present in active CS patients, may have affected brain volume and cognitive function more than cardiovascular risk.

According to the literature, endogenous hypercortisolism exposure causes cognitive dysfunctions and reduces brain volume (Forget *et al.* 2000, Bourdeau *et al.* 2005, Starkman *et al.* 1992, Resmini *et al.* 2012, Andela *et al.* 2015, Mauri *et al.* 1993, Crespo *et al.* 2014). It is possible that in the active population hypercortisolism plays a more important role than cardiovascular risk on cognitive function and brain volume, which may have prevented finding the associations observed in patients in remission of CS.

Regarding vascular age, we did not find any studies correlating it with brain volumes or cognitive function. In clinical practice the concept "vascular age" may be a useful tool for teaching patients, since they could understand better that their vascular age at the moment is higher than their current age, rather than more complicated concepts as 10-year cardiovascular risk. It has been reported that in younger individuals knowing their "heart-age" can have a high emotional impact, leading to a change in their life style (Soureti *et al.* 2010). Furthermore, if patients were informed that increased vascular age has been linked to both reduced brain volumes and poorer cognitive function, this may also encourage them to improve their healthy habits. In fact, Canadian guidelines for diagnosis and treatment of dyslipidemia already recommend communicating vascular age to patients to improve hypertension and lipid control (Anderson *et al.* 2013).

4.6. Cushing's syndrome: Active patients vs patients in remission

According to our results, CS seems to lead to different comorbidities in the different phases of the disease. Active CS patients have a poor memory performance and reduced cerebellar cortex volumes, which do not seem to be

present after cure. They also have a high cardiovascular risk, increased levels of anxiety and depression and smaller total brain and grey matter volumes than controls.

After successful treatment cortisol levels are normalized and patients are considered to be cured. Cerebellar volume and cognitive function seems to normalise. However, other important comorbidities seem to persist or show up. Patients may still have an increased cardiovascular risk, as well as high levels of depression or anxiety and smaller total brain and grey matter volumes than controls. Additionally, they will have a new comorbidity, not present in the active phase: a higher degree of WML than controls, correlated with the level of blood pressure and hypertension duration.

Putting the information of the two studies together, it seems that some of the alterations that we can find in active CS may be at least partially reversible (as cognitive decline and cerebellar volume reduction). However, the cardiovascular risk associated to the disease may lead to further comorbidities in the future, if it is not controlled. Increased depression and anxiety, as well as reduced total brain and grey matter volumes (associated to cardiovascular risk) may also persist after cure.

Therefore, some recommendations may be indicated for both active patients and patients in remission of CS. It would be important to provide psychological support if necessary and to control or even improve their cardiovascular risk in order to prevent brain damage and to reduce the risk of stroke or heart attack. Patient education and communication of their current cardiovascular risk using the “vascular age” may also be helpful to make the patients conscious of their current risk and to be more committed to a change in their life style.

4.7. Limitations of the studies

Both studies had several limitations. The first one was the small sample size and the heterogeneity of the sample, which are difficult to avoid in rare diseases such as CS. This may have led to a low statistical power and possibly prevented finding further significant results, mainly in the active patient population. Confirmatory analysis in larger samples of active CS patients would be of interest. Additionally, the number of patients included was further reduced by strict exclusion criteria, although this restriction avoided the possible influence of multiple factors that could affect the studied parameters, like GH deficiency, older age, brain damage, history of alcohol or drug abuse and severe psychiatric or neurological illnesses. The cross-sectional design is a further potential weakness. Regarding FreeSurfer (the tool for measuring brain volumes), a further limitation is that it is an automated technique that may not be as accurate as manual segmentation. Especially in *study I*, it is feasible that measurement noise may have masked clearer correlations with neuropsychological and clinical parameters. For *study II*, a further limitation was that a standard neurological evaluation was not performed, which might have identified subtle cerebellar dysfunction in CS patients.

4.8. Future lines of investigation

First of all, confirmatory studies of our results with larger samples, mainly including active CS patients, would be of interest. For instance, it would be interesting to confirm if in active CS patients there is also a relationship between cardiovascular risk and both brain volume and neuropsychological performance when including a higher number of patients. New studies in active patients performing a complete neurological examination, including balance

tests may be helpful to establish if the cerebellum is functionally impaired and if neurological performance is related to the cerebellar volume. Further studies on the contribution of dyslipidemia to cerebellar function would also be required. It would also be interesting in the future to prospectively study the possible relationship between cerebellar volume and the number of falls. Longitudinal studies could also confirm if the cerebellar cortex volume increases after cure and, if so, after how long does this occur. Prospective studies would also be necessary to analyse WML progression in active patients, and to investigate the possible role of hypertension control to prevent WML worsening in both active patients and patients in remission of CS. Studies in older patients may also be helpful to establish if WML are associated to cognitive function when patients reach old age. Longitudinal studies could also be helpful to establish if middle-aged WML can predict cognitive performance at a later age. Finally, it would be interesting to analyse the possible benefits of neuropsychological rehabilitation or group therapy for mood, neuropsychological functions and QoL.

5. CONCLUSIONS

The main conclusions of this thesis can be summarized as follows:

I. Cerebellar cortex is reduced in patients with active CS in comparison to normal controls, but not in patients in remission, suggesting initial damage followed by later recovery after cure.

II. Cerebellar cortex volumes are positively correlated to visual memory and QoL and negatively correlated to age at diagnosis, and triglyceride levels.

III. Active CS patients, but not patients in remission, have a poorer memory performace than controls.

IV. Both patient groups have higher anxiety and depression levels than controls.

V. Patients in remission of CS, but not active CS patients have a higher degree of WML than normal controls.

VI. WML are associated with systolic blood pressure and hypertension duration. Patients in remission of CS have longer hypertension exposure than active CS patients, which may explain their higher degree of WML. This also suggests that control of hypertension may be helpful to avoid WML development.

VII. Patients in remission of CS who were taking hydrocortisone had a higher degree of WML than those not taking the drug,

suggesting that substitution therapy should be stopped as soon as possible.

VIII. Cardiovascular risk (measured as 10-year cardiovascular risk and vascular age) is negatively associated to cognitive function and brain volumes in patients in remission, highlighting the importance of cardiovascular risk control in this population.

6. REFERENCES

1. Albiger N, Testa RM, Almoto B, Ferrari M, Bilora F, Petrobelli F, Pagnan A, Mantero F, Scaroni C. **Patients with Cushing's syndrome have increased intimal media thickness at different vascular levels: comparison with a population matched for similar cardiovascular risk factors.** *Horm Metab Res* 2006; 38:405-10.
2. Alexandraki KI, Kaltsas GA, Vouliotis AI, Papaioannou TG, Trisk L, Zilos A, Korbonits M, Besser GM, Anastasakis A, Grossman AB. **Specific electrocardiographic features associated with Cushing's disease.** *Clin Endocrinol (Oxf)* 2011; 74:558-64.
3. American Diabetes Association. **Diagnosis and Classification of Diabetes Mellitus**. *Diabetes Care* 2010; 33(Suppl1): S62-S69.
4. Ambrosi B, Faglia G; Multicenter Pituitary Tumor Study Group. **Epidemiology of pituitary tumors**. In: Faglia G, Beck-Peccoz P, Ambrosi B, Travaglini P, Spada A (eds.). Pituitary Adenomas: New Trends in Basic and Clinical Research. Amsterdam: *Elsevier Science Publishers;* 1991:159–68.
5. Andela CD, van derWerff SJ, Pannekoek JN, van den Berg SM, Meijer OC, van Buchem MA, Rombousts SA, van der Mast RC, Romijn JA, Tiemensma J, Biermasz NR, van der Wee NJ, Pereira AM. **Smaller grey matter volumes in the anterior cingulate cortex and greater cerebellar volumes in patients with long-term remission of Cushing's disease: a case-control study**. *Eur J Endocrinol* 2013; 169:811-819.

6. Andela CD, Niemeijer ND, Sharloo M, Tiemensma J, Kanagasabapathy S, Pereira AM, Kamminga NG, Kaptein AA, Biermasz NR. **Towards a better quality of life (QoL) for patients with pituitary diseases: results from a focus group study exploring QoL**. *Pituitary* 2015; 18:86-100.
7. Anderson TJ, Grégoire J, Hegele RA, Couture P, Mancini GB, McPherson R, Francis GA, Poirier P, Lau DC, Grover S, Genest J Jr, Carpentier AC, Dufour R, Gupta M, Ward R, Leiter LA, Lonn E, Ng DS, Pearson GJ, Yates GM, Stone JA, Ur E. **2012 update of the Canadian Cardiovascular Society guidelines for the diagnosis and treatment of dyslipidemia for the prevention of cardiovascular disease in the adult.** *Can J Cardiol* 2013; 29**:**151-67.
8. Appelman AP, Exalto LG, van der Graaf Y, Biessels GJ, Mali WP, Geerlings MI. **White matter lesions and brain atrophy: more than shared risk factors? A systemiatic review.** *Cerebrovasc Dis* 2009; 28:227-42.
9. Arnaldi G, Angeli A, Atkinson AB, Bertagna X, Cavagnini F, Chrousos GP, Fava GA, Findling JW, Gaillard RC, Grossman AB, Kola B, Lacroix A, Mancini T, Mantero F, Newell-Price J, Nieman LK, Sonino N, Vance ML, Giustina A, Boscaro M. **Diagnosis and complications of Cushing's syndrome: a Consensus Statement**. *J Clin Endocrinol Metab* 2003; 88: 5593–602.
10. Arnaldi G, Boscaro M. **New treatment guidelines on Cushing's disease**. *F1000 Med Rep* 2009; 1:64.

11. Arnaldi G, Scandali VM, Trementino L, Cardinaletti M, Appolloni G, Boscaro M. **Pathophysiology of dyslipidemia in Cushing's syndrome**. *Neuroendocrinology* 2010; 92 Suppl 1:86-90.
12. Baldaçara L, Borgio JG, Lacerda AL, Jackowski AP. **Cerebellum and psychiatric disorders**. *Rev Bras Psiquiatr* 2008; 30:281-289.
13. Balietti M, Fattoretti P, Giorgetti B, Casoli T, Di Stefano G, Platano D, Aicardi G, Lattanzio F, Bertoni-Freddari C. **Effect of two medium chain triglycerides-supplemented diets on synaptic morphology in the cerebellar cortex of late-adult rats.** *Micros Res Tech* 2009; 72:933-938.
14. Barahona MJ, Sucunza E, Resmini, Fernández-Real JM, Ricart W, Moreno-Navarrete JM, Puig T, Farrerons J, Webb SM. **Persistent body fat mass and inflammatory marker increases after long-term cure of Cushing's syndrome**. *J Clin Endocrinol Metab* 2009; 94(9):3365-3371.
15. Barahona MJ, Resmini E, Sucunza N, Webb SM. **Diagnosis of cure in Cushing's syndrome: lessons from long-term follow-up.** *Front Horm Res* 2010; 38:152-7.
16. Barahona MJ, Resmini E, Viladés D, Pons- Lladó G, Leta R, Puig T, Webb SM. **Coronary artery disease detected by multislice computed tomography in patients after long-term cure of Cushing's syndrome**. *J Clin Endocrinol Metab* 2013; 98:1093-9.
17. Bas-Hoogendam JM, Andela CD, van der Werff SJ, Pannekoek JN, van Steenbergen H, Meijer OC, van Buchem MA, Rombouts SA, van der Mast RC, Biermasz NR, van der Wee NJ, Pereira AM. **Altered neural**

processing of emotional faces in remitted Cushing's disease. *Psychoneuroendocrinology* 2015: 59:134-46.

