

Quality of Life and Care in Adrenocortical Carcinoma

Rebecca Valery Steenaard

Propositions belonging to the thesis

Quality of life and care in adrenocortical carcinoma

1. The ACC-QoL questionnaire is a valid instrument to measure quality of life in Dutch patients with adrenocortical carcinoma (this thesis).

2. Quality of life is impacted by adrenocortical carcinoma, especially in patients treated with mitotane and chemotherapy (this thesis).

3. Patients with adrenocortical carcinoma should be treated in an adrenal expert center (this thesis).

4. Adrenalectomy and metastasectomy should be considered in patients with metastatic adrenocortical carcinoma (this thesis).

5. The ACC-QoL can be used to monitor mitotane side-effects, which might help improve quality of life, prevent early discontinuation and thereby improve survival (this thesis).

6. Exercise may have beneficial effects on health-related quality of life and certain cancer-specific concerns: body image/self-esteem, emotional well-being, sexuality, sleep disturbance, social functioning, anxiety, fatigue, and pain (Mishra, et al. Cochrane Database Syst Rev. 2012).

7. Cancer patients that are satisfied with their care experience a better quality of life (Alaloul, et al. Oncol Nurs Forum. 2019).

8. Patient engagement can improve treatment outcomes, patient satisfaction and health (Marzban, et al. J patient experience. 2022).

9. To affect the quality of the day, that is the highest form of arts (Henry David Thoreau).

10. Dokters weten het meest van de ziekte, patiënten van het ziek zijn (prof. dr. A.R.M.M. Hermus).

11. Waar ik me goed voel, ben ik thuis (Desiderius Erasmus).

Rebecca Valery Steenaard Maastricht, 10 september 2026

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Kwaliteit van leven en zorg in bijnierschorscarcinoom

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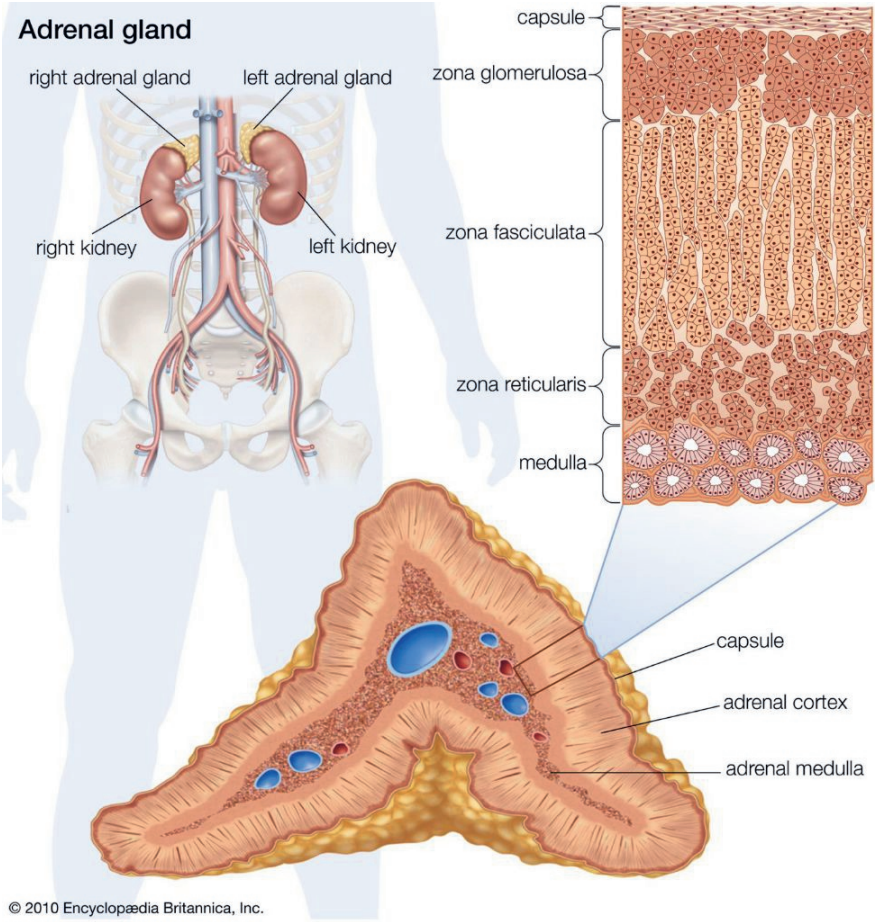


Chapter 1

General Introduction

The adrenal glands are small hormone producing organs situated above both kidneys as shown in figure 1. The gland consists of layers, each producing a hormone. The three layers of the cortex produce aldosterone involved in blood pressure regulation (zona glomerulosa), cortisol involved in metabolic processes (zona fasciculata) and adrenal androgens (zona reticularis), which can be converted into estrogens in fat tissue. The adrenal marrow (medulla) produces the stress hormones adrenaline and noradrenaline. Both benign and malignant tumors can originate from any of the adrenal layers. A malignant tumor originated from adrenal cortex cells is called adrenocortical carcinoma (ACC), which is the focus of this thesis.

Figure 1. Adrenal anatomy and microscopy.



https://www.britannica.com/science/adrenal-gland; Robert D. Utiger; Britannica Editors; Jan. 23, 2026

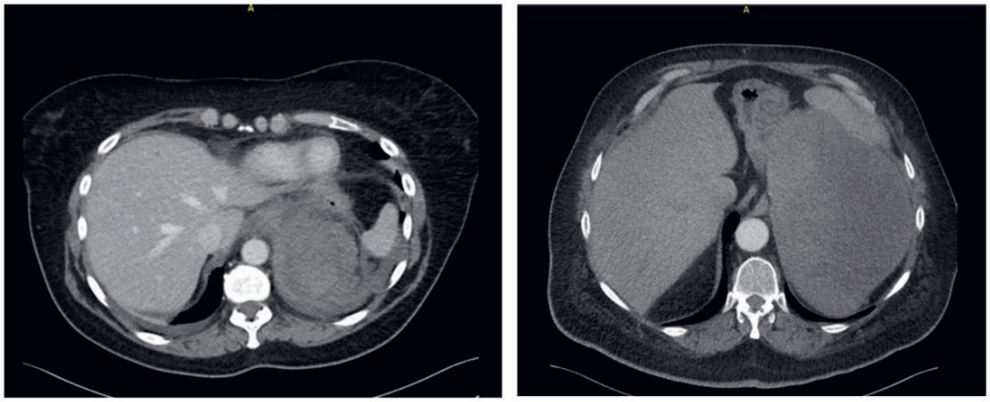
The pathophysiology of ACC is not completely understood. In contrast to other cancers, environmental factors such as smoking, alcohol use or excess sunlight do not appear to play a role in the development of ACC. The majority of ACC is sporadic, however, there are rare genetic tumor disposition syndromes that are associated with an increased risk of ACC, especially in childhood. These include Li-Fraumeni syndrome (TP53), Beckwith-Wiedemann syndrome (11p15 imprinting, IGF2), Lynch syndrome (mismatch repair genes), multiple endocrine neoplasia type 1 (MENIN), familial adenomatous polyposis (APC) and neurofibromatosis type 1 (NF1) (1). From these syndromes and from translational studies, dysfunctional molecular pathways involved in the development of ACC have been identified. These include the TP53 pathway which halts the cell cycle of damaged cells and the insulin-like growth factor (IGF2) pathway involved in adrenal development. This brings us a little closer to understanding how ACC occurs (2).

ACC can originate from any of the three cortex layers and can therefore produce adrenal cortex hormones aldosterone, cortisol, androgens and estrogens. Epidemiological studies have found that 60% of patients with ACC suffer from hormone overproduction (3). Symptoms from cortisol overproduction are most frequent and result in Cushing's syndrome with weight gain, typical Cushingoid facial and body changes, poor diabetes control, muscle wasting, and easy bruising. Adrenal androgen overproduction causes the appearance of male features in females, while estrogen overproduction causes female features in males. Aldosterone production causes hypertension and hypokalemia. Patients without hormone overproduction can present with mechanical complaints such as abdominal pain and difficulties with eating. Some patients have few symptoms and are found incidentally with abdominal imaging performed for another indication. All these symptoms have an important impact on patients' quality of life (QoL) (4). In many patients it takes a long time before a correct diagnosis is made, because the disease is rare and the symptoms are often vague and not always directly recognizable. This period of uncertainty can also have a large impact on patients and their families.

ACC is diagnosed through imaging and hormonal testing. A CT image of a large (>4cm), irregular tumor with unenhanced Hounsfield units above 20 is highly suspected of an ACC. The CT images of two patients with ACC are shown in figure 2. ACC can also be identified through 18F-FDG-PET-CT where ACC has a high tracer

uptake (5). Combined with a suspected urine steroid profile, the diagnostic accuracy increases and complete resection of the tumor (adrenalectomy) is recommended (6). The pathologist can then confirm the diagnosis using the Weiss score. This score consists of nine pathological elements suggestive of ACC (7). A score above 4 is defined as ACC, a score of 2 or 3 as borderline malignant. Furthermore, the rate of cell division (mitosis) can be measured using the Ki-67 index. An index above 10% indicates a more aggressive tumor grade.

Figure 2. CT image of two patients with ACC.



Images are published with permission from the patients.

The recommended treatment for ACC depends on the stage in which the disease presents (8,9). The European network for the study of adrenal tumors (ENSAT) have created a definition of the ACC stages (10). Tumors limited to the adrenal are stage I or II depending on the size. Stage III tumors have involvement of the surrounding lymph nodes or organs. Distant metastases are stage IV.

Adrenalectomy with complete resection is the only curative treatment option for ACC. Even with a complete resection, a large proportion of patients have a high risk of recurrence. Adjuvant treatment for two years with the adrenolytic drug mitotane might lower this risk and therefore improve survival (11). The ADIUVO trial was the first randomized trial in adjuvant setting in ACC. The trial included patients with a completely resected, low grade (Ki-67 index <10%), localized tumor (stage I or II) and randomized between two years of adjuvant mitotane therapy versus observation. Even though the study was discontinued early for lack of recruitment, they were able to conclude that the five year recurrence free and overall survival

was already high in this group of patients and did not improve from adjuvant mitotane therapy (12). The guidelines therefore recommend to treat these patients with surgery only (8,9). Patients that do not meet these criteria, in other words an incomplete resection, a high grade (Ki-67 >10%) or locally advanced (stage III) tumor however, have a high risk of recurrence. The guidelines therefore do recommend to treat these patients adjuvantly with mitotane for two years to lower the risk of recurrence (8,9).

Mitotane is derived from an insecticide DDT and available in tablets of 500mg (13). The dosage is guided by plasma level monitoring. A plasma level above 14mg/L has been associated with better treatment response (14). To reach these levels patients are required to use up to 18 mitotane tablets a day. A plasma level above 20mg/L has been associated with increased toxicity. Toxicity includes gastro-intestinal, neurological, hepatological and hormonal side-effects (15). Some of these side-effects already occur at subtherapeutic plasma levels, which affects the QoL of patients during and after mitotane use.

Mitotane is also recommended to treat patients with metastatic disease, however only 30% of these patients have a positive treatment response. Mitotane is therefore often combined with chemotherapy. The randomized FIRMACT trial concluded that a regimen of etoposide, doxorubicin, cisplatin (EDP) with or without mitotane had a higher response rate and progression-free survival than streptozocine with or without mitotane. The overall survival in this trial with crossover concept, was the same with both regimens (16). EDP-mitotane is therefore recommended as the first-line chemotherapy regimen. Second-line chemotherapy regimen, radiotherapy, local therapies and experimental options such as immunotherapy and targeted therapy can be considered for individual patients. Thus far, the new therapy options have not had successful results in the treatment of ACC. There are known cases with long lasting response or stable disease with the currently existing treatment options. Nevertheless, the overall survival of stage IV ACC is expressed in months, not years (1). During these months, the QoL of patients can be greatly affected by symptoms of the disease, treatment side-effects and emotional distress associated with cancer and end-of-life issues.

The ultimate aim of all ACC research is to improve both the quantity and the quality of life for patients. In other words, increase the survival by improving the quality of

treatment and improving the quality of those months/years gained by relieving symptoms, decreasing side-effects and offering adequate guidance. To determine what we need to do to get there, we first have to know where we are now. The aim of this thesis is therefore to first determine the currently perceived QoL of patients with ACC and the current treatment landscape. The next step is to identify issues with room for improvement and finally to suggest methods to tackle some of these issues.

Part I of the thesis focuses on the QoL of patients with ACC. QoL can be influenced by many factors in life. The part of QoL that is determined by disease is defined as health-related quality of life (HRQoL). We aimed to determine the currently perceived HRQoL in patients with ACC and specific issues that can cause a lower perceived HRQoL. In research, HRQoL is usually measured by questionnaires. To adequately measure HRQoL, a questionnaire is needed that is validated for the specific patient group. Such a questionnaire did not yet exist for ACC. A new questionnaire was therefore developed in this thesis, following the methodology determined by the European organization for research and treatment of cancer (EORTC) (17). Phase I of this method is to determine what is already known on HRQoL in patients with ACC. This is done by a systematic review in **Chapter 2** of this thesis. Phase II consists of focus group interviews with patients to determine which issues matter to them, the results of which are described in **Chapter 3**. The relevant HRQoL issues found through the literature, the focus group interviews and interviews with experts in the field are transformed into a questionnaire. This questionnaire is then validated in the target population in Phase III, which is described in **Chapter 4**. This chapter also shows the currently perceived HRQoL in patients with ACC in the Netherlands and identifies specific issues that can be improved. **Chapter 5** is a specific case of a young girl with ACC, which illustrates how side-effects of treatment can affect HRQoL. This chapter will be the only one discussing ACC in children. The rest of the thesis will be focused on adult patients with ACC. However, a lot of the issues that adult patients face, are also relevant for children. The similarities and differences between adult and childhood cases will therefore also be discussed in Chapter 5.

Part II of the thesis focuses on quality of care for patients with ACC. The recommendations in the current ACC treatment guidelines are mostly based on expert experience (8,9). **Chapter 6** includes data on the current and historical

situation of ACC treatment in the Netherlands, which can support future guideline recommendations. The evolution of ACC incidence, survival, treatment and role of expert centers are described between 1993 and 2020 and the current situation is discussed in more detail. Potential factors for improvement of quality of care and life are identified from this data. A potential issue for improvement is correct identification of patients who can benefit from adjuvant mitotane therapy and patients who can be spared its toxicity. In **Chapter 7**, the feasibility of determining IGF2 methylation in pathology material is tested. IGF2 methylation differs between ACC cells and normal adrenal cells. This diagnostic tool can therefore help to better distinct patients with ACC from patients with a benign adrenal tumor. And finally, **Chapter 8** discusses personalized mitotane dosing with individual dosing strategies and, pharmacokinetic and pharmacogenetic models as potential for improvement of mitotane therapy.

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Part I

Quality of life in adrenocortical carcinoma

Chapter 2

Health-related quality of life in adrenocortical carcinoma

Steenaard RV, Michon LA, Haak HR.

Cancers (Basel). 2019 Oct 8;11(10):1500.

Abstract

Insight into the health-related quality of life (HRQoL) impact of adrenocortical carcinoma (ACC) is important. The disease and its treatment options potentially have an impact on HRQoL. For patients with limited survival, HRQoL research is of the utmost importance. We will therefore provide an overview of HRQoL studies in patients with ACC. We found six studies that measured HRQoL in 323 patients with ACC (3 cross-sectional, 1 cohort, 2 trials), all indicating a reduced HRQoL compared to the general population. The FIRMACT trial found that HRQoL of patients with ACC was reduced compared to the general population, and that chemotherapy-mitotane further reduced HRQoL even though survival improved. Clinical aspects of the disease, including cortisol and aldosterone production, and adrenal insufficiency have shown great impact on HRQoL in benign disease, even after recovery of hormonal status. However, the impact of malignant adrenal disease and treatment options on HRQoL including adrenalectomy, radiotherapy, mitotane therapy, and chemotherapy have not been sufficiently studied in patients with ACC. Although the number of HRQoL studies in patients with ACC is limited, existing literature does indicate that ACC has a large impact on patients' HRQoL, with disease specific aspects. Further HRQoL research in patients with ACC is essential to improve patient-centered care, preferably by using an ACC-specific HRQoL questionnaire.

Introduction

Adrenocortical carcinoma (ACC) is a rare cancer with an incidence between 0.7 – 2.0 per million persons per year (1). The overall median survival ranges from 5 to 159 months, and is strongly dependent on the stage of disease. ACC can present as a functional or non-functional tumor, with the latter often being an additional finding on imaging done for other purposes (incidentaloma finding). Treatment for ACC depends on the stage of disease (2,3). The optimal treatment for solitary tumors is surgical resection. In cases with high risk of recurrence (stage III, R1 or Rx resection, Ki-67>10%), surgery is followed by at least 2 years of adjuvant mitotane monotherapy. For metastatic disease or irresectable tumors, the treatment consists of mitotane monotherapy, radiotherapy or chemotherapy with or without the addition of mitotane.

Symptoms and treatment options of ACC can have a great impact on patients' health-related quality of life (HRQoL). For example, both mitotane and chemotherapy are associated with high toxicity. Interviews with patients with ACC illustrate the importance of healthcare professionals having knowledge of the issues that patients face (4). HRQoL questionnaires can help improve communication about these issues (5). More knowledge on HRQoL issues using a HRQoL questionnaire can further assist in the process of shared-decision making and provide direction for further research (6,7).

