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## Consequences of disease and treatment in ANCA-associated vasculitis

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*Document Version*

Publisher's PDF, also known as Version of record

*Publication date:*

2017

[Link to publication in University of Groningen/UMCG research database](#)

*Citation for published version (APA):*

Tuin, J. (2017). *Consequences of disease and treatment in ANCA-associated vasculitis*. [Thesis fully internal (DIV), University of Groningen]. Rijksuniversiteit Groningen.

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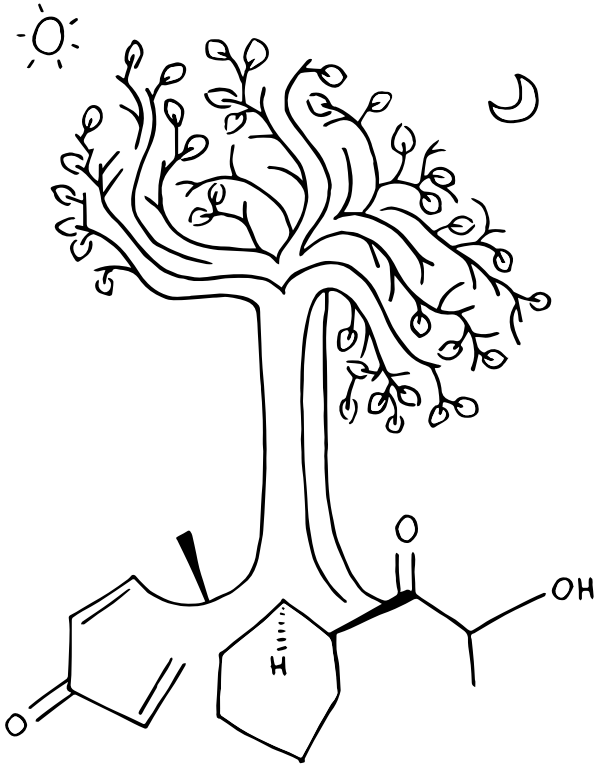
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## Recovery of the hypothalamic-pituitary-adrenal axis during glucocorticoid tapering after the induction of remission in ANCA-associated vasculitis: rationale and design of the CURVE study

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## Abstract

### Background.

Glucocorticoids are the mainstay in the treatment of ANCA-associated vasculitides. High-dose glucocorticoids are administered to control disease activity. These supra-physiological glucocorticoid doses suppress the endogenous cortisol production and disrupt the circadian rhythm of the hypothalamic-pituitary-adrenal (HPA) axis. After induction of remission, a tapering protocol is required to prevent damage secondary to glucocorticoid use and to prevent adrenal insufficiency to occur. There is a high inter-patient variation with respect to tapering and many patients experience complaints suggestive for adrenal insufficiency. No longitudinal studies have investigated the effect of tapering on the recovery of the circadian rhythm of the HPA axis and the relation with complaints compatible with chronic central adrenal insufficiency.

### Study design.

This study is a prospective, longitudinal observational pilot study.

**Participants.** Fifteen patients with a first diagnosis of granulomatosis with polyangiitis and microscopic polyangiitis or a relapse of these diseases on a standard glucocorticoid tapering protocol during induction therapy will be enrolled. Patients should not be exposed to glucocorticoids for more than 4 consecutive weeks 6 months prior to diagnosis (of a relapse). For each patient a healthy age and sex-matched control will be included.

**Outcomes.** The primary study outcome is change in peak cortisol levels at acrophase at 10 mg of prednisolone and after complete withdrawal. Secondary outcomes include: assessments of cortisol ratios or indices of cortisol production at different sampling time points as prognostic markers for impaired recovery of the HPA axis or adrenal insufficiency and the effect of the tapering on somatic and mood complaints, melatonin rhythm, dopaminergic and serotonergic and immunological variations, quality of life, fatigue and sleep quality.