18. Basile AM, Pantoni L, Pracucci G, Asplund K, Chabriat H, Erkinjuntti T, Fazekas F, Ferro JM, Hennerici M, O'Brien J, Scheltens P, Visser MC, Washlund LO, Waldemar G, Wallin A, Inzitari D, LADIS Study Group. **Age, hypertension and lacunar stroke are the major determinants of the severity of age-related white matter changes. The LADIS (Leukoaraiosis and Disability in the Elderly) Study**. *Cerebrovasc Dis* 2006; 21:315-322.
19. Bauer PM, Hanson JL, Pierson RK, Davidson RJ, Pollak SD. **Cerebellar volume and cognitive functioning in children who experienced early deprivation.** *Biol Psychiatry* 2009; 66:1100-1106.
20. Baykan M, Erem C, Gedikli O, Hacihasanoglu A, Erdogan T, Kocak M, Durmus I, Korkmaz L, Celik S. **Impairment of flow-mediated vasodilatation of branchial artery in patients with Cushing's Syndrome**. *Endocrine* 2007; 31:300-4.
21. Bekkelund SI, Pierre-Jerome C, Husby G, Mellgren SI. **Quantitative cerebral MR in rheumatoid arthritis**. AJNR AM J Neuroradiol 1995; 16:767-772.
22. Bhatt AJ, Feng Y, Wang J, Famuyide M, Hersey K. **Dexamethasone induces apoptosis of progenitor cells in the subventricular zone and dentate gyrus of developing rat brain**. *J Neurosci Res* 2013; 91:1191-1202.
23. Biering H, Knappe G, Gerl H, Lochs H. **Prevalence of diabetes in acromegaly and Cushing syndrome.** *Acta Med Austriaca* 2000; 27:27–31.

24. Biller BM, Grossman AB, Stewart PM, Melmed S, Bertagna X, Bertherat J, Buchfelder M, Colao A, Hermus AR, Hofland LJ, Klibanski A, Lacroix A, Lindslay JR, Newell-PriceJ, Nieman LK, Petersenn S, Sonino N, Stalla GK, Swearingen B, Vance ML, Wass JA, Boscaro M. **Treatment of adrenocorticotropin-dependent Cushing's syndrome: a consensus statement**. *J Clin Endocrinol Metab* 2008; 93: 2454-62.
25. Black S, Gao F, Bilbao J. **Understanding white matter disease: imaging pathological correlations in vascular cognitive impairment**. *Stroke* 2009; 40 Suppl 3: S48-52.
26. Boscaro M, Barzon L, Fallo F, Sonino N. **Cushing's syndrome**. *Lancet* 2001; 357(9258):783-91.
27. Bolland MJ, Holdaway IM, Berkeley JE, Lim S, Dransfield WJ, Conaglen JV, Croxson MS, Gamble GD, Hunt PJ, Toomath RJ. **Mortality and morbidity in Cushing's syndrome in New Zealand**. *Clin Endocrinol (Oxf)* 2011; 75(4):436-42.
28. Bourdeau I, Bard C, Noël B, Leclerc I, Cordeau MP, Bélair M, Lesage J, Lafontaine L, Lacroix A. **Loss of brain volume in endogenous Cushing's syndrome and its reversibility after correction of hypercortisolism**. *J Clin Endocrinol Metab* 2002; 87:1949-54.
29. Bourdeau I, Bard C, Forget H, Boulanger Y, Cohen H, Lacroix A. **Cognitive function and cerebral asessment in patients who have Cushing's Syndrome**. *Endocrinol Metab Clin North Am* 2005; 34:357-69.
30. Bujalska IJ, Kumar S, Stewart PM. **Does central obesity reflect "Cushing's disease of the omentum"?** *Lancet* 1997; 349:1210-3.

31. Cameron AJ, Shaw JE, Zimmet PZ. **The metabolic syndrome: prevalence in worldwide population.** *Endocrinol Metab Clin North Am* 2004; 33:351-375.
32. Camozzi V, Sanguin F, Albigier N, Scaroni C, Mantero F, Zaninotto M, Frigo A, Piccolo M, Luisetto G. **Persistent increase of osteoprotegerin levels after cortisol normalization in patients with Cushing's syndrome.** Eur J Endocrinol 2010; 162:85-90.
33. Casonato A, Pontara E, Boscaro M, Sonino N, Sartorello F, Ferasin S, Girolami A. **Abnormalities of von Willebrand factor are also part of the prothrombotic state of Cushing's syndrome**. *Blood Coagul Fibrinolysis 1*999; 10:145-51.
34. Chait A, Bornfeldt KE. **Diabetes and atherosclerosis: is there a role for hyperglycemia?** *J Lipid Res* 2009; 50 Suppl 1:S335-9.
35. Chandran DS, Ali N, Jaryal AK, Jyotsna VP, Deepak KK. **Decreased autonomic modulation of heart rate and altered cardiac sympathovagal balance in patients with Cushing's syndrome: role of endogenous hypercortisolism**. *Neuroendocrinology* 2013; 97:309-17.
36. Chanson P, Salenave S. **Metabolic syndrome in Cushing's syndrome**. *Neuroendocrinology* 2010; 92 Suppl 1:96-101.
37. Cicala MV, Mantero F. **Hypertension in Cushing's syndrome: from pathogenesis to treatment**. *Neuroendocrinology* 2010; 92 Suppl 1:44-9.
38. Cizza G, Lotsikas AJ, Licinio J, Gold PW, Chrousos GP. **Plasma leptin levels do not change in patients with Cushing's disease shortly**

after correction of hypercorticolism. *J Clin Endocrinol Metab* 1997; 82:2747–2750.

39. Clayton RN, Raskauskiene D, Reulen RC, Jones PW. **Mortality and morbidity in Cushing's disease over 50 years in Stoke-on-Trent, UK: audit and meta-analysis of literature**. *J Clin Endocrinol Metab* 2011; 96(3):632-42.
40. Coelho MC, Santos CV, Neto LV, Gadelha MR. **Adverse effects of glucocorticoids: coagulopathy**. Eur J Endocrinol 2015; 173: M11-21.
41. Colao A, Pivonello R, Spiezia S, Faggiano A, Ferone D, Filippella M, Marzullo P, Cerbone G, Siciliani M, Lombardi G. **Persistence of increased cardiovascular risk in patients with Cushing's disease after five years of successful cure**. *J Clin Endocrinol Metab* 1999;84:2664-2672.
42. Crespo I, Granell-Moreno E, Santos A, Valassi E, Vives-Gilabert Y, De Juan-Delago M, Webb SM, Gómez-Ansón B, Resmini E. **Impaired decisión-making and selective cortical frontal thinning in Cushing's syndrome**. *Clin Endocrinol (Oxf)* 2014; 81:826-33.
43. Crowther MA. **Pathogenesis of atherosclerosis**. *Hematology Am Soc Hematol Educ Program* 2005:436-41.
44. Daidone V, Boscaro M, Pontara E, Cattini MG, Occhi G, Scaroni C, Mantero F, Casonato A. **New insight into the hypercoagulability of Cushing's syndrome**. *Neuroendocrinology* 2011; 93:121-5.
45. Davignon J, Ganz P. **Role of endothelial dysfunction in atherosclerosis**. *Circulation* 2004; 109(Suppl 1): III27-32.

46. De Leeuw FE, de Groot JC, Oudkerk M, Witteman JC, Hofman A, van Gjin J, Breteler MM**. Hypertension and cerebral white matter lesions in a prospective cohort study**. *Brain* 2002; 125:765-772.

47. De Leo M, Pivonello R, Auriemma RS, Cozzolino A, Vitale P, Simeoli C, De Martino MC, Lombardi G, Colao A. **Cardiovascular disease in Cushing's syndrome: heart versus vasculature**. *Neuroendocrinology* 2010; 92 Suppl 1: 50-4.

48. Debette S, Markus HS. **The clinical importance of white matter hyperintensities on brain magnetic resonance imaging: systematic review and meta-analysis**. *BMJ* 2010; 331:c3666.

49. Debette S, Seshadri S, Beiser A, Au R, Himali JJ, Palumbo C, Wolf PA, DeCarli C. **Midlife vascular risk factor exposure accelerates structural brain aging and cognitive decline**. *Neurology* 2011; 77:461-468.

50. Dekkers OM, Horváth-Puhó E, Jorgensen JO, Cannegieter SC, Ehrenstein V, Vandenbroucke JP, Pereira AM, Sorensen HT. **Multisystem morbidity ans mortality in Cushing's syndrome: a cohort study**. *J Clin Endocrinol Metab* 2013; 98:2277-84.

51. Dichgans J, Mauritz KH. **Patterns and mechanisms of postural instability in patients with cerebellar lesions**. *Adv neurol* 1983; 39:633-643.

52. Dorn LD, Burgess ES, Friedman TC, Dubbert B, Gold PW, Chrousos GP. **The longitudinal course of psychopathology in Cushing's syndrome after correction of hypercortisolism**. *J Clin Endocrinol Metab* 1997; 82:912-919.

53. Dorn LD, Cerrone P. **Cognitive function in patients with Cushing syndrome: a longitudinal perspective**. *Clin Nurs Res* 2000; 9:420-40.

54. Dovio A, Allasino B, Palmas E, Ventura M, Pia A, Saba L, Aroasio E, Terzolo M, Angeli A. **Increased osteoprotegerin levels in Cushing's syndrome are associated with an adverse cardiovascular risk profile**. *J Clin Endocrinol Metab* 2007; 92:1803-8.

55. Dregan A, Stewart R, Gulliford M. **Cardiovascular risk factors and cognitive decline in adults aged 50 and over: a population-based cohort study.** *Age Ageing* 2013; 42:338-45.

56. Eckert MA, Keren NI, Roberts DR, Calhoun VS, Harris KC. **Age-related changes in processing speed: unique contributions of cerebellar and prefrontal cortex**. *Frontiers in Human Neuroscience* 2010; 4:10.

57. Erem C, Nuhoglu I, Yilmaz M, Kocak M, Demirel A, Ucuncu O, Onder Ersoz H. **Blood coagulation and fibrinolysis in patients with Cushing's syndrome: increased plasminogen activator inhibitor-1, decreased tissue factor pathway inhibitor, and unchanged thrombin-activatable fibrinolysis inhibitor levels**. *J Endocrinol Invest* 2009; 32:169-74.

58. Etxabe J, Vazquez JA. **Morbidity and mortality in Cushing disease: an epidemiological approach**. *Clin Endocrinol (Oxf)* 1994; 40:479–84.

59. Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults. **Executive Summary of the Third Report of**

The National Cholesterol Education Program (NCEP) Expert Panel on Detection, Evaluation, And Treatment of High Blood Cholesterol In Adults (Adult Treatment Panel III). *JAMA* 2001; 285: 2486-97 .

60. Faggiano A, Pivonello R, Spiezia S, De Martino MC, Filippella M, Di Somma C, Lombardi G, Colao A. **Cardiovascular risk factors and common carotid artery caliber and stiffness in patients with Cushing's disease during active disease and 1 year after disease remission**. *J Clin Endocrinol Metab* 2003;88:2527-2533.
61. Fallo F, Maffei P, Dalla Pozza A, Carli M, Della Mea P, Lupia M, Rabbia F, Sonino N. **Cardiovascular autonomic function in Cushing's syndrome**. J Endocrinol Invest 2009; 32:41-5.
62. Fan J, Unoki H, Iwasa S, Watanabe T. **Role of endothelin-1 in atherosclerosis**. *Ann N Y Acad Sci* 2000; 902:84-93.
63. Fatti LM, Bottasso B, Invitti C, Coppola R, Cavagnini F, Mannucci PM. **Markers of activation of coagulation and fibrinolysis in patients with Cushing's syndrome**. *J Endocrinol Invest* 2000; 23:145-50.
64. Feelders RA, Pulgar SJ, Kempel A, Pereira AM. **The burden of Cushing's disease: clinical and health-related quality of life aspects**. *Eur J Endocrinol* 2012; 167:311-26.
65. Ferrau F, Korbonits M. **Metabolic comorbidities in Cushing's syndrome**. *Eur J Endocrinol* 2015; 173:M133-57.
66. Fjeldstad C, Fjeldstad AS, Acree LS, Nickel KJ, Gardener AW**. The influence of obesity on falls and quality of life**. *Dyn Med* 2008; 7:4.