HRQoL focuses on the impact of health on a person's ability to live a fulfilling life. HRQoL questionnaires assess physical, mental, and social health influenced by disease and its treatments, and can be general or disease-specific (8). General HRQoL questionnaires are useful for comparing HRQoL issues to other diseases, but they might result in an underreporting of HRQoL issues. For example, ACC symptoms include general cancer symptoms such as fatigue and weight-loss, but also more specific symptoms such as an increased abdominal size or virilization. A disease specific HRQoL questionnaire could help to better differentiate between issues experienced by different patient groups, at different stages of ACC and during different treatment options. The PROQOLID database is a searchable clinical outcomes measurement database. It contains all existing validated questionnaires and clinical measurement tools for use in research and clinical practice. At the moment, this database does not include a disease specific model that is developed specifically for use in patients with ACC.

As recommended by the recent European Society for Endocrinology (ESE) guideline for ACC management, more knowledge on HRQoL in patients with ACC is necessary to improve patient-centered care (2). This knowledge can firstly be used to aid the shared decision making process (9). Current treatment decisions are mostly based on recurrence risk, expected treatment effect and potential toxicity. However, the potential effect of a treatment on HRQoL is just as important for patients when deciding on a treatment. Secondly, healthcare professionals can use the information to improve patient doctor communication and improve patient satisfaction with care (5). Knowledge on HRQoL can also be used to improve treatment guidance (6,7). When we know which HRQoL issues occur during a specific stage of the treatment, steps can be taken to prevent, detect, and treat these issues. Finally, HRQoL knowledge can be helpful for implementation of quality improvement in clinical care (10).

In this systematic review, we will therefore provide an overview of the current knowledge on HRQoL in patients with ACC. A systematic search for papers in Medline, Cochrane library, Google Scholar, and Embase published in English until July 2019 resulted in a total of six studies that measured HRQoL in 323 patients with ACC, summarized in Table 1. We will present all aspects and treatments of ACC, its potential influence on HRQoL, and the studies measuring HRQoL in patients with ACC.

Materials and Methods

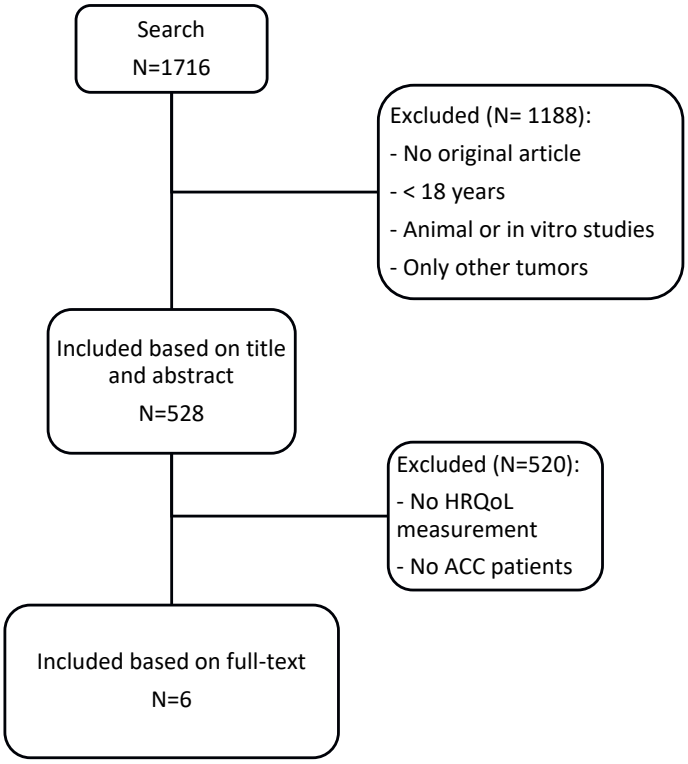
In this systematic review, we provided an overview of HRQoL studies that include patients with ACC. We searched Medline, Cochrane library, Google Scholar, and Embase for articles published in English until July 2019. We used the following keywords: adrenocortical carcinoma or adrenocortical neoplasm, Cushing syndrome or hypercortisolism, hyperaldosteronism, androgens/estrogens or virilizing/feminizing, adrenalectomy, radiotherapy, chemotherapy, mitotane, and adrenal insufficiency. We combined these keywords one or two at the time with the search terms quality of life, or health status, or well-being. For example, we used the search strategy: (adrenocortical carcinoma or adrenocortical neoplasm) and adrenalectomy and (quality of life or health status or well-being).

We included original articles that contained one or more HRQoL measurements and included patients with ACC. Studies were excluded if the participants were below

18 years of age or if the study only included patients with other adrenal tumors such as benign tumors, metastasis, pheochromocytoma, neuroendocrine tumors, and neuroendocrine carcinomas. Studies that researched animals or cell lines were also excluded. The search, exclusion and data extraction procedures were performed by two authors independently, and discrepancies were resolved through discussion.

A total of 1716 articles were identified by the search, of these, 528 met the in-, and exclusion criteria based on title and abstract as illustrated in Figure 1. After full-text review, we included a total of six studies that measured HRQoL in 323 patients with ACC, summarized in Table 1.

Figure 1. Flow diagram of systematic literature search.



ACC: adrenocortical carcinoma; HRQoL: health-related quality of life.

Table 1. Overview of health-related quality of life studies in patients with adrenocortical carcinoma.

Study	Design	Study population	HRQoL measure	HRQoL outcome overall	HRQoL outcome ACC
Pikkarainen 1999 (11)	Cross-sectional, surgery transsphenoidal pituitary or laparotomic adrenalectomy	Overall: 74 CS ACC: 4	VAS	Improved HRQoL after surgery	Not provided (2 alive)
von Essen 2002 (12)	Cross-sectional	Overall: 85 GE tumors ACC: 9	EORTC QLQ-C30, HADS, CASC	Patient satisfaction associated with HRQoL and anxiety	Not provided
Sippel 2008 (13)	Cross-sectional, laparoscopic adrenalectomy	Overall: 60 CS ACC: 2	HRQoL improved yes/no	Improved HRQoL after surgery	Not provided (1 alive)
Fassnacht 2012 (14)	FIRMACT, RCT double blind, EPD-mitotane vs streptozotocine-mitotane	Overall: 304 ACC	EORTC QLQ-C30	No difference in HRQoL between regimes before and after treatment, reduced HRQoL compared to general population	Median EORTC QLQ-C30 score: Baseline 58.3/100 (n=204) After treatment 50.0/100 (n=129, p for change 0.996)
Fleseriu 2012* (15)	Open clinical trial, mifepriston treatment	Overall: 50 CS with DM and/or HT ACC: 3	SF36, BDI	Reduced HRQoL compared to general population, improved HRQoL and reduced depression after treatment	Not provided
Katznelson 2014* (16)	Open clinical trial, mifepriston treatment	Overall: 50 CS with DM and/or HT ACC: 3	SF36, BDI	Reduced HRQoL across all components except body pain	Not provided
Dovirak 2016 (17)	Cohort, laparoscopic adrenalectomy	Overall: 30 ACC: 1	SF12, CARE	Reduced HRQoL and physical activity and increased pain after 2 weeks, recovery of HRQoL after 4 weeks	Not provided

ACC: adrenocortical carcinoma; BDI: Beckwith depression index; CARE: convalescence and recovery evaluation; CASC: comprehensive assessment of satisfaction with care; CS: Cushing's syndrome; DM: diabetes mellitus; EDP: etoposide, doxorubicin, cisplatin; EORTC QLQ-C30: European organization for research and treatment of cancer quality of life questionnaire-C30; GE: gastro-intestinal endocrine tumors; HADS: hospital anxiety and depression scale; HT: hypertension; NEC: neuro-endocrine carcinoma; HRQoL: health-related quality of life; RCT: randomized controlled trial; SF-36/12: Short Form 36/12; VAS: visual analogy scale. * Same research population and measurements.

Results and discussion

Clinical presentation

Functional tumors

ACC presents with autonomous adrenal hormone production in 50-60% of the cases, most commonly with cortisol production or a combination of cortisol and androgen production (18). Symptoms of cortisol producing ACCs are often not comparable to full blown Cushing's syndrome, but mostly include muscle weakness, hypokalemia, wasting, and constitutional symptoms (2,19). Patients with excess cortisol levels experience a reduced HRQoL as recently described by the review of Ho and Druce (20). While treatment is effective in improving HRQoL, patients with long-term remission still report a persistent effect on their HRQoL. This was mostly due to psychological impairments such as an increased risk of anxiety and depressive symptoms (21), negative illness perception, and impaired cognitive functioning with measurable changes in brain morphology due to hypercortisolism (22,23). Furthermore, higher cortisol levels are associated with lower bone mineral density and higher fracture rates, especially in patients with ACC (24).

There are currently two HRQoL instruments in the questionnaire database PROQOLID which are developed specifically for use in patients with Cushing's syndrome, namely the CushingQoL and Tuebingen CD-25 (25,26). Since these questionnaires have not been developed especially for cancer patients, they are not fully applicable for use in patients with cortisol producing ACCs. In our systematic review, we found one study that included three patients with cortisol producing ACC (Table 1) (15). This study reported a reduced HRQoL compared to the general population, even after biochemical remission using mifepristone. Further analysis of the same data showed that all domains of HRQoL were altered except for body pain (16). However, the results of the patients with ACC were not provided separately in both studies, therefore no conclusions on the effect of cortisol producing ACCs on HRQoL can be made based on these two studies.

ACCs presenting with aldosterone production are rare. Patients with hyperaldosteronism usually present with hypertension and hypokalemia or with cardiovascular events including atrial fibrillation (27). Hyperaldosteronism is associated with reduced HRQoL in primary aldosteronism (20). Patients can further experience an increased risk of anxiety and depression (28). However, these studies did not include patients with ACC. A new questionnaire to measure HRQoL in patients with primary aldosteronism is currently being developed, the PA-QOL (29). All patients used in the development phase were diagnosed with primary aldosteronism and no patients with malignant aldosterone producing tumors were invited to participate. Therefore, the questionnaire might not be fully applicable in patients with ACC. In our search, we found no studies on HRQoL including patients with aldosterone producing ACCs.

While ACCs producing both cortisol and androgens occur regularly, pure androgen or estrogen producing ACCs are rare. Female patients with an androgen producing ACC present with hirsutism, menstrual irregularity, and virilization including clitoromegaly, male pattern baldness, deep voice, and acne. Male patients with an estrogen producing ACC can experience gynecomastia, hypogonadism, and diminished libido (30). In our search, we have found no studies that measured the HRQoL in patients with androgen or estrogen producing ACCs.

Non-functional tumors

Non-functional tumors most often present with abdominal pain, weight-loss, and non-specific gastrointestinal symptoms such as nausea and anorexia (18,31). More severe cases can present with invasive growth in internal organs including the adjacent kidney and vena cava. Metastases usually include lymph nodes, lungs, and liver. We found one study on HRQoL in gastro-intestinal endocrine tumors that included nine patients with a non-functional ACC (Table 1) (12). Unfortunately, the HRQoL measurements were not provided. The study only described that patient who were satisfied with care had higher HRQoL and lower anxiety scores. Therefore, no conclusions regarding HRQoL in patients with non-functional ACC can be drawn from this study.

Cancer surveillance

ACC can be discovered accidentally on imaging done for other purposes or during incidentaloma follow-up. Follow-up for incidentaloma can have an impact on

overall HRQoL and anxiety, due to patients' concerns about possible malignancy of the tumor (32,33). Similarly, it can be hypothesized that follow-up for recurrence after initial treatment of the tumor can have the same negative impact on HRQoL. Patients with ACC are seen by their healthcare professional every three months during treatment and for the first two years after treatment, and every three to six months for three more years. When no recurrence occurs, these check-up are recommended to be continued with an adapted surveillance protocol (2). This follow-up visit includes routine physical and laboratory checkups, and imaging of thorax, abdomen and pelvis to search for local disease recurrence or distant metastases. We found no studies on the potential HRQoL impact of this surveillance protocol.

During follow-up after ACC treatment healthcare professional also need to take into account that the impact of cancer on HRQoL does not end when treatment has been completed. It has been demonstrated that long-term cancer survivors still experience an impact on their HRQoL (34). This impact is no longer caused by the acute symptoms of the disease and the adverse effects of treatment. Instead, patients suffer from other issues, such as changes in body image, health behavior, fatigue, negative health outlook, and financial insecurity. These effects on HRQoL have been studied in other types of cancer, but we have found no studies that included patients with ACC.

Surgery

Open adrenalectomy is the standard treatment for localized ACC or locally advanced ACC (2,3). En bloc resection of the periadrenal fat, locoregional lymph nodes, and adjacent organs are recommended in case of suspected invasion. Complications of open adrenalectomy include injury to adjacent organs, bleeding, thrombosis, pneumothorax, and wound infections. Adrenalectomy for malignant tumors and hormonally active tumors has a higher complication rate, and is associated with worse long-term outcome than adrenalectomy for benign adrenal tumors (35-37). Two recent reviews show that laparoscopic adrenalectomy for ACC has similar outcomes in tumor control but faster recovery time and higher patient satisfaction (38,39). It is therefore concluded that a laparoscopic approach can be safe in selected patients with a tumor with a diameter less than six centimeters without evidence of local invasion, if the surgeon has sufficient experience (2).

We found three studies on HRQoL after adrenalectomy for benign and malignant adrenal tumors (Table 1) (11,13,17). These three studies included seven patients with ACC, of whom four were alive during HRQoL measurement. However, none of the HRQoL measures was reported separately for the patients with ACC. One study showed that patients with a benign or malignant non-functional adrenal tumor experience a slight decrease in HRQoL directly after surgery, mostly due to pain and limited physical activity (17). HRQoL fully recovered in the weeks following surgery. This is in line with the findings of a study on adrenalectomy including only benign adrenal tumors (40).

Studies on benign and malignant cortisol producing adrenal tumors show an improvement of HRQoL after surgery due to recovery of hormonal status (11,13,41). However, both studies used a single-question method to measure HRQoL. One used a visual analogue score (ranged from zero to ten) to indicate HRQoL and the other used a change question (has your HRQoL been improved, yes or no). Furthermore, we hypothesize that these results might not be directly generalizable to patients with ACC since the presentation in patients with ACC is often different from patients with benign causes of Cushing's syndrome. Even though studies report no differences in HRQoL between different etiologies of Cushing's syndrome (pituitary, adrenal or ectopic origin), these analysis did not include ACC as an etiology (13,42,43).

Radiotherapy

Radiotherapy has a smaller role in the treatment of primary ACC. Routine adjuvant radiotherapy after complete resection of small tumors is not recommended (2,3). However, a recent systematic review did find a positive effect on local control (44). Radiotherapy can play a role in improving local control of irresectable tumors or symptomatic metastasis (45,46), despite having no effect on overall survival (47). Radiotherapy for ACC causes mostly mild acute toxicity such as nausea and fatigue, and only limited late toxicity (44,48). Furthermore, radiotherapy provides pain relief especially for vertebral and bone metastases, and can therefore have a potential positive effect on HRQoL. We found no studies on the HRQoL of patients who received radiotherapy for ACC, therefore there is no evidence yet supporting this hypothesis.

Mitotane monotherapy

Mitotane therapy is used as adjuvant therapy for two years after surgery in patients without macroscopic residual tumor and a perceived high risk of recurrence (stage III, R1 or Rx resection, Ki-67 >10%). For patients with an irresectable tumor or metastases, mitotane can be used in palliative setting as monotherapy or combined with chemotherapy (2,3). Mitotane dosage is dependent on the performance status of the patient and the tolerability of the drug. The dosage is increased until a blood concentration between 14 mg/L and 20 mg/L is reached. Above a concentration of 20 mg/L the risks of adverse effects are highly increased.

Mitotane therapy has a high prevalence of adverse effects ranging from limited to severe. Table 2 provides an overview of adverse effects adapted from the recent ESE guidelines on the management of ACC (2). Almost all patients experience gastro-intestinal adverse effects such as nausea and diarrhea. Most patients have laboratory disturbances including an increase in hepatic enzymes and hypercholesterolemia. Therefore, the use of supportive medication such as anti-emetics, anti-diarrhea and lipid lowering medication are recommended. All these adverse effects and the effects of supportive medication can potentially have an impact on patients' HRQoL.

At higher serum concentrations, mitotane can cause neuropsychological adverse effects including vertigo, ataxia, impaired speech, and lethargy. More severe neuropsychological effects also occur, including cognitive problems such as impaired concentration and memory problems. There have even been reports of clinical depression with the need for antidepressants. As described in two paper in the early '90s, these neuropsychological effects appear to be partly to completely reversible after cessation of mitotane therapy (49,50). However, after these two papers these effects have not been studied in further research efforts.

In our search, we found no studies on HRQoL in patients who receive mitotane monotherapy. The only study that used a HRQoL questionnaire in mitotane users, is the FIRMACT study, which studies mitotane-chemotherapy combination therapy (Table 1) (14). As it is difficult to interpret the results for mitotane therapy alone, we will describe the results in the chemotherapy section.

Adrenocortical insufficiency

All patients on mitotane therapy develop adrenocortical insufficiency and require glucocorticoid supplementation, mostly in the form of hydrocortisone divided in one to three daily doses (2). The supplementation of hydrocortisone in patients with ACC is different from the other forms of adrenal insufficiency due to CYP450 induction caused by mitotane. This results in high metabolism of several drugs, especially steroid based drugs such as hydrocortisone. Glucocorticoid supplementation in patients with ACC is therefore guided by clinical parameters only. This results, more often than in the other forms of adrenal insufficiency, in under- and overtreatment leading to subsequent complaints affecting HRQoL.