**Measurements.** On six different prednisolone doses participants will collect saliva approximately every one or two hour during a 24-hour period. The 24-hour sampling will take place at prednisolone dosages of 10 mg, 7,5 mg, 5 mg, 2,5 mg and 4 weeks and 12 weeks after complete withdrawal. In addition, subjects will collect 24-hour urine during these 24-hour periods. Blood samples will be drawn at the following visits to the outpatient clinic. Somatic complaints, anxiety, depression, quality of life, fatigue and sleep will be assessed by questionnaires at the outpatient clinic. At the last visit a conventional ACTH stimulation test will be performed to test adrenal function.

**Analysis.** Collected samples will be analyzed with liquid chromatography mass-spectrometry (LCMS-MS). Results will be analyzed with appropriate statistical software.

### Conclusion.

Individual variation in HPA axis recovery might be observed early during tapering. Monitoring recovery by saliva sampling might enable us with a tool to predict recovery of the HPA axis, individualize tapering and improving both safety and efficacy of glucocorticoid treatment.

**Clinical trial register.** NTR4966 (Dutch Trial Register).

## Introduction

ANCA-associated vasculitides (AAV) are a group of chronic auto-immune diseases which cause inflammation of the small-to-medium-sized blood vessels [53]. Until the 1960's no effective treatment was available and patients were faced with dismal prognosis with survival of only several months. Survival improved with the use of glucocorticoids, but only 34% survived beyond the first year [1]. The introduction of oral cyclophosphamide in the 1960's led to a substantial improvement in patient survival [54]. Glucocorticoids are still valued in the treatment of AAV and the combination of cyclophosphamide with glucocorticoids has been the cornerstone of therapy for generalized disease since. Minor relapses of AAV can be treated with glucocorticoids alone. With these treatment options the five-year survival rate is 75% to 83% nowadays [3, 55].

The use of glucocorticoids is limited by their acute and long-term side effects. Metabolic complications, cardiovascular disease and infections frequently develop during and after therapy and are accountable for a high rate of comorbidities in AAV patients [9-11]. Glucocorticoids are therefore tapered and withdrawn after remission is attained.

After treatment with high-dose glucocorticoids a tapering protocol is required to prevent reactivation of disease and to prevent adrenal insufficiency to occur. Since there is a paucity of evidence that supports a particular glucocorticoid tapering protocol, different protocols are used world-wide [4, 7]. Notable, a substantial part of patients do not discontinue glucocorticoids within the time frame of study protocols or guidelines. A large subgroup analysis of several trials initiated by the European Vasculitis Study group (EUVAS) showed that 21% of patients continued prednisolone for 3 to 4.5 years and 28% of patients used prednisolone for a duration of 5 years. For whatever reason, many physicians and patients fail to adhere to the treatment protocol and discontinue glucocorticoids.

In our experience tapering of glucocorticoids can be challenging. A high inter-individual variation with respect to tapering is observed, some tolerate tapering well, whereas others do not. A substantial number of patients report non-specific complaints like myalgias, arthralgias and fatigue during tapering or early after withdrawal. Most of the complaints promptly resolve after increasing the glucocorticoid dose. This gives rise to the hypothesis that a central adrenal insufficiency secondary to glucocorticoids might have developed. In 1965, Graber and colleagues already observed similar complaints in combination with subnormal adrenal tests [33]. Of note, these symptoms are difficult to distinguish from disease activity and this might (falsely) be the motivation for both the physician as well as the patient to not further reduce the glucocorticoid dose.

The hypothalamic-pituitary-adrenal (HPA) axis is a complex feedback mechanism and influenced by many biological and psychological factors. Genetics, inflammation, drugs, psychological stress and also the dopaminergic system are thought to influence the HPA axis [32, 56]. The HPA axis shows a circadian rhythm with peak cortisol levels early in the morning [57]. Exogenous glucocorticoids suppress HPA axis function and the endogenous production of cortisol. Suppression of cortisol production can occur even at doses considered to be physiological and can last for an uncertain period of time [23, 24, 58-60]. Low dose glucocorticoid treatment does not exclude the presence of adrenal insufficiency [23, 24].