67. Forette F, Seux ML, Staessen JA, Thijs L, Birkenhäger WH, Babarskiene MR, Babeanu S, Bossini A, Gil-Extremera B, Girerd X, Laks T, Lilov E, Moisseyev V, Tuomilehto J, Vanhanen H, Webster J, Yodfat Y, Fagard R. **Prevention of dementia in randomised double-blind placebo-controlled Systolic Hypertension in Europe (Syst-Eur) trial.** *Lancet* 1998; 352:1347-51.

68. Forget H, Lacroix A, Somma M, Cohen H. **Cognitive decline in patients with Cushing's syndrome**. *J Int Neuropsychol Soc* 2000; 6:20-29.

69. Forget H, Lacroix A, Cohen H. **Persistent cognitive impairment following surgical treatment of Cushing's syndrome**. *Psychoneuroendocrinology* 2002; 27:367-383.

70. Galvan V, Jin K. **Neurogenesis in the aging brain**. *Clin Interv Aging* 2007; 2:605-610.

71. Geer EB, Shen W, Strohmayer E, Post KD, Freda PU. **Body composition and cardiovascular risk markers after remission of Cushing's disease: a prospective study using whole-body MRI**. *J Clin Endocrinol Metab* 2012;97(5):1702–11.

72. Giordano R, Picu A, Marinazzo E, D'Angelo V, Berardelli R, Karamouzis I, Forno D, Zinnà D, Maccario M, Ghigo E, Arvat E. **Metabolic and cardiovascular outcomes in patients with Cushing's syndrome of different aetiologies during active disease and 1 year after remission**. *Clin Endocrinol (Oxf)* 2011;75(3):354–60.

73. Gnjidic Z, Sajko T, Kudelic N, Manelica M, Vizner B, Vrkljan M, Hat J, Rumboldt Z. **Reversible "brain atrophy" in patients with Cushing's disease**. *Collegium Antropologicum* 2008; 32-1165-1170.

74. Gorelick PB, Scuteri A, Black SE, Decarli C, Greenberg SM, Iadecola C, Launer LJ, Laurent S, Lopez OL, Nyenhuis D, Petersen RC, Schneider JA, Tzourio C, Arnett DK, Bennett DA, Chui HC, Higashida RT, Lindquist R, Nilsson PM, Roman GC, Sellke FW, Seshadri S; American Heart Association Stroke Council, Council on Epidemiology and Prevention, Council on Cardiovascular Nursing, Council on Cardiovascular Radiology and Intervention, and Council of Cardiovascular Surgery and Anesthesia. **Vascular contributions to cognitive impairment and dementia: a statement for healthcare professionals from the american heart association/american stroke association**. *Stroke* 2011; 42:2672-713.
75. Gotch PM. **Cushing's syndrome from the patient perspective**. *Endocrinol Metab Clin North Am* 1994; 23:607-17.
76. Greenman Y. **Management of dyslipidemia in Cushing's syndrome**. *Neuroendocrinology* 2010; 92 Suppl 1:91-5.
77. Grottoli S, Gauna C, Tassone F, Aimaretti G, Corneli G, Wu Z, Strasburger CJ, Diegeuez C, Casanueva FF, Ghigo E, Maccario M. **Both fasting-induced leptin reduction and GH increase are blunted in Cushing's syndrome and in simple obesity**. *Clin Endocrinol* 2003; 58: 220–228.
78. Grundy SM, Cleeman JI, Daniels SR, Donato KA, Eckel RH, Franklin BA, Gordon DJ, Krauss RM, Savage PJ, Smith Jr SC, Spertus JA, Costa F. **Diagnosis and management of the metabolic syndrome: an American Heart Association/National Heart, Lung and Blood Institute Scientific Statement**. *Circulation* 2005; 112:2735-52.

79. Guthikonda S, Haynes WG. **Homocysteine: role and implications in atherosclerosis**. *Curr Atheroscler Rep* 2006; 8:100-6.

80. Hammer GD, Tyrrell JB, Lamborn KR, Applebury CB, Hannegan ET, Bell S, Rahl R, Lu A, Wilson CB. **Transsphenoidal microsurgery for Cushing's disease: initial outcome and long-term results**. J Clin Endocrinol Metab 2004; 89(12):6348-57.

81. Hassan-Smith ZK, Sherlock M, Reulen RC, Arlt W, Ayuk J, Toogood AA, Cooper MS, Johnson AP, Stewart PM. **Outocome of Cushing's disease following transsphenoidal surgery in a single center over 20 years**. *J Clin Endocrinol Metab* 2012; 97(4):1194-201.

82. Hawn MT, Cook D, Deveney C, Sheppard BC. **Quality of life after laparoscopic bilateral adrenalectomy for Cushing's disease**. *Surgery* 2002; 132:1064-8.

83. Heald AH, Ghosh S, Bray S, Gibson C, Anderson SG, Buckler H, Fowler HL. **Long-term negative impact on quality of life in patients with successfully treated Cushing's disease**. *Clinical Endocrinology* 2004: 61: 458-465.

84. Hook JN, Giordani B, Schteingart DE, Guire K, Giles J, Ryan K, Gebarski SS, Langenecker SA, Starkman MN. **Patterns of cognitive change over time and relationship to age following successful treatment of Cushing's disease**. *J Int Neuropsychol Soc* 2007; 13:21-9.

85. Horak FB, Diener HC. **Cerebellar control of postural scaling and central set in stance**. *J Neurophysiol* 1994; 72:479-493.

86. Hu W, Zhang Y, Wu W, Yin Y, Huang D, Wang Y, Li W, Li W. **Chronic glucocorticoids exposure enhances neurodegeneration in the**

frontal cortex and hippocampus via NLRP-1 inflammasome activation in male mice. *Brain Behav Immun* 2015; pii:S0889-1591(15):30026-X.

87. Isidori AM, Minnetti M, Sbardella E, Graziadio C, Grossman AB. **MECHANISMS IN ENDOCRINOLOGY: The spectrum of haemostatic abnormalities in glucocorticoid excess and defect**. *Eur J Endocrinol* 2015; 173: R101-13.

88. Jeraakathil P, Wolf PA, Beiser A, Massaro J, Seshadri S, D'Agostino RB, DeCarli C. **Stroke risk profile predicts white matter hyperintensity volume: The Framingham study**. *Stroke* 2004; 35:1857-61.

89. Johnson AG. NSAIDs and increased blood pressure. **What is the clinical significance?** *Drug Saf* 1997; 17:277-89.

90. Johnson MD, Woodburn CJ, Vance ML. **Quality of life in patients with a pituitary adenoma**. *Pituitary* 2003; 6:81-7.

91. Kaffashian S, Dugravot A, Nabi H, Batty GD, Brunner E, Kivimäki M, Singh-Manoux A. **Predictive utility of the Framingham general cardiovascular disease risk profile for cognitive function: evidence from the Whitehall II study.** *Eur Heart J* 2011; 32: 2326-32.

92. Kalafutova S, Juraskova B, Vlcek J. **The impact of combinations of non steroidal anti-inflammatory drugs and anti-hypertensive agents on blood pressure.** *Adv Clin Exp Med* 2014; 23:993-1000.

93. Kamenicky P, Redheuil A, Roux C, Salenave S, Kachenoura N, Raissouni Z, Macron L, Guignat L, Jublanc C, Azarine A, Brailly S, Young J, Mousseaux E, Chanson P. **Cardiac structure and function in**

Cushing's syndrome: a cardiac magnetic resonance imaging study. *J Clin Endocrinol Metab* 2014:99: E2144-53.

94. Kelly WF. **Psychiatric aspects of Cushing's syndrome**. *QJM* 1996; 89:543-51.

95. Kern PA, Ranganathan S, Li C, Wood L, Ranganathan G. **Adipose tissue tumor necrosis factor and interleukin-6 expression in human obesity and insulin resistance**. *Am J Physiol Endocrinol Metab* 2001; 280:E745-51.

96. Khiat A, Bard C, Lacroix A, Rousseau J, Boulanger Y. **Brain metabolic alterations in Cushing's syndrome as monitored by proton magnetic resonance spectroscopy**. *NMR Biomed* 1999; 12:357-63.

97. Khiat A, Bard C, Lacroix A, Boulanger Y. **Recovery of the brain choline level in treated Cushing's patients as monitored by proton magnetic resonance spectroscopy**. *Brain Res* 2000; 862:301-7.

98. Kirilov G, Tomova A, Dakovska L, Kumanov P, Shinkov A, Alexandrov AS. **Elevated plasma endothelin as an additional cardiovascular risk factor in patients with Cushing's syndrome**. *Eur J Endocrinol* 2003; 149:549-53.

99. Kleen JK, Sitomer MT, Killeen PR, Conrad CD. **Chronic stress impairs spatial memory and motivation for reward without disrupting motor ability and motivation to explore**. *Behav Neurosci* 2006; 120:842–851.

100. Knopman DS, Mosley TH, Catellier DJ, Sharrerr AR; Atherosclerosis Risk in Communities (ARIC) Study. **Cardiovascular risk factors and**

cerebral atrophyin a middle-aged cohort. *Neurology* 2005; 65**:**876-81.

101. Kola B, Christ-Crain M, Lolli F, Arnaldi G, Giacchetti G, Boscaro M, Grossman AB, Korbonits M. **Changes in adenosine 5'-monophosphate-activated protein kinase as a mechanism of visceral obesity in Cushing's syndrome**. *J Clin Endocrinol Metab* 2008; 93:4969-73.

102. Kresk M, Silha JV, Jezkova J, Hana V, Marek J, Weiss V, Stepan JJ, Murphy LJ. **Adipokine levels in Cushing's syndrome; elevated resistin levels in female patients with Cushing's syndrome**. *Clin Endocrinol* 2004; 60: 350–357.

103. Langenecker SA, Weisenbach SL, Giordani B, Briceño EM, Guidotti Breting LM, Schallmo MP, Leon HM, Noll DC, Zubieta JK, Schteingart DE, Starkman MN**. Impact of chronic hypercortisolemia on affective processing**. *Neuropharmacology* 2012; 62:217-25.

104. Lauterbach EC, Harris JB, Bina WF 3rd. **Mood and neurobehavioral correlates of cerebellar lesions**. *Cogn Behav Neurol* 2010; 23:63-73.

105. Leal-Cerro A, Considine RV, Peino R, Venegas E, Astorga R, Casanueva FF, Dieguez C. **Serum immunoreactive-leptin levels are increased in patients with Cushing's syndrome**. *Horm Metab Res* 1996; 28:711–713.

106. León-Carrión J, Atutxa AM, Mangas MA, Soto-Moreno A, Pumar A, Leon-Justel A, Martín-Rodriguez JF, Venegas E, Domínguez-Morales MR, Leal-Cerro A. **A clinical profile of memory impairment in**

humans due to endogenous glucocorticoid excess. *Clinical Endocrinology* 2009; 70: 192–200.

107. Levi Marpillat N, Macquin-Mavier I, Tropeano AI, Bachoud-Levi AC, Maison P. **Antihypertensive classes, cognitive decline and incidende of dementia: a network meta-analysis**. *J Hypertens* 2013; 31:1073-82.

108. Liao D, Cooper L, Cai J, Toole JF, Bryan N, Hutchinson RG, Tyroler HA. **Presence and severity of cerebral white matter lesins and hypertension, its treatment and its control**. **The ARIC Study.** *Stroke* 1996; 27:2262-2270.