Patients with adrenocortical insufficiency experience a reduced HRQoL, increased anxiety, and increased fatigue (20). This HRQoL impact persists during adequate glucocorticoid supplementation. It is hypothesized that this persistent effect on HRQoL is due to the lack of a physiological cortisol rhythm during the day. Efforts to better simulate this rhythm with dual release preparations and three-times per day dosages have not yet resulted in improved HRQoL (20). Studies into the effects of additional mineralocorticoid and androgen replacement on HRQoL in patients with adrenocortical insufficiency have contradictory results (20). The decision to add fludrocortisone or testosterone supplementation should therefore be based on clinical judgement and laboratory disturbances (2).

There is one disease-specific HRQoL questionnaire for use in patients with adrenocortical insufficiency, the AddiQoL (51). This questionnaire was developed using patients with both primary and secondary causes of adrenal insufficiency, however, no patients with ACC were included. Therefore, the questionnaire might not be fully applicable for use in patients with ACC. For example, the impact of adrenal insufficiency on HRQoL in patients with mitotane therapy is unknown. Many of the symptoms of adrenocortical insufficiency overlap with the adverse effects of mitotane therapy, which include fatigue, anorexia, and myalgia (Table 2) (52). It might therefore be difficult to distinguish the impact of adrenocortical insufficiency on HRQoL from the impact of mitotane therapy. We have found no HRQoL studies on adrenocortical insufficiency in our search that include patients with ACC or patients on mitotane therapy.

Table 2. Adverse effects of mitotane monotherapy.

Clinical effects	Laboratory disturbances
Common - very common • Gastrointestinal: nausea, vomiting, diarrhea, anorexia, mucositis • Central nervous system: lethargy, somnolence, vertigo, ataxia, confusion, depression, dizziness, decreased memory, paresthesia, myasthenia • Adrenal insufficiency • Gynecomastia • Skin rash • Primary hypogonadism in men *Rare - very rare* • Liver failure • Autoimmune hepatitis *Incidence not known* • Cardiovascular: hypertension • Ocular: blurred vision, double vision, toxic retinopathy, cataract, macular oedema • Hemorrhagic cystitis	*Common - very common* • Increase of hepatic enzymes (γ-GT) • Increase in hormone binding globulins (CBG, SHBG, TBG, vitamin D binding protein) • Disturbance of thyroid parameters (interference with binding of T4 to TBG, total T4↓) • Hypercholesterolemia, hypertriglyceridemia • Hepatic microsomal enzyme induction with increased metabolism of glucocorticoids and other steroids and barbiturates, phenytoin, warfarin • Leukopenia • Prolonged bleeding time *Rare - very rare* • Thrombocytopenia, anemia • Hematuria, albuminuria

Chemotherapy

Chemotherapy is indicated for rapidly progressive or extensive metastatic disease, and for progression during mitotane monotherapy (2,3). The first-line chemotherapy consists of etoposide, doxorubicin, cisplatin (EDP), with or without the addition of mitotane. As a second-line treatment, streptozotocine-mitotane or gemcitabine-capecitabine can be considered (53,54). Participation in clinical trials after failure of EDP chemotherapy can also be considered (2). Other chemotherapeutic regimens and immunotherapy have shown little effectiveness thus far (18,46).

Patients on chemotherapy-mitotane combination therapy experience the same adverse effects as patients on mitotane monotherapy listed in Table 2 (2). Additional adverse effects that are more specific to chemotherapy include bone-marrow toxicity, cardiovascular or thromboembolic events, loss of hair, and

respiratory events (14,55). These adverse effects can have a significant impact on HRQoL of patients undergoing chemotherapy (56).

The FIRMACT trial measured HRQoL in patients with ACC on chemotherapy-mitotane combination therapy and is the largest study on HRQoL in patients with ACC thus far (Table 1) (14). The study compared the effectiveness of EDP-mitotane to streptozotocine-mitotane in 304 patients with ACC in palliative setting. They used the general cancer HRQoL questionnaire developed by the European Organization for the Research and Treatment of Cancer (EORTC QLQ-C30) to measure HRQoL before and during treatment. They discovered that the EDP-mitotane regime had a better progression-free and overall survival, while HRQoL did not differ between the groups. They found that patients on both regimens had a reduced HRQoL before the start of the treatment (EORTC QLQ-C30 median score 58.3/100, n=204). In comparison, the median score in the general population is 75.0/100 and in patients with metastatic colon cancer 66.7/100 (57). HRQoL was even further affected during treatment (50.0/100, n=129). However, this reduction was not significant due to the limited number of patients still alive during follow-up combined with a response rate of approximately 66%.

A limitation of the FIRMACT study is that they only used a general cancer HRQoL questionnaire. General HRQoL questionnaires do not cover the full range of specific ACC-related problems, which might result in underreporting of HRQoL issues. Furthermore, no data was provided on the different sub-scales of the EORTC-QLQ-C30. We therefore have no information on which domains of HRQoL are affected in patients with ACC on chemotherapy.

Conclusions

Little is known about HRQoL in patients with ACC. There have been six HRQoL studies that included patients with ACC. Four of them included only a few patients with ACC, and did not provide the HRQoL measurements for the patients with ACC separately. One study only provided a positive associations between patient satisfaction with care and HRQoL. The only study thus far with an adequate sample of patients with ACC and a validated HRQoL questionnaire is the FIRMACT study that compared two chemotherapy-mitotane regimes (14). They discovered that the HRQoL of patients with ACC in palliative setting was reduced compared to the general population. The study also showed that even though one of the regimes

had a positive effect on survival, both the treatments had a negative effect on patients' HRQoL.

Although the number of HRQoL studies in patients with ACC is limited, literature does indicate with reasons that the course of the disease and the treatments of ACC can have a large impact on patients' HRQoL. Healthcare professionals can take this potential impact into account when discussing diagnosis and treatment options. For example, patients can experience hypercortisolism, hyperaldosteronism, and adrenocortical insufficiency, which have a large impact on HRQoL in patient with benign diseases as shown in the recent review of Ho and Druce (20). This impact on HRQoL even persists after recovery of hormonal status. However, we hypothesize that patients with ACC can experience different and additional HRQoL issues due to malignancy of the tumor. Cancer and its treatment have major impact on patients' lives. The issues that influence HRQoL in cancer patients are not fully comparable to those in other chronic illnesses (56,58). These issues have not yet been studied in patients with ACC. Furthermore, the HRQoL effects of mitotane therapy, an important part of the ACC treatment with a high toxicity rate, are still unknown.

Directions for further research

The recent ESE guideline on ACC describes the importance of gathering more information on HRQoL in patients with ACC (2). In this review we showed that little is known about HRQoL in patients in ACC and a disease-specific questionnaire does not exist. Knowledge of HRQoL across the course of the disease and treatment options is necessary to understand the impact of the disease on patients' lives. This knowledge will help communication about HRQoL issues and will aid the shared decision making process (5).

We therefore conclude that there is need for more HRQoL research in patients with ACC to improve patient-centered care. To better aid the processes of shared decision making and treatment guidance, we need more information on the HRQoL impact of all stages of the disease and all treatment options. We therefore recommend that all future trials and cohorts include at least a general HRQoL measurement designed for use in cancer patients. In addition, disease specific HRQoL questionnaires are more sensitive to differentiate between different symptoms of the disease and adverse effects of treatment options. We therefore

recommend efforts be taken to develop an ACC-specific HRQoL questionnaire, since patients with ACC can also experience more specific HRQoL issues. The results of this review can be used as a first source of information, in line with the guidelines of questionnaire development set by the EORTC (59).

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Chapter 3

Patient and partner perspective on health-related quality of life in adrenocortical carcinoma

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Abstract

Little is known about the impact of adrenocortical carcinoma (ACC) on health-related quality of life (HRQoL), and no disease-specific questionnaire exists. This qualitative study aimed to identify relevant domains of HRQoL for patients with ACC. In 2 focus group interviews, we discussed concerns regarding living with ACC and its treatments. The first group consisted of 6 patients on mitotane therapy and their partners or relatives, the second group of 4 patients after surgery alone and their partners. Inductive qualitative content analysis was used to analyze the interviews. We identified 4 domains related to HRQoL in patients with ACC, namely physical complaints, mental consequences, social consequences, and functional limitations. For example, physical complaints included symptoms of the disease and side-effects of mitotane therapy; mental consequences included feeling insecure and living from scan to scan; and functional limitations included daily activities and mobility. We further found that patients' experiences with the health care system and health care professionals and partner perspectives influence HRQoL. In conclusion, ACC has a large impact on HRQoL in 4 domains. These results can be used to improve communication about HRQoL issues. We will use our findings to generate a disease-specific questionnaire to measure HRQoL in patients with ACC.

Introduction

Adrenocortical carcinoma (ACC) is a rare cancer with high morbidity and mortality (1). Treatment options and prognosis depend on the stage of the disease (2). Single tumors are treated with surgical adrenalectomy. Depending on the recurrence risk, surgery is followed by at least 2 years of adjuvant mitotane therapy. Irresectable tumors or metastasis can be treated by surgery, radiotherapy, mitotane therapy, or chemotherapy-mitotane combination therapy. Both the symptoms and treatment options of ACC can have an impact on health-related quality of life (HRQoL). For example, tumors can be hormonally active and treatment can induce hormonal deficiencies, which are both associated with impaired HRQoL (3).

We discovered that there have been limited studies on HRQoL in patients with ACC (4). One randomized controlled trial including 294 patients with advanced ACC indicated an impaired HRQoL compared to the general population and a further impairment due to chemotherapy-mitotane combination therapy (5). However, there have been very limited studies in patients after surgical therapy and no studies in patients on mitotane monotherapy. We hypothesize that HRQoL research on benign adrenal tumors might not be fully generalizable to patients with ACC. For example, mitotane therapy is used only for ACC and has a unique spectrum of potentially severe side-effects. Therefore, the most recent guideline on the management of ACC expressed a need for more research on HRQoL in patients with ACC (2).

Further research can provide information on the influence of different treatment options on HRQoL, which is essential to the process of shared decision making. To conduct this research, patient-reported outcome measures (PROM) are increasingly used to measure HRQoL, both general and disease specific. These PROMs can also be used in the clinical setting to improve treatment guidance and thus improve patient-centered care (6). To date, no disease-specific PROM exists for measuring HRQoL in patients with ACC. To determine which issues and domains should be included in an HRQoL PROM for ACC, patient input is essential. In this qualitative study, we therefore conducted focus group interviews with patients with ACC and their partners or relatives, with the aim of identifying relevant domains of HRQoL for patients with ACC.

Methods

Participant Inclusion

Patients with ACC with all stages of disease being treated as outpatients at Máxima MC the Netherlands were eligible for inclusion. Patients were purposefully selected to create a heterogeneous reflection of the ACC population. We contacted patients face to face and by telephone. Eligible patients received a letter with information about the study. In total, 16 patients were contacted. Two patients were unable to attend on the chosen date, 3 patients did not want to bring up the past, and 1 patient who had received chemotherapy-mitotane combination therapy was unable to attend because of physical illness.

The partner or a relative closely involved in the treatment was also invited. Some patients indicated they were more comfortable talking while their partner was present and partners could help with recall. Secondly, we hypothesized that partner perspectives could influence patients' experiences and HRQoL. We therefore included the partner perspectives to be able to investigate this phenomenon.

Two focus group interviews were carried out at Máxima MC. The first group consisted of 6 patients who currently or recently received mitotane treatment and their partners or relatives (1 mother, 1 sister). Two patients received mitotane adjuvant after surgery, 4 for metastatic disease. Two patients had also received radiotherapy. The second group consisted of 4 patients who were treated with surgery only and their partners. The sample was heterogeneous in terms of age (mean 57.1 years; range, 26-71 years), sex (7 female, 3 male), time since diagnosis (mean 4.9 years; range, 1-18 years), hormone production (2 cortisol, 1 cortisol and androgens, 7 none), ENSAT (European Network for the Study of Adrenal Tumors) stage (4 stage I-II, 1 stage III, 5 stage IV), and treatment response (5 complete response, 3 stable disease, 2 progressive disease) (Table 1).

Focus Group Interviews

Both focus group interviews were moderated by a clinical doctor trained at moderating focus groups (M.N.T.K.). The moderator was not involved in ACC treatment and did not know the participants in advance. A co-moderator (R.V.S.) and note-taker were also present during the focus groups. We used a preprepared, semistructured interview guide. The interview questions were inspired by the literature and clinical experience of the research team (4). During the introduction

round, the moderator and co-moderator introduced themselves and explained the aim of the focus group. Patients were then asked to describe their experience with the disease and treatment options, the influence on everyday life, and their concerns regarding HRQoL. The focus group interviews were ended when no new topics came up, which occurred after 2.5 hours in the first group and 2 hours in the second.

The study protocol has been approved by the local Medical Ethics Committee. Written consent was obtained from all patients and all quotations have been anonymized. Partners have been indicated with a "p."

Table 1. Patient characteristics.

	Group 1 Mitotane (N=6)	**Group 2 Non-Mitotane (N=4)**	**Overall (N=10)**
Age[1], mean (range)	55.8 (26-71)	59 (57-61)	57.1 (26-71)
Female, N	4	3	7
Years since diagnosis[1], mean (range)	3.0 (1-4)	7.8 (1-18)	4.9 (1-18)
ENSAT stage[1], N			
I	1	2	3
II	0	1	1
III	1	0	1
IV	4	1	5
Hormone production, N			
None	4	3	7
Cortisol	1	1	2
Cortisol and androgens	1	0	1
Therapy, N			
Surgery	5	4	9
Mitotane	6	1[2]	7
Radiotherapy	2	0	2
Chemotherapy	0	0	0
Response (RECIST) [1], N			
Complete response	2	3	5
Stable disease	3	0	3
Progressive disease	1	1	2

ENSAT: European Network for the Study of Adrenal Tumors; RECIST: Response Evaluation Criteria in Solid Tumors. [1]At time of focus group. [2]Discontinued after 5 weeks because of side-effects.

Analysis

Both focus group interviews were audiotaped and transcribed smooth verbatim with comments on nonverbal observations. A total of 4.5 hours of audiotape resulted in 2218 lines of transcribed text. The participants were not asked to provide feedback on the transcripts or the findings.

Inductive qualitative content analysis was used to analyze the transcripts using QDA Miner Software Lite, v2.0.5 (7-9). Using an open coding method, codes were given to each line of transcription hypothesis free to label the meaning of the phrase. These codes were then grouped in subcategories and categories related to HRQoL (Table 2). The final coding framework was used to establish an overview of HRQoL domains relevant for patients with ACC.

Coding was performed independently by 2 researchers (R.V.S. and L.A.M.) and discrepancies were handled by discussion. After reviewing the initial coding framework, significant overlap between the patient experiences and the existing Cancer Patient Experiences Questionnaire (CPEQ) was discovered (10). Similarly, an overlap existed between the partner perspectives and the existing Cancer Support Person's Unmet Needs Survey—Short Form (SPUNS-SF) questionnaire (11). We therefore used the main categories of these existing questionnaires to recode the patient experiences and partner perspectives.

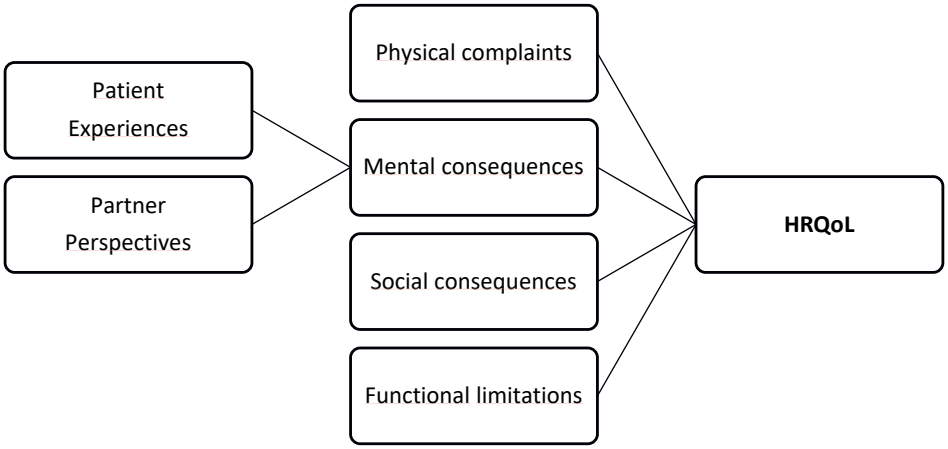
Results

We identified 4 domains related to HRQoL in patients with ACC, namely physical complaints, mental consequences, social consequences, and functional limitations. We further found that patients' experiences with the health care system and health care professionals and partner perspectives influence HRQoL, mostly through effects on the mental consequences domain. A conceptual model of HRQoL in ACC is represented in Figure 1.

Table 2. Example of Inductive Coding Framework.

Examples of translated quotes	Code	Sub-category	Category	Domain
3: I took mitotane only at night, … then I woke up every night with diarrhea, fell back asleep again and functioned better during the day. 5: I get diarrhea attacks. … This morning while traveling here, we got out of traffic just in time for me to run to a bathroom. 6: When the dose (mitotane) is increased, I get diarrhea at night.	Diarrhea	Gastro-intestinal	Side-effects of mitotane	Physical complaints
4: Mitotane makes me nauseous.	Nausea			
3: I thought I was walking straight, but I walked straight into a door. 4: At night I have to shuffle forward. 4p: And you have to hold on to something. 6: It feels like I'm drunk.	Loss of coordination	Neurological		
3: With the mitotane I had neurological complaints, I had no concentrations. 5: It is a lack in concentration, with a newspaper I have to stop every once in a while and read it again.	Loss of concentration			
2p: He had a loss of balance, dizziness. 8: What bothered me most was the dizziness and therefore not being allowed to drive a car.	Balance disturbances and dizziness			

Figure 1. Conceptual model of health-related quality of life domains in adrenocortical carcinoma.