Whether AAV patients experience a delayed recovery of the HPA axis and whether this is related to the observed complaints remains unclear. To prospectively study recovery of the HPA axis during tapering of glucocorticoids in the treatment of AAV, we designed a longitudinal observational study.



Circadian recovery of the HPA axis will be monitored through saliva sampling at various time points and at various doses during tapering. Recovery after discontinuation will be assessed by ACTH stimulation test. Complaints and symptoms will be monitored by questionnaires, laboratory and urine assessments, including assessments of multiple endocrine axes. Individual variation in HPA axis recovery might be observed early during tapering. Monitoring recovery by saliva sampling will enable us with a tool to predict recovery of the HPA axis, individualize tapering and improving both safety and efficacy of glucocorticoid treatment.

### **Study protocol**

#### *Study setting & approval*

This study prospective, longitudinal, observational study will be performed in the University Medical Centre Groningen at the departments of Nephrology and Rheumatology. The study is approved by the Institutional Review Board (IRB) (METc2014/247) and registered in the Dutch Trial register (identifier: NTR 4966). This study will be conducted in accordance with the principles of Helsinki (Brazil, version October 2013) and in accordance with the Medical Research Involving Human Subjects Act (WMO) in the Netherlands.

#### *Participants*

Patients with a new diagnosis or a relapse of granulomatosis with polyangiitis or microscopic polyangiitis diagnosed based on the Chapel Hill Consensus nomenclature, receiving standard induction treatment with cyclophosphamide or rituximab both in combination with glucocorticoid treatment will be enrolled. For each patient a healthy age- and sex-matched control will be enrolled. In order to be eligible to participate in this study, a patient or healthy control must meet all of the listed inclusion criteria presented in table 1. A patient or healthy control meeting any of the exclusion criteria listed in table 1 will be excluded.

#### *Study design*

Individuals meeting the inclusion criteria will collect 24-hour urine and saliva samples at 11 different time points during a 24-hour period during tapering of prednisolone starting at 10 mg. Subsequent sampling will take place at 7,5 mg, 5 mg, 2,5 mg and four and twelve weeks after complete withdrawal (figure 1). The following day after saliva and urine collection, participants will visit the outpatient clinic. Here early morning fasting blood samples will be drawn, 24-hour urine and saliva collection will be handed in and blood pressure, waist-to-hip ratio (WHR) and weight and length will be measured. Participants will complete a short set of questionnaires.

Fifteen healthy age- and sex-matched controls will collect urine and saliva at 11 different time points during one 24-hour period. They will not undergo invasive procedures, e.g. venipuncture. General health information will be collected by a questionnaire.

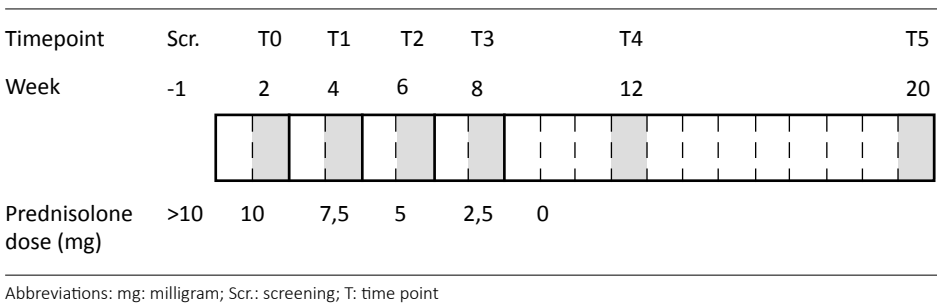
The planned recruitment period is 24 months. A summary of all study procedures is shown in table 2.