109. Lindslay JR, Nansel T, Baid S, Gumowski J, Nieman LK. **Long-term impaired quality of life in Cushing's syndrome despite initial improvement after surgical remission**. *J Clin Endocrinol Metab* 2006; 91:447-453.

110. Lindholm J, Juul S, Jorgensen JO, Astrup J, Bjerre P, Feldt-Rasmussen U, Hagen C, Jorgensen J, Kosteljanetz M, Kristensen L, Laurberg P, Schmidt K, Weeke J. **Incidence and late prognosis of Cushing's syndrome: a population-based study**. *J Clin Endocrinol Metab* 2001; 86:117–123.

111. Longstreth WT Jr, Manolio TA, Arnold A, Burke GL, Bryan N, Jungreis CA, Enright PL, O'Leary D, Fried L. **Clinical correlates of white matter findings on cranial magnetic imaging of 3301 elderly people: The Cardiovascular Health Study**. *Stroke* 1996; 27:1274-1282.

112. Longstreth WT, Arnold AM, Manolio TA, Burke GL, Bryan N, Jungreis CA, O'Leary D, Enright PL, Fried L. **Clinical correlates of**

ventricular and sulcal size on cranial magnetic resonance imaging of 3,301 elderly people. The Cardiovascular Health Study. Collaborative Research Group. *Neuroepidemiology* 2000; 19:30-42.

113. Maheu FS, Mazzone L, Merke DP, Keil MF, Stratakis CA, Pine DS, Ernst M. **Altered amygdala and hippocampus function in adolescents with hypercortisolemia: a functional magnetic resonance imaging study of Cushing syndrome**. *Dev Psychopathol* 2008; 20:1177-89.

114. Mancini T, Kola B, Mantero F, Boscaro M, Arnaldi G. **High cardiovascular risk in patients with Cushing's syndrome according to 1999 WHO/ISH guidelines**. *Clin Endocrinol (Oxf.)* 2004; 61(6):768-77.

115. Manetti L, Bogazzi F, Giovannetti C, Raffaelli V, Genovesi M, Pellegrini G, Ruocco L, Iannelli A, Martino E. **Changes in coagulation indexes and occurrence of venous thromboembolism in patients with Cushing's syndrome: results from a prospective study before and after surgery**. *Eur J Endocrinol* 2010; 163:783-91.

116. Martignoni E, Costa A, Sinforiani E, Liuzzi A, Chiodini P, Mauri M, Bono G, Nappi G. **The brain as a target for adrenocortical steroids: cognitive implications**. *Psychoneuroendocrinology* 1992;17:343-354.

117. Martínez-Momblán MA, Gómez C, Santos A, Porta N, Esteve J, Úbeda I, Halperin I, Campillo B, Guillaumet M, Webb SM, Resmini E. **A specific nursing educational program in patients with Cushing's syndrome**. *Endocrine* 2015 [Epub ahead of print].

118. Masuzaki H, Ogawa Y, Hosoda K, Miyawaki T, Hanakoa I, Yasuno A, Nishimura H, Yoshimasa Y, Nishi S, Nakao K. **Glucocorticoid regulation of leptin synthesis and secretion in humans: elevated plasma leptin levels in Cushing's syndrome**. *J Clin Endocrinol Metab* 1997; 82:2542–2547.

119. Mauri M, Sinforiani E, Bono G, Vignati F, Berselli ME, Attanasio R, Nappi G. **Memory impairment in Cushing's disease**. *Acta Neurol Scand* 1993; 87:52-55.

120. Merke DP, Giedd JN, Keil MF, Mehlinger SL, Wiggs EA, Holzer S, Rawson E, Vaituzis AC, Stratakis CA, Chrousos GP. **Children experience cognitive decline despite reversal of brain atrophy one year after resolution of Cushing's syndrome**. *J Clin Endocrinol Metab* 2005; 90:2531-6.

121. Michaud K, Forget H, Cohen H. **Chronic glucocorticoid hypersecretion in Cushing's syndrome exacerbates cognitive aging**. *Brain Cogn* 2009; 71:1-8.

122. Mogelvang R, Pedersen SH, Flyvbjerg A, Bjerre M, Iversen AZ, Galatius S, Frystyk J, Jensen JS. **Comparison of osteoprotegerin to traditional atherosclerotic risk factors and high-sensitivity C-reactive protein for diagnosis of atherosclerosis**. Am J Cardiol 2012; 109:515-20.

123. Momose KJ, Kjelberg RN, Kliman B. **High incidence of cortical atrophy of the cerebral and cerebellar hemispheres in Cushing's disease**. *Radiology* 1971; 99:341–348.

124. Muiesan ML, Lupia M, Salvetti M, Grigoletto C, Sonino N, Boscaro M, Rosei EA, Mantero F, Fallo F. **Left ventricular structural and**

functional characteristics in Cushing's syndrome. *J Am Coll Cardiol* 2003; 41:2275-9.

125. Neary NM, Booker OJ, Abel BS, Matta JR, Muldoon N, Sinaii N, Pettigrew RI, Nieman LK, Gharib AM. **Hypercortisolism is associated with increased coronary arterial atherosclerosis: analysis of noninvasive coronary angiography using multidetector computerized tomography**. *J Clin Endocrinol Metab* 2013; 98: 2045-52.

126. Newell-Price J, Bertagna X, Grossman AB, Nieman LK. **Cushing's syndrome**. *Lancet* 2006; 367(9522):1605-1617.

127. Nieman LK, Biller BM, Findling JW, Newell-Price J, Savage MO, Stewart PM, Montori VM. **The diagnosis of Cushing's Syndrome: an Endocrine Society clinical Practice Guideline**. *J Clin Endocrinol Metab* 2008; 93:1526-40.

128. Nieman LK, Biller BM, Findling JW, Murad MH, Newell-Price J, Savage MO, Tabarin A. **Treatment of Cushing's Syndrome: An Endocrine Society Clinical Practice Guideline**. *J Clin Endocrinol Metab* 2015; 100(8):2807-31.

129. Nitshala A, Preis SR, Beiser A, Devine S, Hankee L, Seshadri S, Wolf PA, Au R. **Midlife cardiovascular risk impacts executive function: Framingham offspring study**. *Alzheimer Dis Assoc Disord* 2014; 28:16-22.

130. Ntali G, Asimakopoulou A, Siamatras T, Komninos J, Vassiliadi D, Tzanela M, Tsagarakis S, Grossman AB, Wass JA, Karavitaki N. **Mortality in Cushing's syndrome: systematic analysis of a large**

series with prolonged follow-up. *Eur J Endocrinol* 2013; 169(5):715-23.

131. Patrassi GM, Dal Bo Zanon R, Boscaro M, Martinelli S, Girolami A. **Further studies on the hypercoagulable state of patients with Cushing's syndrome**. *Thromb Haemost* 1985; 54:518-20.

132. Pecori Giraldi G, Toja PM, De Martin M, Maronati A, Scacchi M, Omboni S, Cavagnini F, Parati G. **Circadian blood pressure profile in patients with active Cushing's disease and after long-term cure**. *Horm Metab Res* 2007; 39:908-14.

133. Petramala L, D'Elia L, Iannucci G, Concistrè A, Zinnamosca L, Marinelli C, De Vicentis G, Ciardi A, De Toma G, Letizia C. **Subclinical Atherosclerosis in Patients with Cushing's Syndrome: Evaluation with Carotid Intima-Media Thickness and Ankle-Brachial Index**. *Endocrinol Metab (Seoul)* 2015 [Epub ahead of print].

134. Pereira AM, Delgado V, Romijn JA, Smit JW, Bax JJ, Feelders RA. **Cardiac dysfunction is reversed upon successful treatment of Cushing's syndrome**. *Eur J Endocrinol* 2010; 162:331–340.

135. Pikkarainen L, Sane T, Reunanen A. **The survival and well-being of patients treated for Cushing's syndrome**. *J Intern Med* 1999; 245(5):463-8.

136. Pires P, Santos A, Vives-Gilabert Y, Webb SM, Sainz-Ruiz A, Resmini E, Crespo I, De Juan-Delago M, Gómez-Anson B. **White matter alterations in the brains of patients with active, remitted, and cured Cushing syndrome: a DTI study**. *AJNR Am J Neuroradiol* 2015; 36:1043-8.

137. Pivonello R, De Leo M, Vitale P, Cozzolino A, Simeoli C, De Martino MC, Lombardi G, Colao A. **Pathophysiology of diabetes mellitus in Cushing's syndrome**. *Neuroendocrinology* 2010; 92:77-81.

138. Plotz CM, Knowlton AI, Ragan C. **The natural history of Cushing's syndrome**. *Am J Med* 1952; 13(5):597-614.

139. Ragnarsson O, Berglund P, Eder DN, Johannsson G. **Long-term cognitive impairments and attentional deficits in patients with Cushing's disease and cortisol-producing adrenal adenoma in remission**. *J Clin Endocrinol Metab* 2012; 97: 1640-8.

140. Ragnarsson O, Glad CAM, Bergthorsdottir R, Almqvist EG, Ekerstad E, Widell H, Wängberg B, Johannsson G. **Body composition and bone mineral density in women with Cushing's syndrome in remission and the association with common genetic variants influencing glucocorticoid sensitivity**. *Eur J Endocrinol* 2015;172(1):1–10.

141. Rees DA, Hanna FW, Davies JS, Mills RG, Vafidis J, Scanlon MF. **Long-term follow-up results of transsphenoidal surgery for Cushing's disease in a single centre using strict criteria for remission**. *Clin Endocrinol (Oxf)* 2002; 56:541-51.

142. Resmini E, Santos A, Gómez-Ansón B, Vives Y, Pires P, Crespo I, Portella MJ, de Juan-Delago M, Barahona MJ, Webb SM. **Verbal and visual memory performance and hippocampal volumes, measured by 3-Tesla magnetic resonance imaging, in patients with Cushing's syndrome**. *J Clin Endocrinol Metab* 2012; 97: 663-71.

143. Resmini E, Santos A, Gómez-Anson B, López-Mourelo O, Pires P, Vives-Gilabert Y, Crespo I, Portella MJ, de Juan-Delago M, Webb SM.

Hippocampal dysfunction in cured Cushing's syndrome patients, detected by ^{1}H-MR spectroscopy. *Clin Endocrinol (Oxf)*. 2013; 79:700-7.

144. Rubenstein LZ. **Falls in older people: epidemiology, risk factors and strategies for prevention**. *Age Ageing* 2006; 35 (Suppl 2): ii37-ii41.

145. Santos A, Resmini E, Martínez-Momblán MA, Crespo I, Valassi E, Roset M, Badia X, Webb SM. **Psychometric performance of the CushingQoL questionnaire in conditions of real clinical practice**. *Eur J Endocrinol* 2012; 167:337-42.

146. Sapolsky RM, Romero LM, Munck AU. How do glucocorticoids influence stress responses? **Integrating permissive, suppressive, stimulatory and preparative actions**. *Endocr Rev* 2000; 21(1):55-89.

147. Satto T, Hiramatsu R, Iwaoka T, Fujii Y, Shimada T, Umeda T. **Changes of platelets, serum lactic dehydrogenase, gamma-glutamyltranspeptidase, choline esterase and creatine phosphokinase levels in patients with Cushing's syndrome**. *Tohoku J Exp Med* 1984; 142:195-200.

148. Schmahmann JD, Sherman JC. **The cerebellar cognitive affective syndrome**. *Brain* 1998; 121:561-579.

149. Seshadri S, Wolf PA, Beiser A, Elias MF, Au R, Kase CS, D'Agostino RB, DeCarli C. **Stroke risk profile, brain volume, and cognitive function: the Framingham Offspring Study**. *Neurology* 2004; 63:1591-1599.

150. Shimada K, Kawamoto A, Matsubayashi K, Ozawa T. **Silent cerebrovascular disease in the elderly. Correlation with ambulatory pressure**. *Hypertension* 1990; 16:692-699.