HRQoL, health-related quality of life.

Physical Complaints

The physical complaints domain comprised 5 categories: symptoms of the disease, recovery and complications after surgery and radiotherapy, side-effects of mitotane, symptoms of cortisol production and supplementation, and long-term consequences.

The disease presented in most patients with abdominal pain: “1: I thought I had kidney stones, because of the pain I felt in my left abdomen.” Other presenting symptoms included headaches, fatigue, sleep problems, fever, nausea and vomiting, difficulty eating or loss of appetite, and increased abdominal size. One patient had androgen production; she therefore also presented with acne and excessive hair growth. Symptoms due to cortisol production were also mentioned and will be discussed later.

HRQoL was influenced by complications after surgery, side-effects of painkillers, wound problems, scar problems, nausea, pain, and hiccups. Recovery time after surgery varied between the patients. Some made a good recovery, whereas others suffered from the aftermath for weeks: “8: I couldn’t get out of bed, only to eat and shower. It took 6 weeks for me to get out of the house for a walk.” The 2 patients who received radiation therapy made a good recovery.

The patients on mitotane therapy felt a large burden on their HRQoL due to the side-effects. Gastrointestinal side-effects included diarrhea, flatulence, nausea, vomiting, changes in taste, liver toxicity, and elevated cholesterol. Neurological side-effects included loss of coordination, loss of concentration, balance disturbances and dizziness, forgetfulness, slowing, and difficulty speaking: "8: What bothered me most was the dizziness and therefore not being able to drive a car. This makes me dependent on everyone, which I cannot stand." Some patients further reported a loss of sexuality and menstrual disturbances.

The effects of cortisol-producing tumors and cortisol replacement therapy had overlapping effects on HRQoL in our patients and will therefore be discussed together. Patients were mostly hindered by the increased incidence of infections, resulting in the need for immediate medical attention and higher supplementation dosages: "3p: What we find annoying about the cortisol supplementation is that we have to go straight to the hospital when she gets a simple flu." Other symptoms that were reported were poor wound healing, palpitations, rushed feeling, edema, weight gain, and dry, fragile, and itchy skin.

Long-term effects of ACC mostly involved minor to severe fatigue, loss of physical fitness, and weakness: "4: I have to ask my husband to do everything because I am too tired. Even taking a shower exhausts me."

Mental Consequences

All patients and partners reported feeling insecure at some point during the diagnostic procedures, treatment, or follow-up. They felt insecure about the cause and consequences of their physical complaints, about their diagnosis, and mostly about the future. Especially when new scans approach, the feeling of insecurity returns. Patients feel like they are living from scan to scan: "7: When it is time for the tests and scans again, the uncertainty returns. Then I am worried about what the next scan will show."

After receiving the diagnosis patients and partners felt worried or even startled and some had difficulty accepting the situation: "10: My body cannot do what I want it to do anymore, … but accepting that is difficult." Some patients further reported getting emotional or irritated more easily since being diagnosed with cancer. On the other hand, continuing life and staying optimistic was widely expressed in both

groups: “5: I don’t know how long it will take and what I can expect, but at the moment I feel really good, the tumor is gone and I remain positive.”

Some treatment options were viewed with caution or even fear. For example, all patients were afraid of complications of radiation therapy and some partners felt relieved when no radiation therapy was necessary. Patients also reported aversion toward taking their medication, including mitotane therapy, cortisol replacement therapy, and supportive medication. Four patients suffered from mental side-effects of treatment such as mood disturbances, panic attacks, and even burnout: “3p: Before the diagnosis she didn’t recognize herself due to the mood swings and panic attacks. 3: Even now when my supplementation dose is too high, I get a crazy temper.”

Social Consequences

Suffering from ACC had a large impact on family life. Among others, this was due to changes in role functioning such as performing household chores and looking after (grand)children. But their families also provided energy to patients: “10p: The disease had a lot of impact, but together we can live through anything.” Outside the family, patients experienced compassion from their neighbors and friends. However, they sometimes experienced a lack of understanding due to the disease being rare and not clearly visible: “6: I sometimes struggle with the fact that people can’t see that I am sick. Don’t get me wrong, I am glad that I am not bald from chemotherapy, but people don’t notice that I am sick. … People judge me for that, like why can you look good and go to work, but you can’t clean your own windows.” The disease being rare also results in a lack of contact with other patients; this was reported as disappointing by some patients and partners.

Functional Limitations

Patients were limited in their daily living due to consequences of ACC; this included work and daily activities such as housework and babysitting grandchildren. Leisure activities such as holidays, hobbies, and going out for dinner were also limited by the disease. Patients further experienced a lack in mobility due to an inability to walk, cycle, drive, or travel long distances. Staying independent even with physical limitations was perceived as very important.

These functional limitations could be consequences of various aspects of the disease. Some patients were limited by fatigue or (neurological) side-effects of mitotane therapy: “4: I am not allowed to cycle anymore, because I drove right into parked cars.” Others experienced limitations due to mental issues or had to adjust activities and meals based on medication. Owing to these limitations, patients had to make different choices and adjust their lifestyle, which influenced their HRQoL.

Patient Experiences

Patients’ experiences with the health care system and health care professionals influenced their HRQoL. After reviewing the initial coding framework, significant overlap between the patient experiences codes and the existing CPEQ was discovered (10). Experiences that patients valued most were related to good contact with their doctor, proper information about the diagnosis and treatment options, and good organization of care. Less mentioned were the importance of patient safety, good nurse contact, contact with next of kin, and hospital standards. Patients described their experiences mostly in relation to the mental consequences of ACC, for example, a good relation with their doctor and receiving proper information leading to feeling less worried: “3: Between appointments questions start pilling-up and I start worrying, so I send an email and my doctor replies with a reassuring message, then I can relax again.”

Partner Perspectives

During the interviews, partners and relatives shared their perspectives on coping with the disease. An overlap existed between the partner perspectives and the existing SPUNS-SF questionnaire (11). The partners mostly expressed their emotional and personal needs, the importance of proper information, access and continuity of health care, and worries about the future. Work and financial needs were mentioned less by the partners in our groups.

Partners’ perspectives influenced not only their own HRQoL but also the HRQoL of the patient. For example, a partner worrying about treatment side-effects might have led to the patient feeling insecure about the treatment: “2p: I was so opposed to him taking the medication, that I think I might have made it more difficult.”

Discussion

In this qualitative study, we identified 4 domains related to HRQoL in patients with ACC, namely physical complaints, mental consequences, social consequences, and functional limitations. This is in line with established models on HRQoL (12-14). Thus far, there has been limited information about the influence of ACC on HRQoL. This information is important in the process of shared decision making and communication about HRQoL issues so that patient-centered care can be provided.

HRQoL can be measured using general or disease-specific PROMs. One example of a general HRQoL PROM for use in cancer patients is the EORTC-QLQ-C30 (European Organisation for Research and Treatment of Cancer core quality of life questionnaire). This questionnaire includes general issues that cancer patients experience as a result of their disease. We found that our results had a large overlap with the items in the EORTC-QLQ-C30. We therefore hypothesize that this questionnaire can be a good tool to measure HRQoL in patients with ACC to get a general overview of all relevant domains. However, not all relevant issues we found are included in the EORTC-QLQ-C30. This questionnaire does not include issues that are more specific to one type of cancer, such as in ACC the neurological side-effects of mitotane or the effects of cortisol production or supplementation. Therefore, the EORTC has the option to develop disease-specific models that can be used in addition to the EORTC-QLQ-C30 questionnaire to obtain a better understanding of the full range of HRQoL issues patients face (15). The results of our qualitative study can be used as a first effort to create an ACC-specific model to be used in addition to the EORTC-QLQ-C30.

Future research using an HRQoL PROM appropriate for use in patients with ACC will provide much needed information on the effects of different treatment options on HRQoL. This information is essential in the process of shared decision making for health care professionals and patients. The results of this study and a disease-specific HRQoL PROM can also aid patient-centered care by improving guidance of individual therapies (6). For example, almost all patients reported the importance of regaining their physical and mental fitness. Receiving aftercare by a rehabilitation specialist, physical therapist, or psychologist trained in treating cancer patients was viewed as a positive contribution to reaching these goals by our participants. Research has shown that exercise-based or multidimensional rehabilitation programs are beneficial to HRQoL in cancer patients (16, 17). A

change in fatigue or weakness can be used as a signal that referral to a cancer aftercare program might be beneficial.

We found that patient experiences and partner perspectives influence HRQoL, especially through the mental consequences domain. This is in line with other studies that found a positive association between patient experiences and clinical or patient-reported outcomes (18, 19). It is therefore important to also measure patient and partner experiences to gain information about the full range of a patients' well-being or better interpret a change in the mental domain. For example, when a doctor properly tailors the information to a specific patient's needs and creates clear expectations, this might result in less worry about the future and a higher HRQoL (20, 21). Measuring and communicating about these experiences could therefore play a role in improving HRQoL (22). We found a large overlap with the experiences our participants reported and the CPEQ and SPUNS-SF questionnaires (10, 11). We therefore suggest using these or similar questionnaires when measuring patients experiences and partner perspectives in ACC research and practice.

A potential limitation of this study is the limited number of participants in the focus group interviews. For model development, usually inclusion of 20 patients or more is recommended (23). However, ACC is a rare cancer, with an incidence of 1 per million person-years and an overall median survival of 17 months (1). Our study thus included one-tenth of the estimated population of ACC survivors in the Netherlands. Furthermore, the participants were a heterogeneous sample of the population and both focus groups were continued until no new topics came up to improve data saturation.

A second limitation is the potential selection of healthier patients with fewer HRQoL concerns. We aimed to limit this phenomenon by purposefully selecting patients with all disease stages and treatment options. The only exception was that no patient on chemotherapy was included because of physical illness at the time of the focus group interviews. We hypothesize that patients on chemotherapy-mitotane combination therapy experience the same HRQoL issues as discussed during the focus groups but with different intensity (5). We therefore expect the results would not have differed if the patient on chemotherapy had been included. Furthermore, the EORTC-QLQ-C30 has been developed with patients on a wide

range of chemotherapy regimens (15). Most relevant issues for patients on chemotherapy will therefore already be included in the questionnaire.

Finally, the presence of the partners or relatives might result in patients not discussing severe HRQoL concerns to spare the others (24). Although this is a well-known phenomenon, we do not feel this occurred during our focus group interviews. In our experience, patients felt more comfortable in the presence of their partner or relative. In addition, the partners and relatives were helpful with recalling memories and finding the correct words to describe HRQoL concerns, especially for the patients suffering from impaired memory and concentration due to mitotane side-effects.

Conclusion

ACC has a large impact on HRQoL. We identified 4 HRQoL domains relevant to patients with ACC. With this knowledge, doctors can improve communication about relevant HRQoL issues. Furthermore, we will use these domains to generate a disease-specific model as an addition to the EORTC-QLQ-C30 questionnaire to measure HRQoL in patients with ACC. With this we aim to improve patient-centered care in this specific patient group.

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Chapter 4

Health-related quality of life in adrenocortical carcinoma: development of the disease-specific questionnaire ACC-QOL and results from the PROFILES registry

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Abstract

We aimed to develop a disease-specific adrenocortical carcinoma (ACC) health-related quality of life (HRQoL) questionnaire (ACC-QOL) and assess HRQoL in a population-based cohort of patients with ACC. Development was in line with European Organization for Research and Treatment of Cancer (EORTC) guidelines, though not an EORTC product. In phase I and II, we identified 90 potential HRQoL issues using literature and focus groups, which were reduced to 39 by healthcare professionals. Pilot testing resulted in 28 questions, to be used alongside the EORTC QLQ-C30. In Phase III, 100 patients with ACC were asked to complete the questionnaires twice in the PROFILES registry (3-month interval, respondents: first 67, second 51). Confirmatory factor analysis demonstrated the structural validity of 26 questions with their scale structure (mitotane side-effects, hypercortisolism/hydrocortisone effects, emotional effects). Internal consistency and reliability were good (Cronbach's alpha 0.897, Interclass correlation coefficient 0.860). Responsiveness analysis showed good discriminative ability (AUC 0.788). Patients diagnosed more than 5 years ago reported a good HRQoL compared with the Dutch reference population, but experienced residual fatigue and emotional problems. Patients who underwent recent treatment reported a lower HRQoL and problems in several domains. In conclusion, we developed an ACC-specific HRQoL questionnaire with good psychometric properties.

Introduction

Adrenocortical carcinoma (ACC) affects approximately one in a million persons each year (1). When diagnosed at an early stage, surgery can result in long-term survival. However, often the prognosis is poor due to advanced disease or unfavorable tumor characteristics. Treatment options for ACC include surgery, mitotane therapy, chemotherapy and radiotherapy (2,3). Each of these treatment options and the disease itself can have a negative impact on patients' health-related quality of life (HRQoL).

HRQoL is usually assessed with questionnaires, which can be generic or for a specific disease group or issue. The EORTC QLQ-C30 is a well-known questionnaire designed for use among patients with cancer (4). Its items include general effects of cancer on HRQoL such as general physical and emotional functioning, and symptoms such as fatigue. The EORTC also developed disease-specific questionnaires that can be used in addition to the EORTC QLQ-C30 questionnaire, for example, in breast cancer (5). No disease-specific questionnaire has yet been developed for ACC and apart from questionnaires addressing hormone overproduction, there is no HRQoL questionnaire including items specific to ACC-related problems (6).

Even though HRQoL is an important part of cancer care, studies in ACC are limited (6). To date, only the FIRMACT study has assessed HRQoL in a large study of patients with ACC (7). They found a lower HRQoL as assessed with the EORTC QLQ-C30 in patients with advanced ACC compared to the general population. There are no publications yet that report specifically on HRQoL of patients with other stages of ACC or other treatment modalities.

Patients with ACC experience many combinations of issues which can impair HRQoL (8). Examples of these issues are mitotane side-effects, hormone excess, problems with sexuality, and concerns about scan results. These issues are not yet included in the EORTC QLQ-C30 or any other questionnaire. The use of existing questionnaires in ACC research or patient care might consequently result in underreporting of HRQoL issues. We therefore believe that there is an unmet need for a disease-specific HRQoL questionnaire developed for use in patients with ACC.

This study aimed to develop and provide initial validation of a disease-specific HRQoL questionnaire to be used alongside the EORTC QLQ-C30 in patients with ACC (ACC-QOL). The secondary objective is to assess HRQoL in a population-based cohort of patients with ACC.

Methods

The method followed the guidelines published by the EORTC QOL Group for phases I to III of new questionnaire development (5). However, since the study was not initiated by an EORTC working group, and the development of this questionnaire was not set up as an international study, the questionnaire cannot be seen as an official EORTC product. The study has been approved by the Dutch-certified Máxima MC Committee for Ethics (approval number N19.075).

Phase I – Issue identification

In the first phase, a comprehensive list was composed of potential HRQoL issues that patients with ACC might face. This was done using a systematic literature search and focus group interviews, of which the methods and results can be found in more detail in previous publications (6,8). In short, we found 6 studies that assessed HRQoL in patients with ACC and listed the reported issues. We then organized focus group interviews with 10 patients and their partners purposefully selected to form a heterogeneous group (mean age 57.1 years, range 26–71; 7 females; mean time since diagnosis 4.9 years, range 1–18; 2 cortisol production, 1 cortisol and androgens production; 4 ENSAT stage I-II, 1 stage III, 5 stage IV; 10 surgery, 6 mitotane, 2 radiotherapy; 5 complete response, 3 stable disease, 2 progressive disease).

We developed a semi-structured interview guide based on the literature search and clinical expertise of the research group (6). Patients were asked to describe their experiences with the disease and treatment options, the impact on everyday life, and their concerns regarding HRQoL. The interviews were transcribed smooth verbatim and transcripts were analyzed by inductive qualitative content analysis using QDA miner lite software v2.0.5 (9,10,11). Coding was performed by two independent researchers and discrepancies were resolved through discussion. The codes and the results from the literature search were used to form a comprehensive list of 90 HRQoL issues.

Phase II – Question selection

Five healthcare professionals (HCP) were selected based on their experience in treating patients with ACC. The group consisted of two endocrinologists, two oncologists and one surgeon employed at adrenal expert centers in the Netherlands. The HCPs evaluated the list of HRQoL issues produced in phase I and were asked to identify missing issues. They were then asked to rate issues (excluding issues already in the EORTC QLQ-C30) based on relevance using a four-point Likert scale (not relevant–very relevant) and select 10 core issues to be included in a disease-specific HRQoL questionnaire. The most relevant items on the list were selected based on the Likert scale rated by the HCPs (mean score ≥ 2.5) and the core issues selected by the HCPs (selected by ≥1 HCP). This resulted in a list of 39 relevant HRQoL issues.

The list of relevant HRQoL issues was transformed into questions compatible with the EORTC QLQ-C30 format (response categories: “not at all”, “a little”, “quite a bit” and “very much”; time frame: one week for all items, 4 weeks for the sexuality item). Issues already used in other EORTC models, were selected from the EORTC QOL Item Bank, which comprises all existing EORTC QOL questionnaire items and their translations (12). The issues with no corresponding question in the EORTC item bank (n = 13) were converted into questions using the same format after consensus by two authors.

The questions were pilot tested using cognitive interviews with four consecutive patients with ACC at the Máxima MC outpatient clinic. They were asked to complete the questionnaire using the thinking-out-loud method and then answer questions regarding readability, feasibility, relevance, and identification of repeated themes. This resulted in a further reduction of the questionnaire to 28 questions. The four participants needed between 15 and 30 min to complete both the EORTC QLQ-C30 and the ACC-QOL.