**Table 1.** Eligibility criteria CURVE study.

Inclusion criteria	
<b>Patients with GPA or MPA</b>	
Newly diagnosed or relapsing GPA or MPA receiving standard GC induction protocol	
<b>Healthy control</b>	
Age and sex-matched control (max. age difference 5 years)	
<b>Both</b>	
Providing written informed consent	
Exclusion criteria	
<b>Patients with GPA or MPA</b>	
Use of >7,5 mg of GC for >4 consecutive weeks <6 months prior to study entry	
<b>Healthy control</b>	
GC use of any dose, incl. oral, inhaled or nasal administration <6 months prior to study entry	
<b>Both</b>	
Age <18 years	
Premenopausal women	
Postmenopausal women using oral contraceptives or estrogen replacement therapy	
A history of endogenous hypocortisolism or hypercortisolism prior to study entry	
Work in shifts or documented severely disturbed sleep pattern	
Not able to perform saliva sampling	
Traveling through time zones (>2h difference) <1 month prior to study entry	
Use of melatonin <6 months prior to study entry	
Documented depression	
Significant medical condition (e.g. hepatic, respiratory, cardiovascular or gastrointestinal) which, in the opinion of the investigator, may interfere with the interpretation of results	
Stressful situation (i.e., death of a relative), which in the opinion of the investigator, may interfere with the interpretation of results	
Abbreviations: GPA: granulomatosis with polyangiitis; MPA: microscopic polyangiitis; GC: glucocorticoids	



**Figure 1.** Study design.



Abbreviations: mg: milligram; Scr.: screening; T: time point

**Table 2.** Overview of study procedures.

Visit	T-1	T0	T1	T2	T3	T4	T5
Weeks of study		2	4	6	8	12	20
GC dose (mg)	15-20	10	7,5	5	2,5	0	0
Start sampling on day x	Scr. at 15- 20 mg	Day 13 +/- 1 on 10 mg	Day 13 +/- 1 on 7,5 mg	Day 13 +/- 1 on 5 mg	Day 13 +/- 1 on 2,5 mg	Day 27 +/- 1 on 0 mg	Day 81 +/- 2 on 0 mg
Visit outpatient clinic day x after saliva sampling	X	1 (+ max 2)	1 (+ max 2)	1 (+ max 2)	1 (+ max 2)	1 (+ max 2)	1 (+ max 2)
Check inclusion and exclusion criteria	X						
Receive informed consent forms	X						
Study instructions	X						
Demographics		X					
Medical history		X					
VDI		X				X	X
BVAS		X			X	X	X
Concomitant medication		X	X	X	X	X	X
Record Adverse Events		X	X	X	X	X	X
Saliva samples		X	X	X	X	X	X
Blood samples		X	X	X	X	X	X
24-hour urine collection		X	X	X	X	X	X
Physical exam		X	X	X	X	X	X
Questionnaires*		X	X	X	X	X	X
ACTH stimulation test							X

\*Questionnaires: somatic complaints (VAS); anxiety and depression (PHQ-9; GAD-7); fatigue (CIS-20); health-related quality of life (RAND-36); sleep quality (PSQI; GSQS).

### Saliva sampling

Saliva cortisol measurements are highly correlated with serum cortisol levels[61, 62]. Salivary cortisol measurement has the advantages that it reflects the plasma free cortisol, which is considered biologically active[62]. Saliva samples will be collected using the Salivette®. Participants will collect samples during a 24-hour period at the 11 time points: after awakening (before 08.00 a.m.); 08.00 a.m.; 09.00 a.m.; 10.00 a.m.; 12 p.m.; 02.00 p.m.; 04.00 p.m.; 07.00 p.m.; 22.00 p.m.; 03.00 a.m.; after awakening; before blood withdrawal at the outpatient clinic at 08.00 a.m. The prednisolone dose is taken immediately after the 09.00 h sampling.

