Study Outline / Protocol for the Research Project ***HairCo-2*** (*Hair Cortisol Measurement in Mitotane-treated ACC patients*)

1. Investigators

1.1. Recipient Scientist, coordinator of the proposed project

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1.2. Status of the Recipient Scientist

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1.3. Associated Investigators

Elisabeth FC van Rossum (Erasmus MC, Rotterdam, Netherlands), as well as members of the German ACC registry

2. Project

2.1. Name of Project

HairCo-2 (Hair Cortisol Measurement in Mitotane-treated ACC patients)

2.2 Scientific context

Adrenocortical cancer (ACC) is a rare malignancy that occurs in approximately 0.5-2 per million persons per year (1, 2). The only curative treatment is radical surgery (3). However, even after initial curative surgery, 40% of the patients have recurrent disease or distant metastases within 2 years (4). Adrenolytic therapy with mitotane (o'p'-DDD) is the primary treatment for patients with advanced ACC (3), either as monotherapy or in combination with cytotoxic chemotherapy. Mitotane is the only approved drug for ACC and has been shown to extend disease-free survival (5), although this has been questioned (6-9). Mitotane has also been used in an adjuvant setting and an international panel of international experts recently recommended the administration of adjuvant mitotane in all patients at high risk of relapse (10). Mitotane is a steroidogenesis inhibitor at different enzymatic steps, which concentrates in the adrenal glands and is thought to provoke mitochondrial degeneration and destruction of the adrenal cortex(11). However, the precise mechanisms of action remain still unknown. It is suggested that serum concentrations of mitotane should exceed 14 mg/L to increase the likelihood for an anti-tumoral response, and kept below 20mg/l to avoid side effects (12, 13). However, often this concentration is not achieved, due to the low bioavailability caused by its low absorption rate and its lipophilic nature (14). The aim of reaching target serum concentrations is also hampered by many side effects of mitotane treatment. Up to 80% of the patients have gastrointestinal side effects including nausea, vomiting, abdominal discomfort and diarrhoea. About 40% develop neurological symptoms such as dizziness, dysesthesia, sedation and ataxia (15, 16).

Mitotane affects all adrenocortical zones, therefore it is necessary to replace glucocorticoids and sometimes mineralocorticoids during mitotane treatment. However, mitotane is a strong inducer of hepatic CYP3A4 activity (17) and increases cortisol binding globulin (CBG) (15), resulting in an increased cortisol metabolism and reduced free, active cortisol (18-22). Therefore normal hydrocortisone replacement therapy (approximately 20 mg/day) as used in adrenal insufficiency, is thought to be not sufficient in ACC patients on mitotane and higher dosages (40-80mg/day) seem necessary. The monitoring of hydrocortisone substitution under mitotane treatment is difficult. One study suggested that free cortisol measurement may offer

additional information in the follow-up of ACC patients on mitotane, especially in the subgroup of patients with Cushing´s syndrome (15). This marker indeed may overcome some of the complications in the interpretation of measured cortisol in serum; however, it only reflects acute free cortisol levels of minutes to hours. Therefore the monitoring of hydrocortisone dose has relied on clinical symptoms until now. However, the clinical symptoms of cortisol deficiency show extensive overlap with side effects of mitotane. Therefore, the differentiation between symptoms of adrenal insufficiency and mitotane induced side effects is difficult. Measurement of cortisol in serum or urine is not helpful, since these methods measure total cortisol (excretion) and do not reflect unbound, free cortisol levels. In addition, these measurements reflect only a short time frame, and serum levels are also influenced by the time of intake of hydrocortisone and may be affected by acute stress (e.g.venapuncture) in case of some residual adrenal function.

In the past few years, a novel method to measure long-term cortisol has been developed by extraction of cortisol from scalp hair (23). This measurement has been well validated by several groups (23-28). Since scalp hair grows with an average rate of 1 cm per month, a hair segment of 1 cm reflects mean cortisol levels of one month. Cortisol measurements in scalp hair have been shown to represent long-term cortisol levels in health and disease (29, 30). Retrospective timelines of hair cortisol levels corresponded with clinical course in patients with Cushing's syndrome (26, 31, 32), cyclic Cushing's Syndrome (31) and Addison's Disease (26, 33). In healthy individuals, hair cortisol levels were positively correlated with BMI and waist circumference, suggesting that hair cortisol measurements reflect cortisol exposure (26, 34-36). Furthermore, hair cortisol levels were elevated in individuals with chronic stress such as unemployment and chronic pain (28, 37). All these studies together show that the measurement of cortisol in hair is a reliable marker of long-term cortisol levels (30). As hydrocortisone is equivalent to endogenous cortisol, this has also been shown for therapy monitoring of patients using hydrocortisone substitution in physiological doses (33, 38).

2.2. Aim

The aim of this study is to investigate whether hair cortisol measurements could be useful in the evaluation of hydrocortisone substitution in mitotane treated ACC patients and whether high dose hydrocortisone replacement as commonly used in clinical practice results in normal long-term cortisol levels in these patients. By using timepoint measurements as saliva samples at midnight and after hydrocortisone ingestion also short phases with high and low cortisol exposure can be related to hair cortisol levels and provide insight in the metabolism of hydrocortisone.