151. Simmons NE, Do HM, Lipper MH, Laws ER Jr. **Cerebral atrophy in Cushing's disease**. *Surg Neurol* 2000; 53: 72-6.

152. Singh D, Park W, Lewy MS, Jung ES. **The effects of obesity and standing time on postural sway during prolonged quiet standing**. *Ergonomics* 2009; 52:977-986.

153. Small M, Lowe GD, Forbes CD, Thomson JA. **Thromboembolic complications in Cushing's syndrome**. *Clin Endocrinol (Oxf)* 1983; 19:503-11.

154. Sonino N, Fava GA, Raffi AR, Boscaro M, Fallo F**. Clinical correlates of major depression in Cushing's disease**. *Psychopathology* 1998; 31:302-6.

155. Sonino N, Bonnini S, Fallo F, Boscaro M, Fava GA. **Personality characteristics and quality of life in patients treated for Cushing's syndrome**. *Clin Endocrinol* 2006; 64:314-318.

156. Soureti A, Hurling R, Murray P, van Mechelen W, Cobain M. **Evaluation of a cardiovascular disease risk assessment tool for the promotion of healthier lifestyles**. *Eur J Cardiovasc Prev Rehabil* 2010; 17:519-23.

157. Starkman MN, Schteingart DE, Schork MA. **Depressed mood and other psychiatric manifestations of Cushing's syndrome: relationship to hormone levels**. *Psychosomatic Medicine* 1981; 43: 3–18.

158. Starkman MN, Gebarski SS, Berent S, Schteingart DE. **Hippocampal formation volume, memory dysfunction, and cortisol levels in patients with Cushing's syndrome**. *Biol Psychiatry* 1992; 32:756-765.

159. Starkman MN, Giordani B, Gebarski SS, Berent S, Schork MA, Schteingart DE. **Decrease in cortisol reverses human hippocampal atrophy following treatment of Cushing's disease**. *Biol Psychiatry* 1999; 46:1595-1602.

160. Starkman MN, Giordani B, Berent S, Schork MA, Schteingart DE. **Elevated cortisol levels in Cushing's disease are associated with cognitive decrements**. *Psychosomatic Medicine* 2001; 6:3 985–993.

161. Starkman MN, Giordani B, Gebarski SS, Schteingart DE. **Improvement in learning associated with increase in hippocampal formation volume**. *Biol Psychiatry* 2003; 53:233-238.

162. Starkman MN, Giordani B, Gebarski SS, Schteingart DE. **Improvement in mood and ideation associated with increase in right caudate volume**. *J Affect Disord* 2007; 101:139-147.

163. Stewart PM. **Tissue-specific Cushing's syndrome, 11-beta-hydroxysteroid dehydrogenases and the redefinition of corticosteroid hormone action**. *Eur J Endocrinol* 2003; 149:163-8.

164. Suhir K, Jennings GL, Esler MD, Korner PI, Blombery PA, Lambert GW, Scoggins B, Whitworth JA. **Hydrocortisone-induced hypertension in humans: pressor responsiveness and sympathetic function**. *Hypertension* 1989; 13:416-21.

165. Swearingen B, Biller BM, Barker FG 2nd, Katznelson L, Grinspoon S, Klibanski A, Zervas NT. **Long-term mortality after transsphenoidal surgery for Cushing's disease**. *Ann Intern Med* 1999; 130(10):821-4.

166. Tata, DA, Marciano VA, Anderson BJ. **Synapse loss from chronically elevated glucocorticoids: relationship to neuropil volume and cell number in hippocampal area CA3**. *J Comp Neurol* 2006; 498:363-374.

167. Terzolo M, Allasino B, Bosio S, Brusa E, Daffara F, Ventura M, Aroasio E, Sacchetto G, Reimondo G, Angeli A, Camaschella C. **Hyperhomocysteinemia in patients with Cushing's syndrome**. *J Clin Endocrinol Metab* 2004; 89:3745-51.

168. Terzolo M, Allasino B, Pia A, Peraga G, Daffara F, Laino F, Ardito A, Termine A, Paccotti P, Berchialla P, Migliaretti G, Reimondo G. **Surgical remission of Cushing's syndrome reduces cardiovascular risk**. *Eur J Endocrinol* 2014; 171(1):127–36.

169. Tiemensma J, Koksoorn NE, Biermasz NR, Keijser BJ, Wassenaar MJ, Middelkoop HA, Pereira AM, Romijn JA. **Subtle cognitive impairments in patients with long-term cure of Cushing's disease**. *J Clin Endocrinol Metab* 2010; 95: 2699-714.

170. Tiemensma J, Kaptein AA, Pereira AM, Smit JW, Romijn JA, Biermasz NR. **Negative illness perceptions are associated with impaired quality of life in patients after long-term remission of Cushing's syndrome**. *Eur J Endocrinol* 2011; 165:527-35.

171. Tirapu-Ustarroz J, Luna-Lario P, Iglesias-Fernandez MD, Hernaez-Goni P. **Cerebellar contribution to cognitive process: current advances**. *Revue Neurologique* 2011; 53:301–315.

172. Toja PM, Branzi G, Ciambellotti F, Radaelli P, De Martin M, Lonati LM, Scacchi M, Parati G, Cavagnini F, Pecori Giraldi F**. Clinical relevance of cardiac structure and function abnormalities in patients with Cushing's syndrome before and after cure**. *Clin Endocrinol (Oxf)* 2012; 76:332-8.

173. Trethowan WH, Cobb S. **Neuropsychiatric aspects of Cushing's syndrome**. *AMA Arch Neurol Psychiatry* 1952; 67: 283-309.

174. Ueland T, Kristo C, Godang K, Aukrust P, Bollerslev J. **Interleukin-1 receptor antagonist is associated with fat distribution in endogenous Cushing's syndrome: a longitudinal study**. *J Clin Endocrinol Metab* 2003; 88:1492–1496.

175. Valassi E, Santos A, Yaneva M, Tóth M, Strasburger CJ, Chanson P, Wass JA, Chabre O, Pfeifer M, Feelders RA, Tsagarakis S, Trainer PJ, Franz H, Zopf K, Zacharieva S, Lamberts SW, Tabarin A, Webb SM; ERCUSYN Study Group. **The European Registry on Cushing's syndrome: 2-year experience. Baseline demographic and clinical characteristics**. *Eur J Endocrinol* 2011; 165(3):383-92.

176. Valassi E, Biller BM, Klibanski A, Misra M. **Adipokines and cardiovascular risk in Cushing's syndrome**. *Neuroendocrinology* 2012; 95: 187-206.

177. Van Aken MO, Pereira AM, Biermasz NR, van Thiel SW, Hoftijzer HC, Smit JW, Roelfsema F, Lamberts SW, Romijn JA. **Quality of life in**

patients after long-term biochemical cure of Cushing's disease. *J Clin Endocrinol Metab* 2005; 90: 3279-3286.

178. Van der Klaauw AA, Kars M, Biermasz NR, Roelfsema F, Dekkers OM, Corsmitt EP, van Aken MO, Havekes B, Pereira AM, Pijl H, Smit JV, Romijn JA. **Disease-specific impairments in quality of life during long-term follow-up of patients with different pituitary adenomas**. *Clin Endocrinol (Oxf.)* 2008; 69:775-84.

179. Van der Werff SJ, Andela CD, Nienke Pannekoek J, Meijer OC, van Buchem MA, Rombouts SA, van der Mast RC, Biermasz NR, Pereira AM, van der Wee NJ. **Widespread reductions of white matter integrity in patients with long-term remission of Cushing's disease**. *Neuroimage Clin* 2014; 4:659-67.

180. Van Haalen FM, Broersen LH, Jorgensen JO, Pereira AM, Dekkers OM. **Management of endocrine disease: Mortality remains increased in Cushing's disease despite biochemical remission: a systematic review and meta-analysis**. *Eur J Endocrinol* 2015; 172(4): R143-9.

181. Van Nieuwpoort IC, Drent ML. **Cognition in the adult with childhood-onset GH deficiency**. *Eur J Endocrinol* 2008; 159 Suppl 1: S53-7.

182. Van Zaane B, Nur E, Squizzato A, Dekkers OM, Twickler MT, Fliers E, Gerdes VE, Büller HR, Brandjes DP. **Hypercoagulable state in Cushing's syndrome: a systematic review**. *J Clin Endocrinol Metab* 2009; 94:2743-50.

183. Verdelho A, Madureira S, Ferro JM, Basile AM, Chabriat H, Erkinjuntti T, Fazekas F, Hennerici M, O'Brien J, Pantoni L, Salvadori E, Scheltens

P, Visser MC, Wahlund LO, Waldemar G, Wallin A, Inzitari D; LADIS study. **Differential impact of cerebral white matter changes, diabetes, hypertension and stroke on cognitive performance among non-disabled elderly. The LADIS study**. *J Neurol Neurosurg Psychiatry* 2007; 78:1325-30.

184. Verhaaren BF, Vernooij MW, de Boer R, Hofman A, Niessen WJ, van der Lugt A, Ikram MA. **High blood pressure and cerebral white matter lesion progression in the general population**. *Hypertension* 2013; 61**:**1354-9.

185. Vestergaard P, Lindholm J, Jorgensen JO, Hagen C, Hoeck HC, Laurberg P, Rejnmark L, Brixen K, Kristensen LO, Feldt-Rasmussen U, Mosekilde L. **Increased risk of osteoporotic fractures in patients with Cushing's syndrome**. *Eur J Endocrinol* 2002; 146:51-6.

186. Wagenmakers MA, Netea-Maier RT, Prins JB, Dekkers T, den Heijer M, Hermus AR. **Impaired quality of life in patients in long-term remission of Cushing's syndrome of both adrenal and pituitary origin: a remaining effect of long-standing hypercortisolism?** *Eur J Endocrinol* 2012; 167: 687-95.

187. Wang J, Barak LS, Mook RA Jr, Chen W. **Glucocorticoid hedgehog agonists in neurogenesis**. *Vitam Horm* 2011; 87:207-15.

188. Webb SM, Badia X, Barahona MJ, Colao A, Strasburger CJ, Tabarin A, van Aken MO, Pivonello R, Stalla G, Lamberts SW, Glusman JE. **Evaluation of health-related quality of life in patients with Cushing's syndrome with a new questionnaire**. *Eur J Endocrinol* 2008: 158:623-30.

189. Weise M, Abad V, Considine RV, Nieman L, Rother KI. **Leptin secretion in Cushing's syndrome: preservation of diurnal rhythm and absent response to corticotrophin-releasing hormone**. *J Clin Endocrinol Metab* 1999; 84:2075–2079.

190. Whelan TB, Schteingart DE, Starkman MN, Smith A. **Neuropsychological deficits in Cushing's syndrome**. *J Nerv Ment Dis* 1980;168:753-757.

191. Widjaja A, Schurmeyer TH, Von zur Muhlen A, Brabant G. **Determinants of serum leptin levels in Cushing's syndrome**. *J Clin Endocrinol Metab* 1998; 83:600–603.

192. Xiong YY, Mok V. **Age-related white matter changes**. *J Aging Res* 2011; 2011:617927.

193. Yaneva M, Kalinov K, Zacharieva S. **Mortality in Cushing's syndrome: data from 386 patients from a single tertiary referral center**. *Eur J Endocrinol* 2013; 169(5):621-7.

194. Yaffe K, Vittinghoff E, Pletcher MJ, Hoang TD, Launer LJ, Whitmer R, Coker LH, Sidney S. **Early adult to midlife cardiovascular risk factors and cognitive function**. *Circulation* 2014;129:1560-7.

195. Zacharieva S, Orbetzova M, Stoynev A, Shigarminova R, Yaneva M, Kalinov K, Nachev E, Elenkova A. **Circadian blood pressure profile in patients with Cushing's syndrome before and after treatment**. *J Endocrinol Invest* 2004; 27:924-30.