Phase III – Validation

Questionnaires were collected within the Patient Reported Outcomes Following Initial treatment and Long term Evaluation of Survivorship (PROFILES) registry (13). PROFILES is a registry for the study of the physical and psychosocial impact of cancer and its treatment from a dynamic, growing population-based cohort of both short and long-term cancer survivors. The registry allows patients to complete

questionnaires online or on paper. Patients for the questionnaire validation phase were selected from the Netherlands Cancer Registry. This registry includes data on all cancer patients diagnosed in the Netherlands. Inclusion criteria were diagnosis with ACC, age above 18 years at diagnosis, alive in March 2021, and treated in an adrenal expert center in the Netherlands. Principal investigators from each adrenal expert center checked the selection (130 patients) and excluded patients who would not be able to fill in a questionnaire (e.g., language barrier, impaired cognitive status, deceased between selection and questionnaire administration).

The principal investigators sent an invitation letter to the remaining 100 patients with a link to the online informed consent form and the EORTC QLQ-C30 and ACC-QOL questionnaires. Paper informed consent forms and questionnaires were sent upon request. When a patient did not respond within 4 weeks, a reminder was sent. The second questionnaire was sent to the respondents 3 months later and contained a global rating scale assessing change in overall HRQoL to assess responsiveness (how is your quality of life compared to 3 months ago? Response categories: “worse”, “stable”, “better”).

EORTC QLQ-C30 sum scores were calculated using the equations provided by EORTC (14). The EORTC QLQ-C30 overall score, global health score and function scores, including physical, role, emotional, cognitive, and social functioning, were scaled from 0 to 100 where 100 equals perfect HRQoL or function. The symptom scores, including fatigue, nausea and vomiting, pain, dyspnea, insomnia, appetite loss, constipation, diarrhea, and financial difficulties, were scaled from 0 to 100, where 0 equals no symptoms. Clinical data of the respondents, the non-respondents, and excluded patients were collected from the Netherlands Cancer Registry to assess selection bias and to determine the effect of clinical characteristics on HRQoL.

Data Analysis

We used descriptive statistics for Phase I-III to determine item relevance. Differences in patient characteristics between respondents, non-respondents, and excluded patients were determined using ANOVA tests for continuous variables and chi-square (or Fisher’s exact) tests for categorical variables. Analyses were performed in SPSS version 26 when unspecified or in STATA version 17 when specified.

Psychometric properties of the ACC-QOL were determined according to the COSMIN taxonomy and methodology, by means of the classical test theory (15,16). Face validity was determined in phases I and II of the study. In phase III, construct validity was assessed using confirmatory factor analysis to confirm the hypothesized scale structure. Four models with increasing number of scales were tested by standard equation modelling in STATA version 17. Model 1: no scale structure; model 2: physical effects and emotional effects scales; model 3: mitotane side-effects, hypercortisolism/hydrocortisone effects and emotional effects scales; model 4: food, neurology, skin, hypercortisolism/hydrocortisone effects and emotional effects scales. Items with low correlation ($r < 0.2$) to any scales and the overall score were considered for deletion. We hypothesized that either model 3 or 4 would have the best fit. A comparative fit index (CFI) close to 0.95 and a standardized root mean square residual (SRMR) and root mean square error of approximation (RMSEA) close to 0.08 was considered a good-fitting model. The best-fitted model was used to calculate the scales scores and overall score for the ACC-QOL in the same manner as the EORTC QLQ-C30 symptom and overall scores (14). Construct validity was further confirmed by hypothesis testing. A hypothesis was confirmed if a correlation was below <0.4 for scales in the ACC-QOL without relations to scales in the EORTC QLQ-C30, and between 0.4 and 0.9 for scales with a relation. Negative correlations in the same magnitude were considered for scales with opposite direction. Any correlation above 0.9 would be rejected, because a scale would not have an added value to the scale in the EORTC QLQ-C30. Because the scale mitotane side-effects was hypothesized to have many correlations with the EORTC QLQ-C30 subscales, the hypotheses were formed for the scales food, neurology, and skin, respectively, irrespective of the best-fitted model. Validity was determined to be acceptable if 7 out of 10 hypotheses were confirmed.

Reliability of the overall ACC-QOL score was assessed using the interclass correlation coefficient (ICC) for absolute agreement between the overall ACC-QOL scores of the first and second questionnaire in patients who perceived a stable HRQoL. An ICC of >0.7 was considered good. Measurement error of the overall ACC-QOL score was calculated as the standard error of the mean (SEM) for the difference between overall ACC-QOL scores of the first and second questionnaire in patients who perceived a stable HRQoL. Reliability of the individual items was calculated with linear weighted Cohen's Kappa for agreement in the response options between the two assessments of patients who perceived a stable HRQoL

(STATA version 17). Interpretation of the Kappa value was performed according to the definition of Landis and Koch (17). Internal consistency was determined by the Cronbach's alpha of the overall score and the separate scales. A Cronbach's alpha of >0.7 was considered good.

Responsiveness was assessed with the criterion approach using a global rating scale for perceived HRQoL change ("worse", "stable", "better"). The change in overall ACC-QOL score was calculated for each patient who participated in both the first and second questionnaire. The mean change was compared between the categories of improvement by means of an ANOVA analysis and by Spearman's correlation. A Spearman's rho above 0.4 was considered good. A ROC-analysis was used to determine the ability of the change in overall ACC-QOL score to discriminate between patients with deterioration and patients with stable or improved HRQoL. An area under the curve (AUC) above 0.7 was considered good.

To determine the effect of disease stage and treatment options on HRQoL, difference in EORTC QLQ-C30 and ACC-QOL scores between different patient groups were calculated using ANOVA tests.

Results

The first questionnaire was completed by 67 patients, the second by 51 patients. We found no differences between respondents, non-respondents, and excluded patients, except for a high proportion of mitotane users among the respondents (Table 1). The majority of the patients were long-term survivors (>5 years since diagnosis, n = 32) with a median time since diagnosis of 6 years (range 0–38 years). A total of 20 patients had received active treatment within the last 1000 days, including surgery, adjuvant radiotherapy on the tumor bed, mitotane therapy or chemotherapy. The mean age at the time of the questionnaire was 59 years (range 29–83 years).

Psychometric Properties of ACC-QOL

The distribution of the response options for each item in the ACC-QOL is shown in Table S1. Each item had a low number of missing values (0–3.9%). Construct validity was assessed with confirmatory factor analysis using four different models with an increasing number of scales (Table 2). As hypothesized, model 3 with three scales (mitotane side-effects, nine items, hypercortisolism/hydrocortisone effects, four

items, emotional effects, ten items) and three items (physical limitations due to surgery, virilization and need for peer support) had the best fit. However, the CFI was considered moderate (0.667). The items on weight gain and social support had limited correlation with any of the scales and with the overall score (weight gain-overall r 0.08; social support-overall r 0.138) and were therefore deleted from the model. Model 3 was used to calculate the overall score for the ACC-QOL. The construct validity was further confirmed by hypothesis testing. Eight out of ten hypotheses about the relations between the ACC-QOL scales and EORTC QLQ-C30 scales were confirmed, and no correlations above 0.9 were seen (Table 3).

Reliability of the overall ACC-QOL score was good, with an ICC for absolute agreement of 0.860 in the patients who perceived a stable HRQoL. The measurement error of the overall ACC-QOL in stable patients was low (mean difference ±SEM: −1.03 ± 1.27). Reliability of the individual items in the score was fair for two items and moderate to substantial for 24 out of 26 items in patients with perceived stable HRQoL (weighted Cohen's Kappa 0.34–0.86). The overall ACC-QOL score and the three scales had a good internal consistency (Cronbach's alpha overall 0.897, mitotane side-effects 0.763, hypercortisolism/hydrocortisone effects 0.784, emotional effects 0.875).

Table 1. Characteristics of respondents, non-respondents, and excluded patients.

	Respondents ***n*** **= 67**	**Non-Respondents** ***n*** **= 33**	**Excluded Patients** ***n*** **= 30**	***p*-Value**
Age at diagnosis (mean years)	50.9	51.2	48.4	0.75
Female	38	24	22	0.15
ENSAT stage				0.16
I	8	2	1	
II	24	12	5	
III	10	6	5	
IV	6	4	2	
Treatment modality				
Adrenalectomy	59	28	27	0.66
Local surgery	6	4	4	0.81
Additional or metastasis surgery	11	2	4	0.33
Radiotherapy	5	2	0	0.66
Mitotane	28	6	6	0.02
Chemotherapy	5	3	2	0.93

Table 2. Confirmatory factor analysis.

	CFI > 0.95	RMSEA < 0.08	SRMR < 0.08
Model 1: Overall	0.527	0.139	0.124
Model 2: Physical effects + emotional effects + overall	0.613	0.127	0.126
Model 3: Mitotane side-effects + hypercortisolism/hydrocortisone effects + emotional effects + overall	0.667	0.117	0.116
Model 4: Food + neurology + skin + hypercortisolism/hydrocortisone effects + emotional effects + overall	0.511	0.139	0.138

CFI: comparative fit index; RMSEA: root mean square error of approximation (RMSEA); SRMR: standardized root mean square residual.

Table 3. Hypothesis testing of scale structure correlations between ACC-QOL scales and EORTC QLQ-C30 subscales.

ACC-QOL Scale	EORTC QLQ-C30 Subscale	Hypothesis Confirmed If	r	Confirmed?
Overall ACC-QOL	Overall EORTC QLQ-C30	r 0.40–0.90	0.657	Yes
Mitotane-food	Appetite	r 0.40–0.90	0.409	Yes
Mitotane-neurology	Cognitive function	r −0.40–−0.90	−0.523	Yes
Mitotane-skin	None	r < 0.40	<−0.403	No
Hypercortisolism/ hydrocortisone	Emotional functioning	r −0.40–−0.90	−0.462	Yes
Emotional	Emotional functioning	r −0.40–−0.90	−0.613	Yes
Emotional	Social functioning	r −0.40–−0.90	−0.661	Yes
Operation	Physical functioning	r −0.40–−0.90	−0.397	No
Virilization	None	r < 0.40	<0.260	Yes
Peer support	None	r < 0.40	<0.309	Yes

Responsiveness was assessed using a global rating scale for perceived HRQoL change (“worse” n = 11, “stable” n = 29, “better” n = 10, missing n = 1). There was a gradual increase in overall ACC-QOL change score for the categories of improvement (mean change ±SD: “worse” −6.27 ± 6.22, “stable” −1.03 ± 6.82, “better” 5.38 ± 5.45; ANOVA p < 0.001). The correlation between the change scores and the categories of improvement was good (Spearman’s rho 0.548). ROC analysis showed the change in overall ACC-QOL score had a good ability to discriminate

between patients with deterioration (n = 11) and patients with stable or improved HRQoL (n = 39), with an AUC of 0.788.

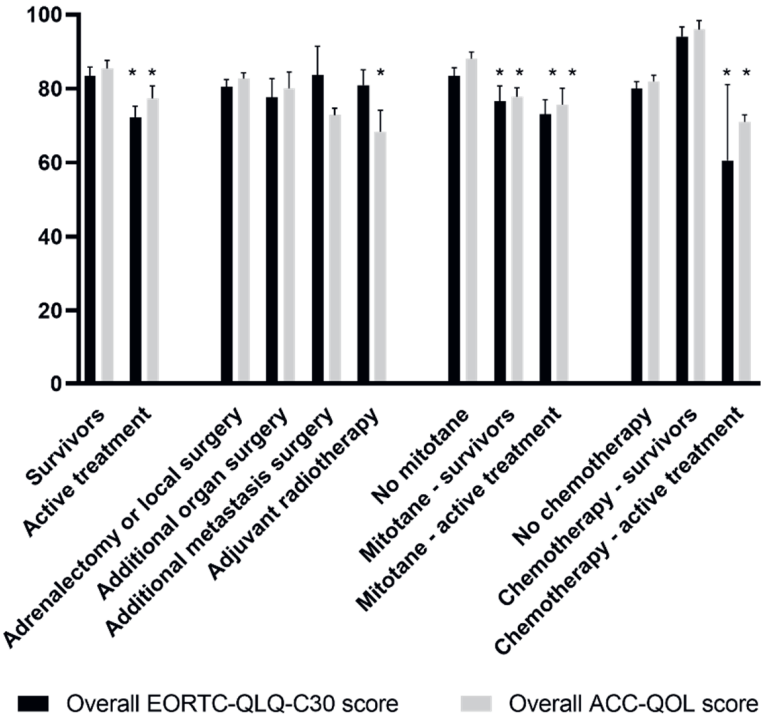
HRQoL Scores in Patient Groups

The mean overall EORTC QLC-C30 score for the total population was 80.5 (Table S2). We found no differences in HRQoL between males and females or between patients with different ENSAT stages at diagnosis. The overall score was significantly lower for patients who underwent active treatment within the last 1000 days, including surgery, adjuvant radiotherapy, mitotane therapy or chemotherapy (mean overall EORTC QLQ-C30 score 72.2) (Figure 1). These patient groups reported a significantly lower HRQoL in several domains, including lower role, emotional and cognitive functioning, and a higher fatigue score on the EORTC QLQ-C30 and higher mitotane and emotional scores on the ACC-QOL.

Patients who underwent additional surgery (e.g., nephrectomy) or metastatic surgery (e.g., liver or lung resection) reported more physical limitations due to surgery on the ACC-QOL than patients who were only treated with an adrenalectomy or local surgery. Patients who were treated with radiotherapy on the primary tumor bed after surgery reported similar physical limitations due to surgery as patients after metastatic surgery. They had the lowest overall ACC-QOL score, with a significantly higher hypercortisolism/hydrocortisone score.

Patients who had received mitotane therapy within the last 1000 days, had significantly lower overall EORTC QLQ-C30 and ACC-QOL scores compared to those never treated with mitotane. These patients indicated problems in many domains, including role, emotional and social functioning and in fatigue, dyspnea, mitotane, emotional and peer support. Patients who had received mitotane therapy at any timepoint during the treatment period, even longer ago, also reported significantly lower scores in these domains compared to those never treated with mitotane. The two patients who had received chemotherapy within the last 1000 days, scored significantly lower on both questionnaires. They had significantly worse scores in physical functioning, fatigue, nausea, constipation on the EORTC QLQ-C30 and the emotional score of the ACC-QOL. The five patients that had received chemotherapy longer ago had similar HRQoL scores to the ACC survivors.

Figure 1. Mean overall EORTC QLQ-C30 and ACC-QOL scores for different patient groups.



Error bars represent standard error of the mean. * $p < 0.05$.

Patients who were diagnosed more than 5 years ago had a relatively good overall EORTC QLQ-C30 score (mean 83.4). However, they scored relatively high on symptom scores fatigue (mean 29.8) and insomnia (mean 23.8) from the EORTC QLQ-C30 and the emotional score (mean 22.6) from the ACC-QOL.

When looking at the individual items from the ACC-QOL, two items had a high percentage of respondents who answered “quite a lot” or “very much”. These items decreased physical capability experienced because of the disease (49.3%), and a negative effect of the disease on sex life (38.8%). Furthermore, the fatigue and emotional scores were worse in all patient groups. This indicates that the majority of ACC patients experience fatigue and emotional problems, which can persist for a long time after diagnosis and the completion of therapy.

Discussion

We presented the first disease-specific HRQoL questionnaire to be used in patients with ACC, the ACC-QOL. This questionnaire can be used alongside the EORTC QLQ-C30 to obtain a complete picture of the HRQoL of a patient with ACC. The proposed version of the ACC-QOL consists of 26 questions and includes 3 scales (mitotane side-effects, hypercortisolism/ hydrocortisone effects and emotional effects) and three single items (physical limitations due to surgery, virilization, need for peer support). The questionnaire has good psychometric properties in terms of face and construct validity, reliability, and responsiveness.

HRQoL assessments are important for both research and clinical care of patients with ACC. In trials, HRQoL is often used as a secondary outcome. A questionnaire should be sensitive to all HRQoL issues that can be caused by the disease and treatment under study. The currently available HRQoL questionnaires did not include effects of mitotane side-effects, hormone production or hydrocortisone substitution and might therefore lack sensitivity to detect all HRQoL issues relevant for patients with ACC. HRQoL questionnaires can also be used to monitor side-effects during treatment, to improve patient–doctor communication, and to aid in treatment decisions (18,19). A disease-specific questionnaire is useful for detecting all issues relevant for the patient. The ACC-QOL is designed to be used alongside the EORTC QLQ-C30 and together the two questionnaires provide a more precise evaluation of HRQoL in patients with ACC, especially during active treatment. It can therefore be used in research setting and patient follow-up, without the added use of a general HRQoL questionnaire to reduce patient burden.

Besides the development and initial validation of the first disease-specific HRQoL questionnaire for patients with ACC, this study is also the first to assess HRQoL in a population-based cohort of ACC patients. We discovered that the overall HRQoL of ACC survivors is good, but slightly lower compared to other cancer survivors in a Dutch reference population (83.4 versus 88.3, respectively) (20). Despite the relatively good overall score, ACC survivors and patients during all treatment modalities score high on fatigue and emotional problems. These patients might benefit from early referral to rehabilitation or psychology services for extra support (21). Patients who had received recent active treatment, mostly mitotane therapy and chemotherapy, experienced additional issues in many HRQoL domains. In the current study we cannot discriminate between complaints due to tumor

progression and actual treatment side-effects. The data, however, indicate that patients with recent active treatment experience many issues that influence their HRQoL. Using a HRQoL questionnaire, and especially a disease-specific questionnaire such as the ACC-QOL, can help identify these issues during treatment and follow-up. This can lead to better patient–doctor communication, treatment guidance and shared decision making (22).