### Laboratory measurements

Samples are stored at -80°C and analyses will be performed after completion of the study, except for plasma adrenocorticotrophic hormone (ATCH), which will be analyzed immediately with luminescence-immunoassay. All stored samples will be analyzed in one run to minimize inter-assay variation. Cortisol and melatonin in saliva and urine will be analyzed with liquid chromatography

mass-spectrometry (LCMS-MS). This method can differentiate between endogenous cortisol production and exogenous glucocorticoid administration. A steroid profile, catecholamines such as dopamine and tryptophan and its metabolites including kynurenine will be measured in 24-hour urine. Blood measurements include serum cortisol, ACTH, corticosteroid-binding globulin (CBG), Hemoglobin A1C (HbA1C), fasting glucose, C-reactive protein (CRP) and a full blood count. At three time points T cell and B cell subsets distribution will be measured.

**Questionnaires**

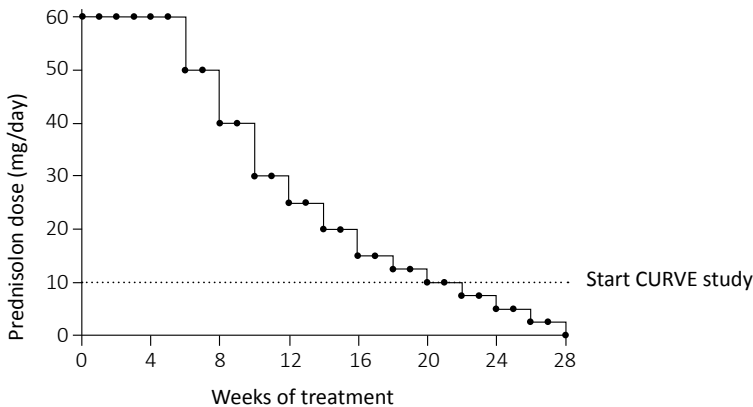
Somatic complaints and symptoms will be assessed by a questionnaire derived from the PHQ-15. Depression and anxiety will be assessed by validated questionnaires, the PHQ-9 and GAD-7 respectively. Health-related quality of life will be assessed by the RAND-36 version 2. Fatigue will be assessed by the Checklist Individual Strength. Quality of sleep will be assessed by two questionnaires the extensively validated Pittsburgh Sleep Quality Index and the Groningen Sleep Quality Scale.

**Physical examination**

Patients will undergo a short physical examination at the outpatient clinic. Physical examination includes measurement of length (only at T0), weight, WHR and blood pressure. Blood pressure will be measured twice in supine position.

**ACTH stimulation test**

The conventional ACTH stimulation test will be conducted 12 weeks after complete glucocorticoid withdrawal. The conventional ACTH stimulation test is the standard diagnostic test to diagnose adrenal insufficiency in the UMCG. The adrenal glands will be stimulated with 250 µg synthetic ACTH (Synacthen®) to study the capacity of the glands to produce a maximum level of cortisol. After infusion, cortisol levels will be measured after 30 and 60 minutes. The standard protocol used at the outpatient clinic in the UMCG will be used, see appendix 1 for the standard protocol. This test will take one and a half to two hours.



**Figure 2.** Overview of the CURVE glucocorticoid protocol.





### Assessments for healthy controls

Healthy controls will collect saliva and urine during one 24-hour period. Demographic data and general health will be assessed by a short questionnaire.

### *Standard care*

Patients with active disease start with prednisolone 1 mg/kg/day once daily in the morning with a maximum of 60 mg until six weeks of treatment. When complete remission is attained and sustained for 2 weeks during the first 6 weeks of therapy, tapering can start earlier. Thereafter, prednisolone will be tapered with 10 mg every two weeks until a daily dose of 30 mg/d, subsequently with 5 mg every two weeks until 15 mg daily and with 2.5 mg every two weeks until complete withdrawal (Figure 2). In case of severe disease activity, methylprednisolone 1000mg can be administered on 3 consecutive days at start of therapy followed by the standard tapering protocol.