196. Zografos GN, Markou A, Ageli C, Kopanakis N, Koutmos S, Kaltsas G, Piaditis G, Papastratis G. **Laparoscopic surgery for adrenal tumors. A retrospective analysis**. *Hormones (Athens)* 2006; 5:52-6.

7. ANNEX: SUMMARY (SPANISH VERSION)

GLOSARIO DE ABREVIACIONES

ACTH: Hormona adrenocorticotropa

CRH: Hormona liberadora de corticotropina

RM: Resonancia magnética

SC: Síndrome de Cushing

TC: Tomografía computerizada

1. INTRODUCCIÓN

1.1. El síndrome de Cushing: prevalencia, etiología y manifestaciones clínicas

Esta tesis trata sobre el síndrome de Cushing (SC) endógeno, una enfermedad rara de muy baja prevalencia (39,1 casos por millón de habitantes, Etxabe *et al.* 1994). Esta enfermedad afecta con mayor frecuencia a mujeres que a hombres (Newell-Price *et al.* 2006).

La causa más común del SC es un adenoma hipofisario productor de hormona adrenocorticotropa (ACTH). Esta forma de la enfermedad es conocida como enfermedad de Cushing y afecta al 70% de los pacientes. Aproximadamente el 20% de los casos son ACTH-independientes y son debidos a un exceso de cortisol por tumores adrenocorticales (10%), carcinomas (8%) o hiperplasia adrenal bilateral. Por último, el 10-12% de los casos son debidos a una secreción ectópica de ACTH o a una producción de hormona liberadora de corticotropina (CRH) por un tumor neuroendocrino (Boscaro *et al.* 2001).

Los pacientes presentan múltiples síntomas, incluyendo plétora facial (debida a la deposición de grasa en las mejillas y la fosa temporal), obesidad central, alteraciones en la composición corporal (incluyendo aumento de masa grasa, reducción de masa ósea con consecuente osteoporosis y disminución de masa muscular), alteraciones cutáneas (estrías rojo-vinosas, facilidad para hacerse moratones, úlceras, dificultades de cicatrización), hirsutismo, amenorrea, debilidad muscular (o miopatía), hipogonadismo, hipertensión, insulinorresistencia y/o diabetes mellitus, dislipidemia, estado protrombótico, enfermedad vascular, arterosclerosis, depresión, ansiedad, déficits cognitivos y calidad de vida alterada (Arnaldi *et al.* 2003, Nieman *et al.* 2008, Bourdeau *et al.* 2005, Starkman *et al.* 1981, Starkman *et al.* 2001, León-Carrión *et al.* 2009, Dorn *et al.* 2000).

1.2. Síndrome de Cushing: Diagnóstico y tratamiento

El primer paso para establecer el diagnóstico de síndrome de Cushing incluye la exploración de la historia farmacológica para excluir la posible exposición a glucocorticoides exógenos. En caso de excluirse la posible causa exógena se deberá proceder a las pruebas bioquímicas. Siguiendo las recomendaciones de las guías (Arnaldi *et al.* 2003; Nieman *et al.* 2008), los primeros tests de screening serán:

- Cortisol en orina de 24 horas (como mínimo dos medidas)
- Test de supresión rápida con 1 mg de dexametasona a medianoche y determinación de cortisol a la mañana siguiente. Alternativamente puede realizarse este test de supresión con 2 mg de dexametasona al día durante 48 horas.
- Cortisol en saliva a medianoche (dos medidas)

Cuando al menos uno de los tests resulte alterado, será necesaria una evaluación endocrinológica más extensa para confirmar el diagnóstico.

En una segunda fase, una vez establecido el diagnóstico se procederá a determinar el diagnóstico diferencial (diagnóstico etiológico). En caso de no haber supresión de ACTH plasmática deberán investigarse las causas ACTH-dependientes (causa hipofisaria y ectópica). La resonancia magnética (RM) hipofisaria puede evidenciar un adenoma en aproximadamente el 60% de los pacientes. Si no es posible identificar la lesión mediante RM, el cateterismo de senos petrosos puede resultar útil para confirmar o excluir el origen hipofisario. Para las posibles causas ectópicas se recomienda realizar una tomografía computerizada (TC) o RM en el cuello, tórax o abdomen, o una gammagrafía con octreotide (Octreoscan). Si existe supresión de ACTH plasmática, se recomienda realizar una TC o RM adrenal para identificar el tipo de lesión (Arnaldi *et al.* 2003).

El tipo de tratamiento diferirá dependiendo de la causa del SC. Este puede incluir cirugía (adenomectomía hipofisaria, adrenalectomía o escisión de la causa ectópica de ACTH), irradiación o medicación para reducir los niveles de cortisol.

1.3. Síndrome de Cushing: mortalidad, riesgo cardiovascular y enfermedad cardiovascular

En los años 50 se estimaba un 50% de mortalidad en los pacientes con SC cinco años tras el diagnóstico. Las opciones terapéuticas actuales han llevado a una mejora en el pronóstico, aunque en algunos casos puede no llegar a ser óptimo. Existe controversia respecto a las tasas de mortalidad en el SC debidas a un aumento de riesgo cardiovascular, ya que en algunos estudios se ha

encontrado una mortalidad por encima de la población normal (Etxabe *et al.* 1994, Lindholm *et al.* 2001, Bolland *et al.* 2011, Clayton *et al.* 2011, Hassan-Smith *et al.* 2012, Ntali *et al.* 2013, Yaneva *et al.* 2013), aunque no en otros (Swearingen *et al.* 1999, Pikkarainen *et al.* 1999, Hammer *et al.* 2004). De todos modos, la mayoría de estudios en pacientes con hipercortisolismo elevado persistente han hallado una tasa de mortalidad estandarizada incrementada en comparación a la población normal (Etxabe *et al.* 1994, Lindholm *et al.* 2001, Hammer *et al.* 2004, Clayton *et al.* 2011, Ntali *et al.* 2013).

El SC conlleva un alto riesgo cardiovascular relacionado con el exceso de cortisol, que a menudo persiste tras la remisión de la enfermedad. Esto es el resultado de una combinación de distintos factores de riesgo, que incluyen factores metabólicos (obesidad central, hipercolesterolemia, hipertrigliceridemia y diabetes), vasculares (hipertensión o arteriosclerosis), cardiacos (relacionados con la funcionalidad y ritmo del corazón) o trombóticos (estado protrombótico).

1.4. Riesgo cardiovascular y lesiones de sustancia blanca

Como ya se ha establecido en la sección previa, el SC se ha relacionado con un mayor riesgo cardiovascular, que habitualmente persiste tras la curación (Colao *et al.* 1999, Barahona *et al.* 2009, de Leo *et al.* 2010). El riesgo cardiovascular elevado parece estar asociado a la presencia de lesiones de sustancia blanca cerebrales (Gorelick *et al.* 2011). Estas lesiones probablemente tengan un origen isquémico, y se evidencian en RM como hiperintensidades en imágenes T2 o FLAIR, correspondiendo habitualmente a áreas de leve gliosis y pérdida de mielina (Debette *et al.* 2010, Mataró *et al.* 2014).

Las lesiones de sustancia blanca son habituales en poblaciones ancianas, y tienden a aumentar con la edad. También son habituales en poblaciones con un elevado riesgo cardiovascular, como pacientes con hipercolesterolemia, hipertensión o diabetes (Verdelho *et al.* 2007). Dado su inherente riesgo cardiovascular, los pacientes con SC podrían tener un alto grado de lesiones de sustancia blanca. Sin embargo, hasta la fecha no se han realizado estudios sobre lesiones de sustancia blanca en pacientes con SC. Este tema requeriría ser investigado, ya que las lesiones de sustancia blanca no son inocuas y pueden tener importantes implicaciones clínicas. Se han asociado a alteraciones cognitivas (principalmente en funciones ejecutivas, aunque también en velocidad de procesamiento de la información, memoria, atención y velocidad psicomotora), así como a un elevado riesgo de demencia, apoplejía e incluso muerte (Debette *et al.* 2010, Mataró *et al.* 2014, Gorelick *et al.* 2011).

1.5. Síndrome de Cushing: cerebro y cognición

Varios estudios han encontrado aumento del tamaño de los ventrículos, atrofia cerebral y menores volúmenes cerebrales en pacientes con SC, en comparación con los controles (Trethowan *et al.* 1952, Momose *et al.* 1971, Bourdeau *et al.* 2002, Simmons *et al.* 2002, Resmini *et al.* 2012). En 1971, mediante pneumoencefalografía se detectó atrofia cerebelar en los pacientes con SC activo. La técnica no era tan precisa como las disponibles actualmente, y no se valoró por separado los volúmenes de sustancia gris y blanca, ni se realizó una evaluación neuropsicológica. El cerebelo tiene implicaciones en el control emocional y la cognición, aunque sorprendentemente hasta la fecha no existen estudios que hayan analizado el volumen cerebelar y sus correlatos neuropsicológicos en el SC. Este sería otro tema que requeriría ser investigado.

El hipocampo ha sido una de las estructuras más estudiadas en el SC. Parece ser que durante la fase activa de la enfermedad su volumen se encuentra reducido en comparación a los controles sanos, y correlaciona con los niveles plasmáticos de cortisol (aunque no con los niveles de cortisol libre urinario) (Starkman *et al.* 1992). Tras tratamiento se puede producir una mejora parcial (Starkman *et al.* 1999, Starkman *et al.* 2003). Los estudios realizados en pacientes en remisión no han hallado diferencias en los volúmenes hipocampales (Resmini *et al.* 2012, Andela *et al.* 2013), aunque sí se han hallado alteraciones funcionales mediante espectroscopia (Resmini *et al.* 2013).

Existen también alteraciones en otras áreas. En niños con SC se encontró una reducción de la amígdala en comparación con el grupo control, que no parecía remitir tras curación (Merke *et al.* 2005). Sin embargo, en pacientes adultos con SC en remisión no se hallaron diferencias en el volumen de la amígdala en comparación con los controles (Andela *et al.* 2013). Por otro lado, algunos estudios han descrito afectación en lóbulo frontal y el tálamo, evidenciados mediante alteraciones espectroscópicas o disminución del grosor cortical en el caso del lóbulo frontal (Khiat *et al.* 1999, Khiat *et al.* 2000, Crespo *et al.* 2014). Otros estudios han hallado alteraciones en la integridad de la sustancia blanca medidas mediante tensor de difusión (van der Werff *et al.* 2014, Pires *et al.* 2014), así como alteraciones en resonancia magnética funcional (Langenecker *et al.* 2012, Maheu *et al.* 2008, Bas-Hoogendam *et al.* 2015).

Respecto a la cognición, la mayoría de estudios han descrito alteraciones en la memoria, tanto a nivel verbal como visual (Whelan *et al.* 1980, Forget *et al.* 2000, León-Carrión *et al.* 2009, Mauri *et al.* 1993, Starkman *et al.* 2001, Michaud *et al.* 2009). Existe controversia respecto a la reversibilidad de estas

alteraciones; mayoritariamente se ha descrito cierta mejora tras la curación de la enfermedad (Mauri *et al.* 1993, Martignoni *et al.* 1992, Starkman *et al.* 2003, Hook *et al.* 2007), aunque en los estudios en pacientes en remisión parece continuar existiendo algún tipo de déficit (Resmini *et al.* 2012, Tiemensma *et al.* 2010).

Respecto al resto de funciones cognitivas existe controversia respecto a las funciones afectadas. Se han hallado alteraciones en funciones ejecutivas, atención, funciones visoconstructivas, lenguaje, velocidad de procesamiento de la información y funciones motoras, aunque de forma menos consistente (Forget *et al.* 2000, Michaud 2009, Starkman 1992, Crespo *et al.* 2014, Whelan 1980).