The current study has some potential limitations. The number of patients in this study is low due to the rarity of ACC. Every patient ever diagnosed with ACC in the Netherlands who wase eligible for the study was invited to participate. The number of included patients was sufficient for the planned analyses, according to the methodology of classical test theory (15). The questionnaire developed in this study needs further international validation following the phase IV methodology, in agreement with the EORTC guidelines (5). Another limitation is the potential survivor bias (23). The population included a relatively high number of patients who were free from disease after treatment, which accounts for the high mean overall HRQoL scores. However, one-third of the population had recent treatment and the findings for the total population are comparable to HRQoL found in other cancer survivors (20). In addition, no difference was found in the patient characteristics between respondents, non-respondents and excluded patients, which indicates the results can be extrapolated to the entire Dutch population of patients with ACC.

Conclusion

We generated the first disease-specific questionnaire for assessing HRQoL in patients with ACC with good psychometric properties, to be used in addition to the EORTC QLQ-C30 questionnaire. We found that ACC survivors reported a good HRQoL with residual fatigue and emotional problems. Patients who underwent recent treatment for ACC reported a lower HRQoL and experienced problems in many domains.

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Supplemental materials

Table S1. Distribution of response options per ACC-QOL item.

Questionnaire 1 (N=67 responders)

Item	Missing	Not at all	A little	Quite a bit	Very much
Have you been restricted in your performance due to the extent of surgery?	0%	65.7%	19.4%	10.4%	4.5%
Have food and drink tasted different from usual?	0%	74.6%	16.4%	6.0%	3.0%
Have you had problems eating?	0%	82.1%	14.9%	3.0%	0%
Have you gained weight?	0%	44.8%	32.8%	19.4%	3.0%
Did you have trouble with your coordination?	0%	52.2%	37.3%	6.0%	4.5%
Have you been dizzy?	0%	64.2%	29.9%	4.5%	1.5%
Did you feel slow?	1.5%	52.2%	23.9%	22.4%	0%
Have you had problems speaking?	1.5%	74.6%	16.4%	6.0%	1.5%
Have you had swelling in any part of your body (for example ankles, legs, arms, hands or fingers)?	0%	70.1%	17.9%	7.5%	4.5%
Have you had skin problems (for example itchy, dry, fragile)?	0%	47.8%	34.3%	11.9%	6.0%
Have you had acne/pimpels and/or excess hair (for example on your face or body)?	0%	83.6%	14.9%	1.5%	0%
Have you had problems with wounds healing?	0%	88.1%	7.5%	3.0%	1.5%
Did you worry about picking up an infection?	0%	76.1%	17.9%	4.5%	1.5%
Have you had mood changes/swings?	0%	50.7%	34.3%	14.9%	0%
Have you felt panic?	0%	82.1%	13.4%	4.5%	0%
Have you had palpitations (faster or irregular heartbeat)?	0%	67.2%	22.4%	9.0%	1.5%
Did you feel aggitated?	1.5%	58.2%	31.3%	7.5%	1.5%
Has the disease or treatment affected your sex life (for the worse)?	0%	46.3%	14.9%	25.4%	13.4%
Have you experienced a decrease in your physical capabilities?	0%	23.9%	26.9%	38.8%	10.4%
Have you had problems planning activities in advance (for example meeting friends)?	0%	40.3%	35.8%	14.9%	9.0%
Have you worried about loss of mobility because of your illness?	0%	56.7%	25.4%	13.4%	4.5%
Have you worried about becoming dependent on others?	0%	55.2%	32.8%	9.0%	3.0%
Did you feel uncertain about the future?	0%	46.3%	34.3%	14.9%	4.5%
Have you worried about the results of examinations and tests?	0%	40.3%	44.8%	10.4%	4.5%

Hafe you felt misunderstood (for example by relatives, friends or healthcare professionals)?	0%	56.7%	34.3%	9.0%	0%
Have you felt supported by your family members or friends?	0%	10.4%	16.4%	29.9%	43.3%
Did you feel the need for peer support?	0%	59.7%	20.9%	13.4%	6.0%
Have you felt motivated to continue with your normal hobbies and activities?	0%	13.4%	25.4%	34.3%	26.9%

Questionnaire 2 (N=51 responders)

Item	**Missing**	**Not at all**	**A little**	**Quite a bit**	**Very much**
Have you been restricted in your performance due to the extent of surgery?	0%	64.7%	21.6%	13.7%	0%
Have food and drink tasted different from usual?	0%	70.6%	21.6%	5.9%	2.0%
Have you had problems eating?	0%	68.6%	21.6%	9.8%	0%
Have you gained weight?	2.0%	54.9%	27.5%	15.7%	0%
Did you have trouble with your coordination?	0%	62.7%	27.5%	9.8%	0%
Have you been dizzy?	0%	64.7%	27.5%	7.8%	0%
Did you feel slow?	0%	52.9%	29.4%	15.7%	2.0%
Have you had problems speaking?	0%	68.6%	21.6%	7.8%	2.0%
Have you had swelling in any part of your body (for example ankles, legs, arms, hands or fingers)?	0%	68.6%	23.5%	7.8%	0%
Have you had skin problems (for example itchy, dry, fragile)?	0%	64.7%	25.5%	5.9%	3.9%
Have you had acne/pimpels and/or excess hair (for example on your face or body)?	0%	84.3%	13.7%	2.0%	0%
Have you had problems with wounds healing?	0%	82.4%	15.7%	2.0%	0%
Did you worry about picking up an infection?	0%	76.5%	15.7%	3.9%	3.9%
Have you had mood changes/swings?	0%	62.7%	31.4%	3.9%	2.0%
Have you felt panic?	0%	76.5%	11.8%	9.8%	2.0%
Have you had palpitations (faster or irregular heartbeat)?	0%	70.6%	19.6%	9.8%	0%
Did you feel aggitated?	2.0%	64.7%	23.5%	7.8%	2.0%
Has the disease or treatment affected your sex life (for the worse)?	3.9%	43.1%	17.6%	25.5%	9.8%
Have you experienced a decrease in your physical capabilities?	0%	29.4%	27.5%	29.4%	13.7%
Have you had problems planning activities in advance (for example meeting friends)?	0%	51.0%	27.5%	15.7%	5.9%
Have you worried about loss of mobility because of your illness?	0%	54.9%	23.5%	19.6%	2.0%

Have you worried about becoming dependent on others?	0%	49.0%	33.3%	11.8%	5.9%
Did you feel uncertain about the future?	0%	47.1%	35.3%	9.8%	7.8%
Have you worried about the results of examinations and tests?	0%	47.1%	37.3%	11.8%	3.9%
Hafe you felt misunderstood (for example by relatives, friends or healthcare professionals)?	0%	62.7%	23.5%	11.8%	2.0%
Have you felt supported by your family members or friends?	0%	7.8%	19.6%	27.5%	45.1%
Did you feel the need for peer support?	0%	58.8%	27.5%	11.8%	2.0%
Have you felt motivated to continue with your normal hobbies and activities?	0%	25.5%	17.6%	29.4%	27.5%

Table S2. Group differences in mean EORTC-QLQ-C30 and ACC-QOL scores.

EORTC-QLQ-C30

Group	**N**	**OV**	**GH**	**PF**	**RF**	**EF**	**CF**	**SF**	**FA**
Total	67	80.5	69.7	82.6	70.1	79.2	76.1	72.9	35.3
ACC survivors > 5 years	35	83.4	69.8	83.8	77.1	82.9	81.4 *	77.1	29.8
Active treatment <1000 days	20	72.2 *	67.9	77.7	58.3 *	67.9 *	64.2 *	65.0	50.0 *
Male	29	83.1	72.7	85.1	71.3	78.4	79.3	81.0	33.7
Female	38	78.5	67.3	80.7	69.3	79.8	73.7	66.7	36.5
ENSAT I	8	83.6	65.6	80.0	75.0	87.5	81.3	85.4	26.4
ESNAT II	24	81.5	69.4	85.8	75.0	74.0	76.4	70.1	33.3
ENSAT III	10	77.4	80.0	80.7	63.3	76.7	75.0	75.0	37.8
ENSATIV	9	70.8	59.3	70.4	51.9	71.3	64.8	61.1	56.8
Adrenalectomy or local surgery	67	80.5	69.7	82.6	70.1	79.2	76.1	72.9	35.3
Additional organ surgery	11	77.7	80.3	81.8	57.6	76.5	84.8	75.8	36.4
Metastasis surgery	3	83.7	77.8	84.4	88.9	88.9	66.7	72.2	40.7
Adjuvant radiotherapy	6	80.9	61.1	78.9	72.2	77.8	75.0	55.6	40.7
No mitotane	35	85.3	74.0	87.0	79.0	85.2	79.5	82.9	27.7
Mitotane - survivors	19	76.6 *	62.3	76.8	61.4 *	75.4 *	78.1	62.3 *	40.4 *
Mitotane - recent	13	73.1 *	68.6	79.0	59.0 *	68.6 *	64.1	61.5 *	48.7 *
No chemotherapy	60	80.0	69.2	82.2	68.3	78.6	75.6	71.1	36.7
Chemotherapy - survivors	5	94.1	81.7	97.3	93.3	95.0	90.0	93.3	8.9

Chemotherapy - recent	2	60.6 *	54.2	56.7 *	66.7	58.3	58.3	75.0	61.1 *
Group	**N**	**NV**	**PA**	**DY**	**IN**	**AL**	**CO**	**DI**	**FD**
Total	67	10.4	17.9	18.4	22.4	12.4	8.5	9.5	11.9
ACC survivors > 5 years	35	7.1	19.5	17.1	23.8	8.6	6.7	5.7	7.6
Active treatment <1000 days	20	15.8	20.0	25.0	33.3	23.3	15.0	11.7	20.0
Male	29	5.2	17.2	18.4	16.1	9.2	6.9	8.0	8.0
Female	38	14.5	18.4	18.4	27.2	14.9	9.6	10.5	14.9
ENSAT I	8	4.2	20.8	16.7	33.3	16.7	4.2	0.0	8.3
ESNAT II	24	10.4	6.9	19.4	19.4	6.9	8.3	16.7	5.6
ENSAT III	10	23.3	33.3	20.0	20.0	16.7	3.3	10.0	13.3
ENSAT IV	9	13.0	25.9	25.9	37.0	14.8	18.5	7.4	25.9
Adrenalectomy or local surgery	67	10.4	17.9	18.4	22.4	12.4	8.5	9.5	11.9
Additional organ surgery	11	10.6	22.7	24.2	15.2	21.2	15.2	21.2	15.2
Metastasis surgery	3	5.6	11.1	11.1	11.1	11.1	11.1	11.1	33.3
Adjuvant radiotherapy	6	0.0	22.2	16.7	11.1	5.6	5.6	5.6	27.8
No mitotane	35	7.7	14.8	11.4	23.8	9.5	5.7	3.9	6.7
Mitotane - survivors	19	14.0	25.4	22.8 *	17.5	12.3	10.5	15.8	15.8
Mitotane - recent	13	12.8	15.4	30.8 *	25.6	20.5	12.8	15.4	20.5
No chemotherapy	60	9.7	17.8	18.3	22.8	12.2	8.3	10.0	12.8
Chemotherapy - survivors	5	0.0	10.0	6.7	6.7	13.3	0.0	0.0	0.0
Chemotherapy - recent	2	58.3 *	41.7	50.0	50.0	16.7	33.3 *	16.7	16.7

ACC-QOL

Group	N	OV	ME	CE	EE	O	V	PS
Total	67	82.7	15.2	15.3	27.5	17.9	6.0	21.9
ACC survivors > 5 years	35	85.5	14.1	15.0	22.6*	17.1	2.9	15.2
Active treatment <1000 days	20	77.4*	22.3*	18.5	36.5*	25.0	6.7	26.7
Male	29	85.2	13.9	13.3	24.9	13.8	3.4	19.5
Female	38	80.8	16.2	16.9	29.5	21.1	7.9	23.7
ENSAT I	8	84.3	15.7	14.6	22.5	8.3	8.3	25.0
ENSAT II	24	82.7	11.7	15.6	27.9	13.9	8.3	26.4
ENSAT III	10	81.3	16.1	19.4	30.0	23.3	3.3	20.0
ENSAT IV	9	75.0	23.0	14.8	45.2	29.6	7.4	29.6
Adrenalectomy or local surgery	67	82.7	15.2	15.3	27.5	17.9	6.0	21.9
Additional organ surgery	11	80.1	14.1	9.8	34.8	33.3*	3.0	24.2
Metastasis surgery	3	72.8	19.8	22.2	32.2	44.4	0.0	44.4
Adjuvant radiotherapy	6	68.3*	21.0	29.2*	40.0	44.4*	11.1	44.4
No mitotane	35	88.0	12.3	11.4	17.8	13.3	2.9	14.3
Mitotane - survivors	19	77.8*	13.8	19.7	36.7*	19.3	10.5	33.3*
Mitotane - recent	13	75.6*	24.9*	19.4	40.3*	28.2	7.7	25.6*
No chemotherapy	60	82.0	15.3	16.7	28.3	17.8	6.7	23.3
Chemotherapy - survivors	5	96.0	7.4	3.3	6.7	6.7	0.0	0.0
Chemotherapy - recent	2	71.0*	31.5	4.2	55.0*	50.0	0.0	33.3

*P<0.05. OV: overall, GH: general health; PF: physical functioning; RF: role functioning; EF: emotional functioning; CF: cognitive functioning; SF: social functioning; FA: fatigue; NV: nausea and vomiting; PA: pain; DY: dyspnea; IN: insomnia; AL: appetite loss; CO: constipation; DI: diarrea; FD: financial difficulties. ME: mitotane side-effects. CE: Cushing/hydrocortisone effects. EE: emotional effects. O: operation. V: virilization. PS: peer support.

Chapter 5

EDP-mitotane in children: reassuring evidence of reversible side-effects and neurotoxicity

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Abstract

Adrenocortical carcinoma affects one in 5 million children each year. Since prognosis for children older than 4 years is limited, clinicians often choose aggressive treatment with etoposide, doxorubicin, cisplatin (EDP) and mitotane after resection. However, little is known about the impact of EDP-mitotane in children. We provide an overview of case-reports and case series listing side-effects and neurotoxicity of EDP-mitotane in children. Fourteen studies were identified describing a range of gastro-intestinal, endocrine, developmental and neuropsychological side-effects. Neurotoxicity included motor- and speech delay, decreased concentration and lower school performance. These side-effects appear to be reversible after mitotane discontinuation. We have added our own experience with a 10 year old girl with advanced adrenocortical carcinoma treated with EDP and 2 years of mitotane after irradical resection. She developed an impactful, but reversible, decrease in cognitive development measured by a standardized neuropsychological assessment before, during and after mitotane therapy. This decrease was mostly measurable in terms of decreased processing speed and concentration and a significant drop in school performance. Combined with fatigue and insecurity, this caused problems in short-term memory and the need to change her school type. In conclusion, EDP-mitotane is associated with several side-effects including neurotoxicity in pediatric cases, all reversible after mitotane discontinuation.

Introduction

One in 5 million children is affected by adrenocortical carcinoma (ACC) each year Worldwide (1). The majority is younger than 4 years of age at the time of the diagnosis. Children most often present with signs of virilization (> 80%), and less often with signs of Cushing or Conn syndrome. Abdominal pain is also reported as presenting symptom (2).

The pathogenesis appears to be different in children than in adults. TP53 mutations are more common in children compared to adults, both sporadic and as germline Li-Fraumeni syndrome (3). Mutations in this gene are very common in the South of Brazil resulting in an incidence of 3–4 per million children (4). Other syndromes related to childhood ACC are Beckwith-Wiedemann syndrome (11p15 imprinting), Lynch syndrome (mismatch repair genes), multiple endocrine neoplasia type 1 (MEN1), familial adenomatous polyposis (APC), neurofibromatosis type 1 (NF1) and Carney complex (2p16 and 17q22-24) (5,6). In children without germline mutations, somatic mutations in these chromosomal regions have also been discovered. From these syndromes different pathways have been identified in relation to ACC pathogenesis in both children and adults, such as p53/Rb signaling, insulin-like growth factor, β-catenin pathway and chromatin remodeling (7). Differences between child and adult pathogenesis have been identified in pathways related to development of the adrenal cortex (DAX1/SF1/Sonic hedgehog) (8). The clinical consequences of these differences have not yet been clarified.

In children the prognosis for survival is known to be better than in adults. The 5-year survival of children up to the age of 4 years is 91%, compared to 30% in older children (1, 2, 9). Factors influencing prognosis are age, tumor size, distant metastases, endocrine overproduction and Ki-67 index (1, 9, 10). The majority of children younger than 4 years present with localized (70%) and small tumors (90% <10 cm), which explains their favorable prognosis. However, the prognosis in advanced ACC is unfavorable in children as well. Additional treatment with etoposide, doxorubicin, cisplatin (EDP) and mitotane is therefore advised, even though there is no exclusive evidence that treatment with mitotane or EDP gives a survival advantage in children (2, 9, 11–14).

Mitotane is the cornerstone in treating aggressive forms of ACC in both children and adults. This drug is a metabolite of the insecticide DDT (dichloro-diphenyl-

trichloroethane) and is known for its high toxicity. Monitoring of mitotane plasma levels has been shown to be very important, as kinetic properties are widely variable between patients. The therapeutic lower limit is defined as 14 mg/L, the toxic upper limit is set on 20 mg/L (15–17). The most common side-effects are complaints of nausea and diarrhea. Neurotoxic side-effects can also occur, which often present as an attention deficit, disturbed sense of balance or memory dysfunction (15). The side-effects of EDP are well described in children and include hearing loss, pancytopenia and neutropenic fever. The side-effects of mitotane on the other hand, are mostly based on studies in adults (18). We do know that mitotane is often discontinued due to intolerance in both children and adults [19, 20). Little is known though about the impact of mitotane on a developing child and especially on cognitive development. We therefore aimed to provide an overview of reported side-effects and neurotoxicity of EDP-mitotane in children with ACC. We have added our own experience with a 10 year old girl who developed an impactful, but reversible, delay in cognitive development during mitotane treatment.