2.3. Methods

Hair samples will be collected and analysed for cortisol levels and compared to saliva and urine samples (collected 1 month before the collection of the hair sample). (Explanation: since hair grows on average 1 cm per month, a hair sample of 2 cm will reflect the mean systemic cortisol levels of the past 2 months – so, this will correspond to the ± 1 month collection of the urine and saliva sample). Hormone measurements will be correlated to mitotane levels, hydrocortisone dose, BMI, etc.

2.4. Requested clinical annotations (type, justification)

Inclusion criteria:

- proven adrenocortical carcinoma (based on histological criteria)
- ENSAT stage I-IV
- Current mitotane treatment for at least 3 months
- Current stable hydrocortisone replacement therapy

Minimum clinical annotations:

- Age (at diagnosis)
- sex
- tumor size
- ENSAT stage at diagnosis and current status
- Type of ACC hormone excess
- BMI (kg/m2) via height and weight
- Waist and hip circumferences
- Hydrocortisone daily dosage (mg/day)
- Mitotane daily dose (g/day) and mitotane blood level (mg/l) (done via Lysosafe service)
- Information, if (and what kind of) additional antitumor therapy was given

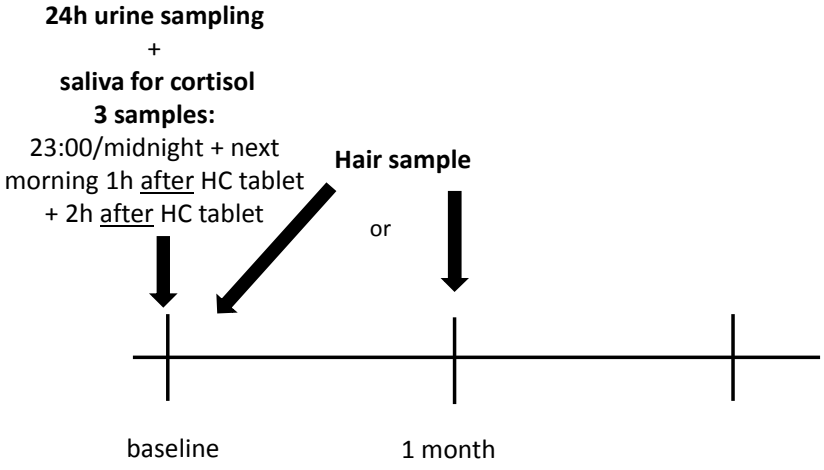
Total number of patients included: 100 (Europe via ENS@T)
(Minimum expected patient cohort per ENS@T center: 10)

Minimum clinical annotations/data acquisition (done via ENS@T registry): Data is entered into ENS@T data base (Age at diagnosis, sex, tumor size, ENSAT stage at diagnosis and current status, type of ACC hormone excess, information on additional antitumor therapy)

2.5. **Requested samples (type, justification of sample size); sample storage according to ENS@T guidelines:**

- 24h urine sample (3 vials a 10ml)
- 3 saliva samples (midnight + 1h and 2h after hydrocortisone intake in the morning)
- hair sample (optimal time point: ***1 month after saliva and urine samples***) (**but also possible at same time point as urine an saliva**)
- one short questionnaire for patients concerning factors which may affect hair cortisol measurements, e.g. frequency of hair washing, use of hair products etc.

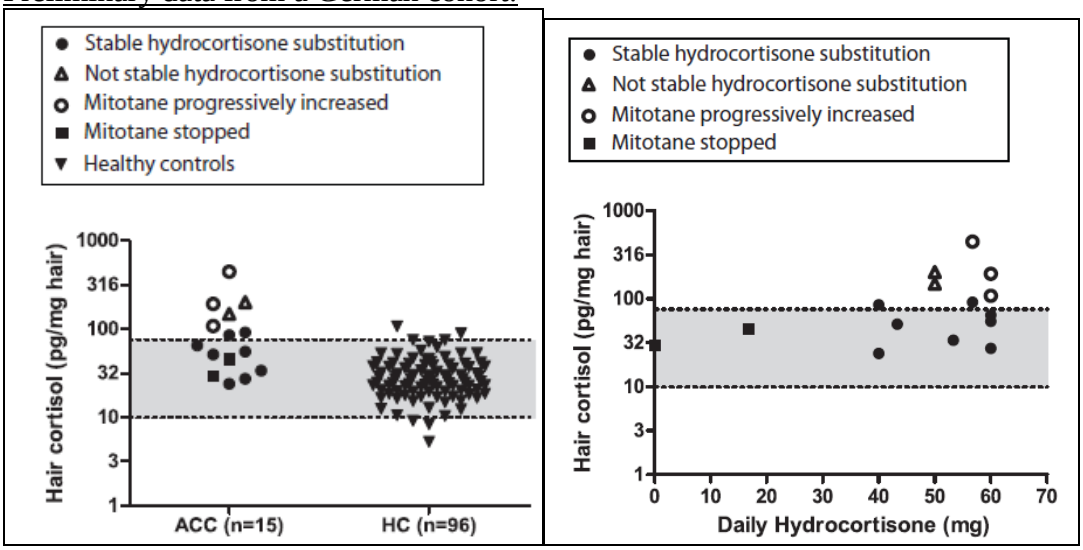
Usually, patients on mitotane have a blood test for liver enzymes and mitotane level every month (4 weeks) – use these visits for:



2.6. Expected results

Based on preliminary data from a German cohort we expect that hair cortisol levels indicate retrospectively if the hydrocortisone dose of the patient was sufficient or too low.

Preliminary data from a German cohort:



Laura Manenschijn, Marcus Quinkler, Elisabeth F.C. van Rossum Horm Metab Res 2014

2.7. Projected time frame

Deadline for affirmation of participation in the study (e-mail to marcusquinkler@t-online.de): **30.12.2014**
Deadline for data acquisition (i.e. input in ENS@T registry) and biosamples:
31.09.2015
Deadline for data analysis and measurements:
31.12.2015
Expected manuscript submission:
31.03.2016
Targeted Journals (depending on outcome): Journal of Clinical Oncology, Lancet Oncology, JCEM

3. Publication policy

1. Recipient scientist formally agrees with the provider(s) - at the time of the request or soon after the provider(s) have accepted the collaboration - the(ir) presence (if any) as co-authors in the publications originating from the collaboration.

2. Number of authors per centre and order of authorships are specified as follows:
   1-3 co-authors per center depending on patient numbers provided as defined in the minimum clinical annotations and projected time frame.
   Depending on the contribution of each centre or if number of authors has to be limited on the basis of a specific journal style co-authors will be represented as “on behalf of ENS@T” and placed in an appropriate list depending on the Journal format (acknowledgment or collaborators list). In this case the recipient scientist will contact all provider(s) and obtain agreement. However, due to the significant work already done the key authorship positions are already predefined: Quinkler, ….van Rossum.

3. The authors agree to acknowledge ENS@T contribution: “This project has been supported by the European Network for the Study of Adrenal Tumors (ENS@T).” (For ENS@T-CANCER related projects: “The research leading to these results has received funding from the Seventh Framework Programme (FP7/2007-2013) under grant agreement n° 259735.”)

4. Conditions of data usage

1. The Recipient scientist will use the data for research purposes only.

2. The data will be used by the Recipient scientist solely in connection with the Research Project as outlined above.

3. The Recipient scientist shall use the data in compliance with all applicable laws and government regulations of the Recipient's country.

4. The Recipient Scientist shall not release the data to any person other than the personnel under the Recipient Scientist’s direct supervision.

5. When the Research Project is completed, a detailed description of the use of the data will be made available to the appropriate ENS@T Working Group

6. In the event that a journal publication or scientific article is published based on use of the data, the Recipient Scientist will send a copy of such publication, or the publication cite, promptly after it becomes available to the Recipient, to the appropriate ENS@T Working Group

5. Conditions of biomaterial usage

Erasmus MC
Universitair Medisch Centrum Rotterdam

Hair sample collection - Instructions

Materials

- Small surgical scissors
- Tape
- Blank paper
- Envelope

Method of sample collection:

- Isolate a bundle of approximately 100-150 strands of hair from the posterior vertex (Figure 1)
- Cut the bundle as close to the scalp as possible
- Tape the bundle of hair strands to a blank paper and try to keep the hairs on the scalp end straight.
- Mark the scalp end on the paper and write down the date of sample collection (Figure 2)
- Put the paper with the hair sample attached in the envelope and store it at room temperature

Figure 1. Cut the bundle of hair strands as close to the scalp as possible

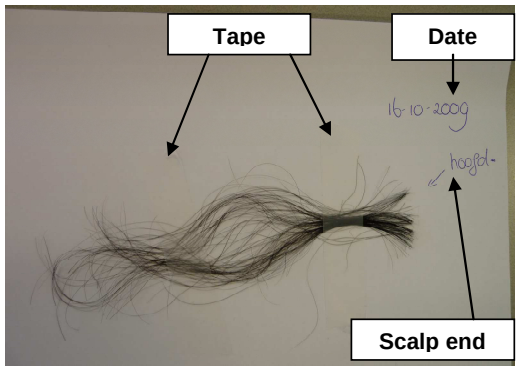


Figure 2. Tape the hair sample to the blanc paper and clearly mark the scalp end.

Important:

1. **The hair sample needs to be collected from the <u>posterior vertex</u> of the scalp**
2. **The hair sample needs to be cut off as close to the scalp as possible**
3. **Clearly mark the scalp end of the hair sample after taping it to the paper**

The hair samples can be stored at room temperature in an envelope.
The saliva and urine samples should be handled and stored according to ENSAT guidelines.

6. References

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