Induction treatment further consists of oral cyclophosphamide 2 mg/kg/d (age >65 or diminished bone marrow reserve start with 1.5 mg/kg/d) or rituximab 4 doses of 375 mg/m<sup>2</sup> BSA at weekly intervals. All patients will be switched to maintenance therapy with azathioprine (1.5 mg/kg/d) after three months of stable remission and will continue on azathioprine until 12 months. After 12 months the dose will be tapered with 25 mg every 3 months.

### *Outcome*

The primary outcome is change in acrophase saliva cortisol concentration during and after glucocorticoid withdrawal. Secondary outcomes include assessments of cortisol ratios or indices of cortisol production at different sampling time points as prognostic markers for impaired recovery of the HPA axis or adrenal insufficiency. Adrenal insufficiency is defined as a morning cortisol level <175 nmol/L after complete withdrawal or a cortisol level of < 375 nmol/L after the conventional ACTH stimulation test with 250 µg ACTH, based on out in-house references validated using the LCMS-MS. Secondary end points include the relation between somatic complaints, mood complaints and sleep disturbance and the recovery of the HPA axis and melatonin rhythm during and after glucocorticoid withdrawal. Further investigations include analyses of dopaminergic and serotonergic variations and immunological variations during glucocorticoid withdrawal. The CURVE study offers the possibility to perform several post-hoc studies assessing recovery of the HPA axis functioning and biological changes during glucocorticoid withdrawal.

### *Statistical analyses and sample size*

The data will be presented quantitative. Participants will not be excluded and data will not be imputed in case of missing data. The change in cortisol levels at the acrophase, nadir and the change in amplitude will be described with descriptive statistics, area under the curve and analysed in relation to change in other parameters especially somatic and mood complaints. This will be analysed with appropriate statistical analyses for longitudinal data with repeated measures, which takes into account the correlation of different measurements within one subject. Repeated measurements for laboratory measurements and scores of the questionnaires will be analysed with appropriate statistical analyses for longitudinal data with repeated measures.

This study is a hypothesis generating pilot study and will be used as a fundament for future studies

and sample size calculations. Until now, no longitudinal data regarding the recovery of the circadian rhythm of the hypothalamic-pituitary-adrenal axis during glucocorticoid tapering in patients with AAV, or other diseases, are known in literature. During a two year period, we will include 15 patients and 15 healthy controls and estimate that 20% will withdraw from study participation during the study. The small sample size is considered justifiable, since this is a pilot study and sequential measures will be collected which increases statistical power.

### ***Safety***

The risk of participating in this study is considered low. Urine and saliva collection is safe and non-invasive. Subjects will undergo additional blood sampling at two time points, the others are in concurrence with standard patient care.

Adrenal function will be assessed once by a conventional ACTH stimulation test (Synacthen) using 250 µm ACTH. This ACTH stimulation test is the standard diagnostic test to diagnose adrenal insufficiency. It is commonly used in general clinical practice and in research. The test will be performed under strict conditions. Participants will be under supervision during the complete test by specialized trained staff. Most common side-effects are of allergic nature: skin reaction at infusion site, rash, dizziness, nausea, vomiting or anaphylactic reaction. Severe side-effects develop rarely. Subjects will be instructed to what to do when complaints or symptoms develop. The test will give insight in the capacity of the adrenal glands to produce a maximum level of cortisol. An insufficient response to the ACTH stimulation test might warrant extra patient care to prevent consequences of a secondary adrenal insufficiency, especially in situations of stress.

### ***Study status***

Patient enrollment started March 2015 and is still recruiting. At time of this writing, seven participants completed the study protocol and 1 age and sex-matched healthy control completed the study protocol. We expect this study to end by the end of 2017. Results are expected in the second half of 2018. Results will be presented at international congresses and results will be submitted for publication in peer-reviewed scientific journals.

### **ACKNOWLEDGEMENTS**

We thank prof. I.P. Kema for involvement in the cortisol measurements using the LCMS/MS and Ms E.P. Herder for her help conducting the study and supporting the participants.

### **DISCLOSURES**

The authors report no competing interest or financial interest which could create a potential conflict of interest with regard to the work.

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