Methods

We performed a literature search into case reports and case series describing side-effects and neurotoxicity in children with ACC or Cushing treated with EDP and/or mitotane. We searched Medline for articles published in English until January 2021. We used the following keywords: adrenocortical carcinoma, adrenocortical neoplasm, Cushing syndrome, chemotherapy, mitotane, child or pediatric.

We included case reports and case series that described side-effects, neurotoxicity or cognitive development in pediatric patients with ACC or Cushing syndrome treated with either mitotane and/or EDP or specific components of EDP. Studies were excluded if the participants were older than 18 years of age. The search, exclusion and data extraction procedures were performed by two authors independently, and discrepancies were resolved through discussion. A total of 989 articles were identified by the search. After full-text review, we included 14 studies, summarized in Table 1. We have compared the results from these studies to reported side-effects and neurotoxicity in adults during EDP-mitotane therapy.

We have added our own experience with a 10 year old girl with advanced ACC treated with EDP-mitotane after irradical resection. All data within this case report

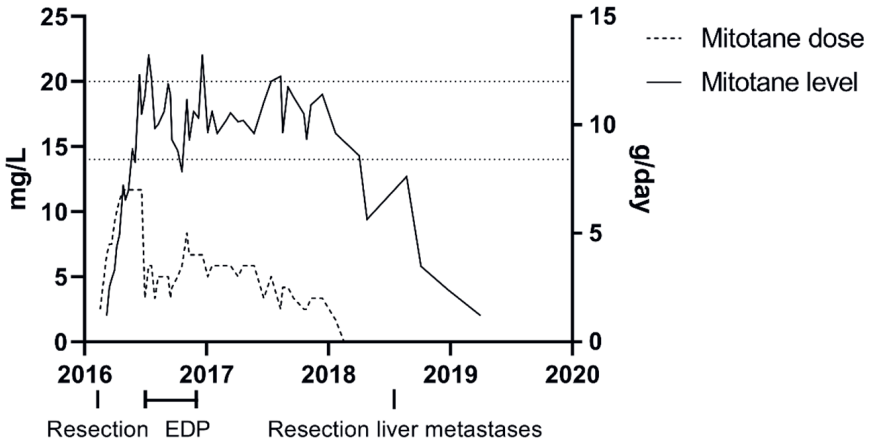
are presented in consultation and with the explicit consent of the patient and her parents. The study has been reviewed by the local Ethical Committee.

Case report

A Caucasian 10-year-old girl presented with abdominal pain, unexplained weight gain, typical cushingoid appearance, severe acne and excessive hair growth on the arms, legs and pubic area (Tanner B1, P3). Imaging showed a 12 cm right sided adrenal tumor with no distant metastases. During surgery, tumor depositions on the peritoneum and around the tumor were seen. Pathology was consistent with ACC, as defined in the Wieneke criteria, with a Ki-67 proliferation index of 10% (21). The resection was determined as irradical with microscopic residues (R1). Genetic testing showed no germline mutations.

She completed 8 cycles of adjuvant EPD and mitotane, with hydrocortisone and fludrocortisone supplementation. In total, she received 2 years of mitotane treatment at adequate plasma levels with an average dose of 3 g per day (Figure 1). Six months after completion of treatment, she developed a liver metastasis of 1.1 cm, which was completely resected through wedge excision. No additional treatment was started afterward. In the three years since, no new lesions have been detected.

Figure 1. Timeline with treatments, mitotane level (mg/L) and mitotane dose (g/day).



EDP etoposide, doxorubicin, cisplatin in eight cycles.

During both EDP and mitotane treatment she experienced nausea, which was controlled with various antiemetics. She developed central hypothyroidism and hypocalcemia, requiring levothyroxine, calcium and vitamin D. She experienced well known side-effects of EDP such as neutropenia, anemia and mouth sores. Emotionally she had mood swings and difficulty taking oral medication.

Her growth slowed during mitotane use from SDS + 1 (148.9 cm) to SDS-1 (158.5 cm). After discontinuation of mitotane she had a slow catch-up growth to SDS − 0.4 (166 cm). Her weight started above average due to Cushing syndrome (45.6 kg, SDS + 2 weight for height). During therapy, she maintained an above average weight for height, which returned to normal after discontinuation of treatment (55.8 kg, SDS + 0). Menarche was present at age 12 during mitotane therapy, after which her menses remained irregular, even after mitotane discontinuation. The adrenal function returned to normal one year after discontinuation of mitotane and all supportive medication were successfully discontinued.

A standardized neuropsychological assessment was performed three times during follow-up: at diagnosis (age 10), during mitotane treatment (age 12) and one year after mitotane discontinuation (age 14). Test scores were age-normalized and categorized into “below average”, “average” and “above average”. An impactful interruption of cognitive development occurred during mitotane therapy. At 12 years, her above average performance at school dropped to below average performance. Socially, it was difficult to keep up with classmates and friends, resulting in a social isolation. She needed to alter the type of high school, which impacted both herself and her family. Compared to the first measurement, she had the same verbal and performance intelligence, however, her test scores were limited by processing speed. Her processing speed and concentration had decreased significantly and fatigue during the test limited her performance. She complained of decreased short-term memory. However, when provided with enough time, no objective memory problems were found and memory was adequate for her school level. It was therefore concluded that her school performance and memory problems were secondary to decreased processing speed and concentration, worsened by fatigue and insecurity.

At age 14, after cessation of treatment, she recovered (nearly) completely, regained a higher school level and socially abilities were restored. Most importantly, her intelligence and short-term memory improved to above average. Her processing speed and concentration had improved, but remained slightly below average. Fatigue and insecurity were no longer an issue. It was concluded that she had shown a significant improvement on multiple cognitive levels.

Review of literature

We identified an additional 14 case reports and case series describing side-effects and neurotoxicity in 94 children with ACC or Cushing treated with EDP and/or mitotane (Table 1). Gastro-intestinal side-effects including nausea, vomiting, anorexia, requirement of a nasopharyngeal tube, diarrhea and abdominal pain were reported in nine studies (20, 22–28). These side-effects were completely reversible after dose reduction or cessation of mitotane.

Mitotane was related to impaired development and endocrine function. Mitotane is known to cause adrenal insufficiency, requiring in most cases hydrocortisone and incidentally fludrocortisone supplementation. Two cases had an acute presentation of adrenal insufficiency and two cases had need for continued hydrocortisone supplementation after mitotane discontinuation (25, 27–29). One case of central hypothyroidism was mentioned (25). Mitotane was reported to have an effect on physical and hormonal development in pediatric patients resulting in growth impairment, increased bone age, gynecomastia, thelarche and central pubertal praecox (23, 25–27, 30, 31). These effects were reversible after dose reduction or cessation of treatment in all but one case (25). In this case, central pubertal praecox continued until one year after mitotane discontinuation for which leuproreline therapy was needed. The effect of endocrine activity of ACC itself also has an important effect on growth and development, especially the negative effects of cortisol production and the effects of sex hormones on pubertal development. However, it is generally observed that hormone levels return to normal after initial resection and adjuvant treatment (28, 29).

Table 1. Studies describing short- and long-term general side-effects and neurotoxicity in children treated with mitotane and/or EDP.

Study	Population	Treatment	General side-effects	Neurotoxicity	Recovery
Case reports					
This report	10–year–old girl Stage IV ACC Cushing and virilization	Resection, EDP–mitotane 3 g/day; 14–20 mg/L 2 years, metastasectomy	Nausea, hypothyroidism, hypocalcemia, fluor vaginalis, irregular menses, growth impairment, fatigue EDP–related: neutropenia, anemia, mouth sores	Mood swings, memory impairment, reduced processing speed and concentration, decreased school performance	5 years disease free, normal school performance, mild concentration issues, irregular menses
Montgomery 1965	3–year–old girl Stage IV ACC Cushing	Mitotane 8g/day	Nausea, vomiting		Good response, died from measles
Becker 1975	3.5–year–old boy Stage III ACC Virilization	Resection, mitotane 1g/day 2.5 years	Gynecomastia, growth impairment, increased bone–age		4 years disease free, growth recovery unknown
De León 2002	2–month–old boy Stage III ACC rapid progression Cushing	Resection, mitotane 2.5 g/day >10 mg/L, re–resection	Gynecomastia, anorexia, growth impairment	Motor and speech delay	Catch–up growth, average intellect, normal speech and motor function, weak organization and attention 15 years
Goto 2008	4–year–old boy Stage I ACC Virilization	Resection, mitotane 5 g/day 13.4–34.6 mg/L 6 months, leuproreline	Anorexia	Encephalopathy, apathy, speech and memory impairment, mild quadriplegia and ataxia	Complete recovery 6 months after mitotane discontinuation, 3 years disease free, normal school performance

Study	Population	Treatment	General side-effects	Neurotoxicity	Recovery
Arai 2008	10–year–old boy Stage IV ACC Virilization	Resection, EDcarboplatin–mitotane 3 g/day, metastasectomy	No severe side–effects		2 years disease free
Rashed 2017	3–year–old boy ALL + Stage II ACC rapid progression	Resection, re–resection, EDP–mitotane 4 g/day, metastasectomy ALL: 6–MCP/MTX	Arachnoid cyst with hydrocephalus	Speech delay	Recovery of speech after mitotane discontinuation, died from progression of ACC
Oddie 2018	4–year–old girl Stage III ACC Virilization	Resection, EDP–mitotane 2.5 g/day	Gastro–intestinal, electrolyte abnormalities, febrile neutropenia, skin pigmentation, central pubertal praecox (gynecomastia, increased bone–age), central hypothyroidism		After 1 year continued hydrocortisone dependence, continued central pubertal praecox after mitotane discontinuation needing leuproreline
Takeuchi 2018	4.5–year–old boy Stage III ACC Gynecomastia	Resection, EDP–mitotane 3 months presurgery GPOH–MET97–mitotane 6 months post surgery	Unknown		After 2 years complete recovery of growth and gynecomastia, continued hydrocortisone/fludrocortisone dependence

Study	Population	Treatment	General side-effects	Neurotoxicity	Recovery
Case series					
Ribeiro 2000	54 children Median age 3 years Stage I–IV ACC	Resection, mitotane, EDP–mitotane	Nausea, vomiting, diarrhea, abdominal pain, gynecomastia/ thelarche	Somnolence, lethargy, ataxic gait, depression, vertigo	After 18 years: 24 died, 30 disease free
Wajchenberg 2000	22 children Age 0–18 years Stage I–IV ACC 22 virilization, 9 Cushing	Resection, 3 mitotane	Nausea, vomiting	Myalgia	After 5 years: 3/3 on mitotane died 19/22 free from disease
Zancanella 2006	11 children Age 2.5–15 years Stage III–IV 11 virilization, 3 Cushing	11 EDP–mitotane 1.8–5.3 g/day 12–29.8 mg/L, 7 resection, 5 metastasectomy	Nausea, vomiting, 2/11 requiring nasogastric tube, diarrhea, abdominal pain, gynecomastia, 1/11 died from untreated acute adrenal insufficiency EDP–related: pancytopenia (WHO1–4), hearing problems (WHO1), 1/11 renal clearance and liver function decrease (WHO1)	Speech delay, lethargy, ataxia, vertigo, 1/11 hypertensive encephalopathy	All side–effects were reversible between 1–3 weeks after mitotane discontinuation or dose reduction After 7.5 years: 1/11 free from disease; 2/11 progression; 8/11 died from progression
Kostiainen 2019	4 children Age 4 months – 5 years Stage I–II 1 Cushing, 2 virilization	EP	1/4 Central pubertal preacox requiring GnRH analogue		Between 1.5 and 12 years: disease free, normal growth and development

Study	Population	Treatment	General side-effects	Neurotoxicity	Recovery
Zekri 2020	18 children Median age 4 years Stage I–IV ACC 16 hormone production	14 resection (2 presurgery EDP), 8 EDP–mitotane 1–2 g/day 4g not tolerated	4/8 Nausea, vomiting, abdominal pain 1/8 Died from severe myelosuppression, cardiac toxicity, chest infection and sepsis	1/8 Speech delay	Recovery of speech after mitotane discontinuation 8/8 stage III–IV died from progression 10/10 stage I–II remain disease free
Motte 2018	6 children Mean age 12 years Juvenile Cushing disease	Mitotane >6 months >10mg/L	6/6 Nausea, vomiting 2/6 Adrenal insufficiency (1/6 acute 27 mg/L) 1/6 Acute severe hepatitis (36 mg/L)	4/6 Asthenia 2/6 Decreased school performance	Recovery after dose reduction or discontinuation

GPOH-MET97 study (13). ACC adrenocortical carcinoma, ALL acute lymphoid lymphoma, EDP etoposide, doxorubicin, cisplatin; EP etoposide, cisplatin, 6MCP/MTX 6-mercaptopurine/methotrexate, WHO World Health Organization classification for adverse events

We found seven case reports and case series in children describing neurotoxicity of mitotane, with or without EDP. A case report from 2002 described a 2-month-old boy, who developed a motor and speech delay while treated with mitotane (levels > 10 mg/L). The neurological damage was reversible in this boy, with a normal intellect after 15 years, with only a slight weakness in organization and concentration (23). Reversible speech delay was also found in four other reports (20, 24, 27, 32). Two of these report side-effects with mitotane levels between 12 and 34.55 mg/L, the other two unfortunately only provided the mitotane dosage (1–2 and 4 g/day). In the case of Rashed et al., further diagnostic efforts into the speech delay let to the discovery of an arachnoid cyst with early hydrocephalic changes and no evidence for cerebral metastasis. The speech delay however, was more likely related to the mitotane, since improvement occurred after mitotane discontinuation (32). Additionally reported neurotoxic side-effects were somnolence, lethargy, ataxia, asthenia, myalgia, vertigo and depression (12, 26–

28). Reported mitotane levels ranged from 10 to 29.8 mg/L. These effects were all reversible after mitotane dose reduction or discontinuation.

Two incidences of encephalopathy were described, both at toxic levels of mitotane (29.8 and 34.55 mg/L) (24, 27). One of these cases presented with apathy, speech delay, memory disturbance, ataxia and mild quadriplegia. This patient fully recovered 6 months after mitotane discontinuation and had normal school performance three years after diagnosis (24). The second patient developed high blood pressure followed by encephalopathy. This resolved after mitotane was temporarily discontinued. The patient made a complete recovery and resumed mitotane at a lower daily dosage (27).

Cognitive side-effects have also been reported at lower doses of mitotane. A study into the effect of mitotane as a treatment for juvenile Cushing Disease, found reduced school performance in two of the six patients. Both patients have fully recovered (28). All of these case reports and case series suggest that neurotoxicity of mitotane is reversible in pediatric cases.

In addition to mitotane, EDP chemotherapy is also commonly applied in pediatric ACC. The side-effects of EDP are well described and include hearing loss, pancytopenia and neutropenic fever. Neuro-cognitive side-effects as a result of EPD have not been described for ACC or other pediatric solid tumor patients treated with similar combinations of chemotherapy (23, 26, 31).

Comparison to side-effects and neurotoxicity in adults

In adults, similar side-effects and neurotoxicity have been described during EDP-mitotane, but differences exist (18, 33). The most common side-effects of mitotane in adults are gastro-intestinal effects such as nausea, vomiting, diarrhea, constipation, flatulence, different taste and anorexia, similar to children. We have found two pediatric case of liver toxicity of mitotane, while in adults, this side-effect has been described more often (27, 28). Endocrinological effects are also reported in both children and adults, such as adrenal insufficiency, hypogonadism, gynecomastia, irregular menses, ovary cysts and hypothyroidism (34). While in children increased bone age has been described in two cases, in adults decreased bone mass has been reported (35). However, this phenomenon in adults is mostly linked to cortisol excess, not to EDP-mitotane. Some known side-effects of

mitotane have not (yet) been described in children, including skin rash and a high cholesterol. Side-effects related to EDP are comparable between adults and children, and include pancytopenia, hair loss, hearing loss, renal and liver toxicity (36).

Neurotoxicity of EDP-mitotane in adults includes somnolence, lethargy, ataxia, paresthesia, peripheral neuropathy, myasthenia, speech impairment (aphasia and dysarthria). Decreased coordination, vertigo and dizziness are also common. Cognitive impairment in adults usually presents with confusion, decreased concentration and memory impairment. Psychological effects have been reported with mood swings, panic attacks, burn-out and even clinical depression (37). These neurotoxic effects appear to be partly or completely reversible after cessation of mitotane, same as in children (15, 38, 39). In adults, the occurrence of neurotoxic and cognitive side-effects of mitotane, are described to be correlated to toxic plasma levels of > 20 mg/L (16). Especially encephalopathy has been described only in children and adults with very high mitotane plasma levels (> 30 mg/l) (24, 27, 40). Remarkably, our patient and most patients in the case reports had no plasma levels above the toxic limit and still had a significant impact on cognitive development during treatment. This warrants specific monitoring of cognitive development in children during treatment, even when plasma levels are within limits. However, there is insufficient data to justify a specific therapeutic target level for children based on the current review.