1.6. Síndrome de Cushing: comorbilidades psicológicas y calidad de vida

El SC ha sido asociado habitualmente a psicopatología. La mayoría de estudios describen presencia de ansiedad y depresión en los pacientes con SC, pudiendo persistir tras la curación (Starkman *et al.* 1981, Whelan *et al.* 1980, Dorn *et al.* 2000, Kelly 1996, Sonino *et al.* 1998, Tiemensma *et al.* 2010, Ragnarsson *et al.* 2012, Andela *et al.* 2013). También son habituales la labilidad emocional, la irritabilidad o la apatía (Starkman *et al.* 1981, Andela *et al.* 2013). Tras el tratamiento, la sintomatología suele mejorar, en paralelo con la recuperación del eje hipotálamo- hipofisario (Dorn *et al.* 1997).

La calidad de vida está también afectada, siendo peor en comparación a controles sanos y pacientes con otros tumores hipofisarios (Lindslay *et al.* 2006, Lindholm *et al.* 2001, Hawn *et al.* 2002, Johnson *et al.* 2003, van der Klaauw *et al.* 2008). Los pacientes activos presentan peor calidad de vida que

los pacientes en remisión (Lindslay *et al.* 2006, Lindholm *et al.* 2001, Webb *et al.* 2008, Santos *et al.* 2012), aunque tras la curación persiste cierto grado de deterioro (van Aken *et al.* 2002, Heald *et al.* 2004, Lindslay *et al.* 2006). Esto se verá influenciado tanto por factores físicos como psicológicos.

2. OBJETIVOS

Los objetivos generales de esta tesis fueron investigar los volúmenes cerebelares y las lesiones de sustancia blanca cerebral en los pacientes con SC, y su relación con el rendimiento neuropsicológico y los parámetros clínicos y hormonales. Los objetivos específicos fueron:

I. Analizar el volumen cerebelar en los pacientes con SC (separando sustancia blanca y sustancia gris) **(Estudio I)**.
II. Estudiar la relación entre el volumen cerebelar, el rendimiento neuropsicológico, los niveles de cortisol y otros parámetros **(Estudio I)**.
III. Investigar la presencia de lesiones de sustancia blanca en pacientes con SC **(Estudio II)**.
IV. Investigar la relación entre el riesgo cardiovascular, las lesiones de sustancia blanca, el rendimiento neuropsicológico y el volumen cerebral en los pacientes con SC **(Estudio II)**.

3. MÉTODOS Y RESULTADOS

Los detalles sobre los métodos y resultados pueden hallarse en los dos estudios que componen esta tesis (ver páginas 55-76).

Estudio I:

Santos A, Resmini E, Crespo I, Pires I, Vives-Gilabert Y, Granell E, Valassi E, Gómez-Ansón B, Martínez-Momblán MA, Mataró M, Webb SM. **Small cerebellar cortex volume in patients with active Cushing's syndrome.** *Eur J Endocrinol*, 2014 Oct; 171(4):461-9.

Estudio II:

Santos A, Resmini E, Gómez-Ansón B, Crespo I, Granell E, Valassi E, Pires I, Vives-Gilabert Y, Martínez-Momblán MA, de Juan M, Mataró M, Webb SM. **Cardiovascular risk and white matter lesions after endocrine control of Cushing's syndrome.** *Eur J Endocrinol* 2015 Dec; 173(6):765-75.

4. RESUMEN DE LOS RESULTADOS Y DISCUSIÓN

4.1. Volúmenes cerebelares

La atrofia cerebelar había sido descrita en poblaciones donde el hipercortisolismo parece tener un rol importante, como la depresión y el estrés postraumático (Baldaçara *et al.* 2008), así como en pacientes con artritis reumatoide, población que requiere tratamiento crónico con glucocorticoides (Bekkelund *et al.* 1995).

Los resultados del *estudio I* confirman el posible rol del hipercortisolismo en el volumen cerebelar. Los pacientes activos presentaron menores volúmenes en el córtex cerebelar bilateral que los controles, aunque no se hallaron diferencias para la sustancia blanca o el volumen total. Estos resultados sugieren que la exposición a glucocorticoides podría llevar a una reducción selectiva de la sustancia gris cerebelar. Diferentes mecanismos podrían estar implicados, incluyendo la neurodegeneración y muerte celular, la reducción de

la neurogénesis o los cambios en la estructura dendrítica (Bhatt *et al.* 2013, Hu *et al.* 2015, Wang *et al.* 2011, Tata *et al.* 2006, Kleen *et al.* 2006).

Los pacientes en remisión no presentaron volúmenes cerebelares distintos a los controles. Estos datos nos llevan a la hipótesis de que el hipercortisolismo produce una reducción cerebelar que podría ser parcialmente reversible tras la normalización del cortisol. En pacientes en remisión otros autores han hallado mayor volumen en el lóbulo posterior izquierdo del cerebelo en comparación con el grupo control. Como posible explicación sugieren que la reorganización neuronal tras el estrés crónico puede llevar a atrofia dendrítica en algunas partes del cerebro y a hipertrofia dendrítica en otras (Andela *et al.* 2013). Esto podría explicar las diferencias entre pacientes activos y curados en nuestro estudio, aunque serían necesarios estudios longitudinales para confirmar esta hipótesis.

A nivel clínico, una reducción del cerebelo puede causar alteraciones en el equilibrio y el control postural (Horak *et al.* 1994, Dichgans *et al.* 1983). Los déficits pueden ser más severos en presencia de obesidad, que se ha relacionado tanto con alteraciones de equilibrio y control postural (Singh *et al.* 2009), como con mayor número de caídas (Fjeldstad *et al.* 2008), en comparación con personas que presentaban normopeso. El riesgo de caídas debe ser tomado en consideración en los pacientes con SC, que presentan un riesgo elevado de fracturas osteoporóticas (Vestergaard *et al.* 2002), pero sobre todo en los pacientes mayores, ya que las caídas se asocian a mayor uso de recursos sanitarios y mayor morbilidad y mortalidad (Rubenstein 2006).

4.2. Cerebelo, correlación con rendimiento neuropsicológico y parámetros clínicos

En el *estudio I*, el volumen del córtex cerebelar se asoció a diversas variables. En primer lugar, la edad al diagnóstico correlacionó negativamente con el volumen del córtex cerebelar bilateral. La capacidad de neurogénesis disminuye a lo largo de la vida (Galvan *et al.* 2007), por lo que la exposición a glucocorticoides a una edad más tardía podría ser un factor de riesgo para mayor reducción cerebelar.

En el mismo estudio se halló también una correlación negativa entre el nivel de triglicéridos y el volumen de córtex cerebelar bilateral. En estudios animales en ratas alimentadas con una dieta rica en triglicéridos, se ha encontrado un efecto parecido al envejecimiento en el córtex cerebelar, con una reducción sináptica (Balietti *et al.* 2009). El control de los niveles de triglicéridos podría ser beneficioso para evitar el deterioro cerebelar, aunque serían necesarios nuevos estudios para confirmar esta hipótesis.

La calidad de vida mostró una correlación positiva con el córtex cerebelar derecho. Esta relación entre calidad de vida y córtex cerebelar no había sido descrita previamente, aunque ya que el cerebelo tiene un rol como modulador de las respuestas emocionales (Schmahmann *et al.* 1998) sería factible que estuviera involucrado en la autopercepción de la calidad de vida.

El rendimiento en memoria visual correlacionó positivamente con el córtex cerebelar izquierdo. A parte del hipocampo, el cerebelo tiene también un papel en la memoria visual (Tirapu-Ustarroz *et al.* 2011, Schmahmann et al. 1998). Estos datos tienen implicaciones clínicas relevantes, principalmente en la fase activa de la enfermedad, ya que los pacientes podrían presentar alteraciones neuropsicológicas. Sería importante realizar una evaluación neuropsicológica

completa en estos pacientes así como un examen neurológico detallado para identificar posibles alteraciones.

4.3. Neuropsicología y estado de ánimo

Los resultados del *estudio I*, indican que los pacientes con SC activo tenían peor rendimiento en memoria visual que los controles, lo que podría conllevar problemas para recordar este tipo de información en su vida diaria. En el *estudio II*, el dominio de memoria incluía tanto memoria visual como verbal, demostrando que los pacientes con SC activo presentaban también peor rendimiento que los controles. En la práctica clínica esto implicaría que los pacientes podrían tener también problemas para recordar la información verbal aportada por los médicos, como las dosis de fármacos o las recomendaciones clínicas. Consecuentemente, podría resultar de ayuda acompañar las explicaciones médicas con información escrita que los pacientes pudieran revisar en casa.

Los resultados de los tests neuropsicológicos en ambos estudios indican que todas las puntuaciones Z se hallan alrededor de una desviación estándard, implicando que no existen déficits cognitivos severos, en línea con estudios previos en pacientes en remisión (Tiemensma *et al.* 2010). En los pacientes con SC activo habría sido previsible hallar mayores déficits, aunque el uso de medicación para reducir los niveles de cortisol (en la mayoría de pacientes), podría haber prevenido que se evidenciara mayor deterioro. De todos modos, la mayoría de pacientes refería tener peor rendimiento cognitivo en comparación con sus capacidades previas.

El estado de ánimo también resultó alterado los pacientes con SC, tanto activos como en remisión. Ambos grupos de pacientes presentaron mayores

puntuaciones en depresión y ansiedad (tanto estado como rasgo), sin correlacionar con los parámetros clínicos. Estos resultados concuerdan con la literatura (Starkman *et al.* 1981, Whelan *et al.* 1980, Dorn *et al.* 2000, Kelly 1996, Sonino *et al.* 1998, Tiemensma *et al.* 2010, Ragnarsson *et al.* 2012, Andela *et al.* 2013). Tal y como establecen las guías sería importante ofrecer una monitorización y tratamiento adecuado de las posibles alteraciones psiquiátricas en estos pacientes (Nieman *et al.* 2015).

4.4. Lesiones de sustancia blanca

Al analizar las lesiones de sustancia blanca en el *estudio II*, los pacientes en remisión presentaron un mayor grado de lesiones (valoradas con la escala de Scheltens) en comparación con los controles y los pacientes con enfermedad activa. Los factores de riesgo cardiovascular se han relacionado habitualmente con este tipo de lesiones en población general (Jeerakathil *et al.* 2004, Gorelick *et al.* 2011). El hecho de que el mayor grado de lesiones no se hallara en los pacientes activos podría explicarse por el menor tiempo de exposición a un elevado riesgo cardiovascular. Las correlaciones positivas entre el grado de lesiones de sustancia blanca y la duración de la hipertensión concuerdan con esta hipótesis. Es importante remarcar que estos datos implican que si el riesgo cardiovascular elevado se mantiene los pacientes con enfermedad activa podrían también desarrollar un mayor grado de lesiones de sustancia blanca.

La relación entre los niveles de tensión arterial y las lesiones de sustancia blanca no es sorprendente, ya que la hipertensión es un conocido predictor de estas lesiones (Longstreth *et al.* 1996, Basile *et al.* 2006, Xiong *et al.* 2011). La severidad de la hipertensión y el bajo grado de control de la misma se han relacionado con la presencia de lesiones de sustancia blanca en población

hipertensa (Shimada *et al.* 1990, Liao *et al.* 1996, De Leeuw *et al.* 2002). En casos de hipertensión no controlada, la progresión de las lesiones de sustancia blanca es mayor en pacientes no tratados que en pacientes tratados, lo que sugiere que el tratamiento de la hipertensión puede ser una importante herramienta para reducir la progresión de las lesiones (Verhaaren *et al.* 2013). La hipertensión evaluada 5 o 20 años atrás también se ha asociado con la presencia de lesiones de sustancia blanca en la vejez (De Leeuw *et al.* 2002, Debette *et al.* 2011). Los datos implican que la causa de las lesiones de sustancia blanca podría no ser directamente el SC, sino la exposición a largo plazo a hipertensión arterial. Esto tiene gran importancia ya que la hipertensión es un factor de riesgo modificable.

Las lesiones de sustancia blanca se han asociado habitualmente a reducciones de volumen cerebral y funciones cognitivas alteradas (Debette *et al.* 2010, Mataró *et al*, 2014, Appelman *et al.* 2009), aunque en nuestro estudio no se halló esa relación. La edad de los pacientes podría explicar estos resultados, ya que la mayoría de estudios se han realizado en pacientes ancianos donde la atrofia cerebral y el deterioro cognitivo son más pronunciados. Ya que las lesiones de sustancia blanca son factores de riesgo para el deterioro cognitivo, la demencia y la apoplejía (Debette *et al.* 2010, Mataró *et al.* 2010), hay que tener en cuenta que esta población podría estar en riesgo de padecer deterioro cognitivo en el futuro. Este sería un factor a evaluar mediante estudios prospectivos. De todos modos, es posible que el control de la hipertensión pudiera ayudar a prevenir la progresión de las lesiones de sustancia blanca (Liao *et al.* 1996, De Leeuw *et al.* 2002) y a reducir el riesgo de desarrollar deterioro cognitivo y demencia (Forette *et al.* 1998, Levi *et al.* 2013).

Por otro lado, proporcionar educación en relación a los fármacos que pueden empeorar la hipertensión, como los anti-inflamatorios no esteroideos (Johnson 1997, Kalatufova *et al.* 2014) sería recomendable. Los pacientes en remisión que tomaban hidrocortisona tenían un mayor grado de lesiones de sustancia blanca que los pacientes en remisión que no estaban siendo tratados con el fármaco. Estos hallazgos sugieren que se debería tratar de limitar el uso de hidrocortisona lo máximo posible. En algunas situaciones los pacientes pueden presentar dificultades para diferenciar los síntomas relacionados con la insuficiencia suprarrenal de otras causas, y pueden tomar dosis extra de hidrocortisona, que pueden empeorar la hipertensión (Sudhir *et al.* 1989). Sería importante alentar a los pacientes a revisar tu presión arterial regularmente para identificar posibles incrementos y evitar tomar dosis extra de hidrocortisona regularmente en caso de presentar síntomas como dolores de cabeza o cansancio.

4.5. Riesgo cardiovascular, volúmenes cerebrales y rendimiento neuropsicológico

Los resultados del *estudio II* demuestran que, confirmando los hallazgos de estudios previos, el riesgo cardiovascular es mayor en pacientes activos y curados en comparación con el grupo control (Colao *et al.* 1999, Mancini *et al.* 2004, Faggiano *et al.* 2003). Por otro lado, estos resultados muestran que ambos grupos de pacientes tienen menores volúmenes cerebrales (tanto a nivel de sustancia gris como de volumen total) en comparación con los controles, en consonancia con estudios previos (Resmini *et al.* 2012, Simmons *et al.* 2000, Momose *et al.* 1971).

El *estudio II* también demuestra que el riesgo cardiovascular (medido como riesgo cardiovascular a 10 años) presenta una correlación inversa con los volúmenes cerebrales y la función cognitiva en los pacientes con SC en remisión. Estas asociaciones concuerdan con estudios previos en población normal (Knopman *et al.* 2005, Longstreth *et al.* 2000, Seshadri *et al.* 2004, Nitshala *et al.* 2014, Yaffe *et al.* 2014, Dregan *et al.* 2013, Kaffashian *et al.* 2011). Los resultados ponen en relieve la necesidad de prestar una especial atención a los pacientes con alto riesgo cardiovascular a 10 años, ya que esto podría implicar volúmenes cerebrales reducidos y disminución de las funciones cognitivas. Por otro lado, el riesgo cardiovascular a 10 años también puede tener implicaciones en el rendimiento cognitivo futuro, ya que se ha asociado a disminución de las funciones cognitivas transcurridos entre 10 y 25 años (Nitshala *et al.* 2014, Yaffe *et al.* 2014). Esto remarca la importancia de controlar el riesgo cardiovascular incluso a edades tempranas para prevenir futuras alteraciones cognitivas.

En los pacientes con SC activo no se halló correlación entre riesgo cardiovascular, volúmenes cerebrales y funciones cognitivas. El reducido tamaño de la muestra podría ser una posible explicación, aunque otra hipótesis sería que el riesgo cardiovascular puede tardar un tiempo en afectar al cerebro y al rendimiento cognitivo. Siguiendo esta hipótesis, el hecho de que los pacientes activos hayan estado expuestos a un elevado riesgo cardiovascular durante menos tiempo podría no haber permitido encontrar asociaciones. Por otro lado los efectos del hipercortisolismo crónico, aún presentes en los pacientes activos, podrían haber afectado al volumen cerebral y a las funciones cognitivas más que el riesgo cardiovascular, previniendo encontrar asociaciones.

Respecto a la edad vascular, no hallamos estudios que la correlacionaran con los volúmenes cerebrales o la función cognitiva. En la práctica clínica, el

concepto de edad vascular puede ser una herramienta útil para la comunicación del riesgo cardiovascular al paciente, ya que se trata de un concepto sencillo de entender en comparación con otros como el riesgo cardiovascular a 10 años. En individuos jóvenes, conocer la "edad del corazón" puede tener un alto impacto emocional y llevar a cambios en el estilo de vida (Soureti *et al.* 2010). De hecho, las guías canadienses para el diagnóstico y tratamiento de la dislipidemia recomiendan comunicar la edad vascular a los pacientes para mejorar el control de la hipertensión y la dislipidemia (Anderson *et al.* 2013).

4.6. Síndrome de Cushing: Pacientes activos vs pacientes en remisión

Según nuestros resultados, parece ser que el SC lleva a diferentes comorbilidades en las distintas fases de la enfermedad. Los pacientes activos tienen un bajo rendimiento a nivel de memoria y un volumen del córtex cerebelar reducido. También presentan un alto riesgo cardiovascular, altos niveles de ansiedad y depresión y menores volúmenes cerebrales que los controles (tanto a nivel de sustancia gris como de volumen total).

En los pacientes en remisión, los volúmenes cerebelares y el rendimiento neuropsicológico parecen normalizarse, aunque otras comorbilidades persisten o incluso pueden aparecer algunas distintas. Los pacientes pueden seguir teniendo un elevado riesgo cardiovascular, así como altos niveles de depresión o ansiedad, y menores volúmenes totales cerebrales o de sustancia gris que los controles. Además pueden presentar una comorbilidad distinta a las halladas en la fase activa: un mayor grado de lesiones de sustancia blanca, correlacionado con los niveles de presión arterial y la duración de la hipertensión.

Parece ser que algunas de las alteraciones halladas en los pacientes con SC pueden ser al menos parcialmente reversibles. Sin embargo, el riesgo cardiovascular, asociado a la enfermedad, puede llevar a otras comorbilidades en el futuro si no se controla. Los niveles elevados de depresión y ansiedad, así como los volúmenes cerebrales reducidos pueden persistir también tras la curación.

En consecuencia, sería importante proporcionar soporte psicológico a los pacientes en caso necesario y controlar o incluso mejorar el riesgo cardiovascular, para prevenir la posible afectación cerebral y reducir el riesgo de infarto o ataque al corazón. La educación al paciente y la comunicación de su riesgo cardiovascular actual utilizando el concepto de edad vascular puede también ser útil para concienciar a los pacientes de la necesidad de un cambio en su estilo de vida.

4.7. Limitaciones de los estudios

Ambos estudios tienen varias limitaciones. Estas incluyen el tamaño de la muestra reducido así como la heterogeneidad de la muestra, limitaciones difíciles de evitar en estudios con enfermedades raras. Por otro lado, los estrictos criterios de inclusión también contribuyeron a la reducción de la muestra final, aunque evitaron la posible influencia en los resultados de múltiples factores como el déficit de GH, la edad avanzada, el daño cerebral, una posible historia de abuso de alcohol o de drogas y las enfermedades neurológicas o psiquiátricas severas. Otras limitaciones fueron el diseño transversal del estudio o el uso de una técnica de segmentación cerebral automática, posiblemente no tan precisa como las segmentaciones manuales.

Para el *estudio II*, otra limitación fue la no realización de una evaluación neurológica complementaria.

4.8. Futuras líneas de investigación

En primer lugar, sería importante realizar estudios confirmatorios de nuestros resultados con mayores muestras, principalmente incluyendo pacientes activos. Por ejemplo, sería importante confirmar si en los pacientes activos existe una relación entre el riesgo cardiovascular y los volúmenes cerebrales y rendimiento neuropsicológico al incluir un mayor número de pacientes. Otros estudios en pacientes activos incorporando una evaluación neurológica (incluyendo tests de equilibrio) serían útiles para establecer si el cerebelo está alterado a nivel funcional y si el rendimiento neurológico está relacionado con el volumen cerebelar. La realización de nuevos estudios sobre la contribución de la dislipidemia a la función cerebelar sería también importante. También sería interesante estudiar de forma prospectiva la posible relación entre el volumen cerebelar y el número de caídas. Los estudios longitudinales podrían también confirmar si el córtex cerebelar aumenta tras la curación, y si es así, en cuánto tiempo sucede. Nuevos estudios prospectivos serían también necesarios para analizar la progresión de las lesiones de sustancia blanca en los pacientes activos e investigar el rol del control de la hipertensión para prevenir el empeoramiento de las lesiones en los pacientes activos y curados. La realización de nuevos estudios en pacientes mayores también puede ser importante para establecer si las lesiones de sustancia blanca están asociadas con el rendimiento cognitivo cuando los pacientes llegan a una edad anciana. Los estudios longitudinales pueden también ser de ayuda para establecer si las lesiones de sustancia blanca en pacientes de mediana edad pueden predecir el

rendimiento cognitivo a una edad más tardía. Finalmente, sería interesante analizar los posibles beneficios de la rehabilitación neuropsicológica o la terapia de grupo para mejorar el estado de ánimo, el rendimiento neuropsicológico y la calidad de vida de los pacientes.

5. CONCLUSIONES

Las principales conclusiones de esta tesis se pueden resumir de la siguiente forma:

I. El córtex cerebelar está reducido en los pacientes con SC activo en comparación con el grupo control, pero no en los pacientes con SC en remisión, sugiriendo una posible afectación inicial seguida de una recuperación posterior tras la curación hormonal.

II. El volumen del córtex cerebelar está positivamente correlacionado con la memoria visual y la calidad de vida y negativamente correlacionado con la edad al momento del diagnóstico, y el nivel de triglicéridos circulantes.

III. Los pacientes con SC activo, pero no los pacientes en remisión, tienen peor rendimiento a nivel de memoria que los controles.

IV. Ambos grupos de pacientes presentan mayores niveles de ansiedad y depresión que los controles.

V. Los pacientes en remisión, pero no los que aún presentan un SC activo, tienen un mayor grado de lesiones de sustancia blanca que el grupo control.

VI. Las lesiones de sustancia blanca están asociadas a la tensión arterial sistólica y a la duración de la hipertensión. Los pacientes con SC remisión presentan mayor duración de la hipertensión, lo que podría explicar su mayor grado de lesiones de sustancia blanca. Esto sugiere que el control de la hipertensión podría resultar beneficioso para evitar la progresión de las lesiones de sustancia blanca.

VII. Los pacientes con SC en remisión e inhibición del eje hipotálamo-hipófiso-suprarrenal que precisaban la toma de hidrocortisona sustitutiva tenían un mayor grado de lesiones de sustancia blanca que aquéllos que no tomaban del fármaco.

VIII. El riesgo cardiovascular (medido como riesgo vascular a 10 años y como edad vascular) está asociado negativamente a la función cognitiva y los volúmenes cerebrales en los pacientes con SC en remisión, remarcando la importancia del control del riesgo cardiovascular en esta población.