Conclusion

EPD-mitotane therapy in children is associated with a range of side-effects including gastro-intestinal, developmental, endocrine and neuropsychological. Especially the gastro-intestinal and neuro-cognitive side-effects are attributable to mitotane and appear to be reversible after mitotane discontinuation. We have added our experience with a 10 year old girl who developed an impactful but reversible delay in cognitive development, measured with a standardized neuropsychological assessment multiple times during EDP-mitotane treatment. This case and the reports in the literature describe a similar course with sometimes severe, but reversible effects of EDP-mitotane, even in young children before the completion of normal development. This should provide reassurance to physicians, parents and patients in case EDP-mitotane treatment is indicated.

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Part II

Quality of care in adrenocortical carcinoma

Chapter 6

Time trends in incidence, treatment and survival of patients with adrenocortical carcinoma, a nationwide study

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Abstract

Purpose: We aimed to determine time trends in incidence, treatment and survival of patients with adrenocortical carcinoma in the Netherlands.

Methods: All 685 adult patients with adrenocortical carcinoma diagnosed between 1993 and 2020 in the Netherlands were included, using the nation-wide prospective Netherlands Cancer Registry.

Results: The median age-adjusted incidence rate based on the European Standard Population was 1.62 per million person-years [0.83-2.11] and was stable over time. We saw a gradual increase in stage III on diagnosis (13 to 25%) with a stable proportion of stage IV (40%). The five-year survival remained stable over time for stage I-II at 65% and stage III at 35%, while the survival for stage IV increased from 3% in 1993-1996 to 11% in 2017-2020. Since the Dutch Adrenal Network was founded in 2004, more patients were referred to an expert center ($p<0.001$), which was associated with increased survival (adjusted HR 0.70, 95%CI[0.57-0.85]). Multivariate cox-regression showed increased survival in all stages when treated with adrenalectomy (adjusted HR 0.53, 95%CI[0.43-0.65]) and mitotane therapy (adjusted HR 0.73, 95%CI[0.55-0.98]). In stage IV disease, adrenalectomy, surgical control of disease, chemotherapy and mitotane therapy were associated with increased survival. However, only 58% of mitotane users reached a therapeutic drug level and 59.5% discontinued treatment prematurely.

Conclusions: The incidence of adrenocortical carcinoma is stable over time. The five-year survival for stage I-III remained stable, while the survival for stage IV increased. Factors associated with increased survival are centralization of care, adrenalectomy, surgical control of disease, chemotherapy and mitotane therapy.

Introduction

Adrenocortical carcinoma (ACC) is a cancer originating from the adrenal cortex. Since the adrenal cortex produces cortisol, aldosterone and adrenal androgens, a subset of patients with ACC presents with hormonal overproduction such as Cushing syndrome, hyperaldosteronism, virilization or feminization (1,2). Patients with non-functional ACC can present with symptoms of tumor mass effect or complaints caused by metastases. A proportion of patients is found incidentally with abdominal imaging for another indication. Treatment modalities for ACC include surgery, mitotane therapy, chemotherapy and radiotherapy (1,2). Even with these treatment modalities and the introduction of new anti-cancer treatments in recent years, prognosis is still poor, especially for patients diagnosed with metastatic disease (3).

ACC is a rare disease, which makes reliable epidemiological studies on incidence, survival and effects of treatment more difficult. The nation-wide prospective Netherlands Cancer Registry (NCR) includes information on every patient in the Netherlands ever diagnosed with ACC since 1989. This provides unique real-life data on this rare disease. A previous study using this registry determined that the incidence of ACC in the Netherlands varied between 0.8 and 2.0 between 1993 and 2010 (4). Our aim is to determine whether the incidence and survival of ACC changed over time, with an update until 2020. We further aimed to determine the current use of treatment strategies and their effect on survival in this real-life population-based cohort using newly added disease specific data to the registry from 2014 onwards. Lastly, it is recommended to treat patients with ACC in an expert center (1,2). In 2004 the Dutch Adrenal Network (DAN) was founded to unite all clinical and research expert centers on adrenal diseases in the Netherlands. We aimed to determine whether this had an effect on centralization of care and more importantly on survival.

Methods

The NCR is a prospective registry with data on all cancer patients diagnosed in the Netherlands from 1989 onward. All pathology diagnoses of malignancy are recorded in a national pathology database. Diagnoses without pathology confirmation are collected through hospital declaration data. Survival is documented through national death records. Treatment and referral hospital are collected from the hospital declaration data. Additional data is collected by trained

data collectors directly from the hospital records. Use of the data for this specific study was approved by the NCR board of directors and the NCR adrenal scientific committee consisting of Dutch experts on ACC.

From 1989 to 2013 the NCR includes data on extend of disease (EoD), treatment modality (including surgery, chemotherapy, radiotherapy, immunotherapy) and hospital for patients with ACC. Together with experts on ACC within DAN, we determined which new items specific to ACC were helpful to record from 2014 onwards. These items are: ENSAT stage, ASA status (1-5), ALAT (U/L), creatinine (umol/L), hormone production (cortisol, adrenal androgens, estrogens, aldosterone from medical records or laboratory measurements), 24-hour urine cortisol (elevated or normal compared to local laboratory normal range), dexamethasone suppression test (not-suppressed or suppressed compared to local laboratory normal range), ENSAT stage (I-IV), surgical approach to adrenalectomy (open or laparoscopic), resection status (complete or not complete as stated in pathology and surgery reports), vena cava infiltration (from pathology, surgery and radiology reports), Weiss score (0-9 from pathology reports), Ki-67 index (% from pathology reports), mitotic index (number of mitosis per high power field or mm2 from pathology reports, converted to mitosis per 50 high power fields), maximum mitotane dose (mg/day), maximum mitotane level (mg/L), mitotane discontinuation or adjustment other than for plasma levels (from medical records), reason for mitotane discontinuation or adjustment (from medical records) and imaging modality (from radiology reports).

Before 2014, only EoD stage localized disease in the tissue of origin was registered, without information on tumor size. Therefore, before 2014 no distinction can be made between ENSAT stage I localized disease less than 5 centimeters and ENSAT stage II localized disease more than 5 centimeters. Therefore, in analysis using all data between 1989 and 2020, we used ENSAT stages I and II combined. There was no difference between the EoD and ENSAT system in the registration of stage III locally advanced disease and IV metastatic disease. Additional surgery was defined as resection of tumor growth in surrounding organs during adrenalectomy, resection of local recurrence or additional lymph node resection other than routine locoregional lymphadenectomy during adrenalectomy. Treatment in an expert center was defined as one or more treatments performed in one of the DAN hospitals. These hospitals did not change over the course of the study.

Statistical analysis was done using STATA version 17 and SPSS version 29. Incidence was calculated as age-standardized incidence per million person-years using the European Reference Population on the 1993 to 2020 data for all patients aged 18 and over upon diagnosis. Childhood ACC in this population was described in a previous project and not included in the current analysis (5). Data between 1989 and 1992 was discarded for this analysis because only a few cases were registered in those years. Evaluation of the trend in incidence was performed by calculating the estimated annual percentage of change (EAPC). Survival was determined using Kaplan Meier curves and life tables on the 1993 to 2020 data. Time trends in ENSAT stage, one-year survival and five-year survival were calculated by comparing means of 8 predetermined time periods (1993-1996, 1997-2000, 2001-2004, 2005-2008, 2009-2012, 2013-2016, 2017-2020).

Multivariate cox regression was used to determine adjusted hazard ratios (HR) on the 1989 to 2020 data. The proportional hazard assumption was met and treatment covariates were not time-dependent. Presented adjusted HR for all stages were adjusted for age, sex, ENSAT stage (I-II, III, IV), adrenalectomy, additional surgery, mitotane therapy, adjuvant radiotherapy and referral center. Presented adjusted HR for stage IV disease were adjusted for age, sex, adrenalectomy, additional surgery, metastasis surgery, mitotane therapy, chemotherapy and radiotherapy. Ki-67, resection status and hormone overproduction are also known determinants of survival. These data however, were only available from 2014 to 2019 and had missing data due to poor hospital record keeping (3). These parameters were therefore not used in the adjusted HR since there was insufficient data for a robust survival analyzes.

The current situation in adrenocortical carcinoma treatment in the Netherlands was analyzed using descriptive statistics, chi-square, ANOVA analysis and T-tests on the 2014 to 2019 data. This time-period was chosen because it contained the complete detailed data on the new ACC-specific items. At the time of data-analyses, the detailed data of 2020 had not been recorded yet.

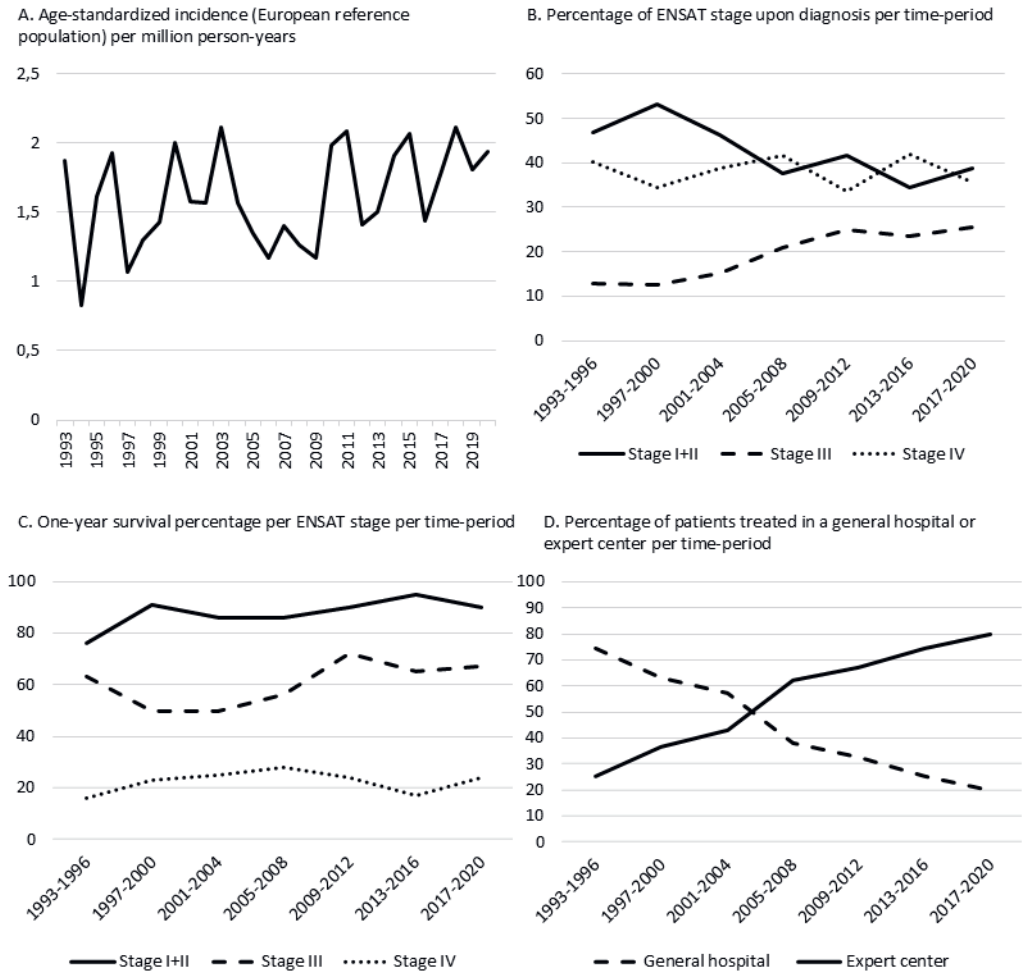
Results

Time trends in ACC

All 685 adult patients with ACC diagnosed between 1993 and 2020 in the Netherlands were included in the analyses of time trends in ACC. Median age at

diagnosis was 58 years (range 18-89 years) and 56.8% was female. The age-adjusted incidence rate based on the European Standard Population varied between 0.83 and 2.11 per million person-years with a median of 1.62 per million person-years (Figure 1A). This incidence was stable over time (EAPC -1.52%, 95% CI[-16.7%-13.4%]).

Figure 1. Time trends in adrenocortical carcinoma between 1993 and 2020.



We saw a gradual increase in the proportion of ENSAT stage III upon diagnosis (13% to 25%), with a similar decrease in stage I-II and a stable proportion of stage IV (40%) (p for trend 0.001) (Figure 1B). Even with this change in ENSAT stage upon diagnosis, the survival for stage I-II and III remained stable over time (one-year

survival stage I-II 90%, stage III 67%; five-year survival stage I-II 65%, stage III 35%; p for trend 0.43) (Figure 1C). However, while the one-year survival for stage IV remained stable at 24% (p of trend 0.77), the five-year survival increased from 3% to 11% in the last time-period from 2017 to 2020 (p for trend 0.01).

Since the founding of the Dutch Adrenal Network in 2004, more patients were referred to an expert center (p<0.001) (Figure 1D). Between 2014 and 2019, 85% of adrenalectomies and 95% of medical treatments were performed in an expert center. Treatment in an expert center was associated with a survival benefit for the total population (adjusted HR 0.70, 95%CI[0.57-0.85]) (Figure 2A).

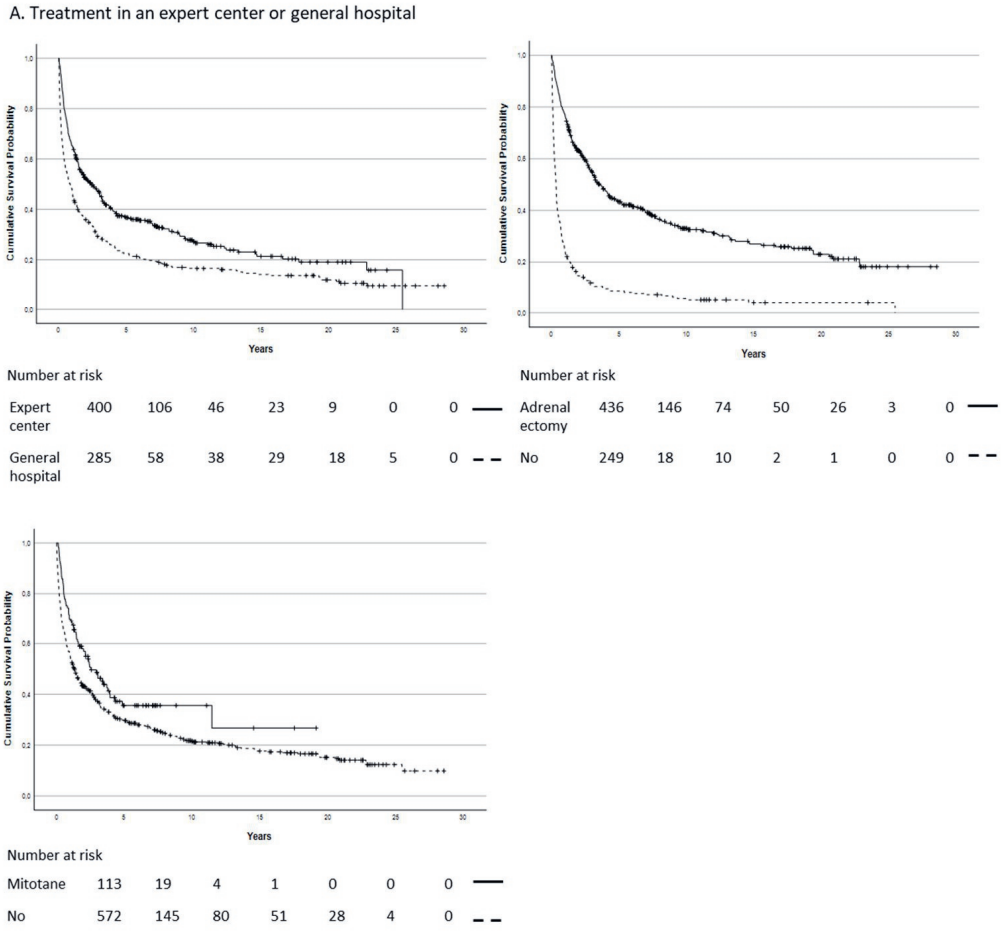
Current situation

Localized and locally advanced disease, stage I-III

Between 2014 and 2019, 89 patients were diagnosed with localized and locally advanced ACC stage I-III. Of the patients diagnosed in this period, 6.6% had ENSAT stage I, 33.8% stage II and 25.0% stage III disease upon diagnosis. The diagnostic details such as hormone production, pathology data and performance status are shown in Table 1. Adrenalectomy was performed in 85 patients. The majority of adrenalectomies was performed by open resection (91.6%), with the exception of stage I disease (77.8% laparoscopic surgery). Complete resection was accomplished in 86.1% of adrenalectomies for stage I-II disease and only 46.4% for stage III disease. Surgery for ACC was performed in DAN centers 85.1% of the time. There was no difference in resection status between DAN centers and non-DAN centers (p-value 0.363). Additional surgical resection in stage III disease included additional lymph node resection other than lymph node resection as part of the adrenalectomy (n=5) and surrounding organ resection (n=34, various combinations of diaphragm, kidney, liver, gallbladder, pancreas, stomach, spleen, colon, omentum, peritoneum resection and unspecified abdominal surgery). Adjuvant radiotherapy was administered to 6 patients with ENSAT stage II or III. Multivariate cox-regression showed a survival benefit for adrenalectomy (adjusted HR 0.53, 95%CI[0.43-0.65]) in all stages (Figure 2B). This survival benefit was not found for adjuvant radiotherapy (adjusted HR 0.94, 95%CI[0.61-1.46]). The survival benefit from additional surgery did not reach significance (adjusted HR 0.67, 95%CI[0.42-1.07]).

Adjuvant mitotane was given to 17 patients with stage II and 18 patients with stage III disease. One patient with stage I was prescribed mitotane due to a Ki-67 index of 20% and later proved to have rapidly progressive disease. Multivariate cox-regression showed a survival benefit from adjuvant mitotane therapy (adjusted HR 0.73, 95%CI[0.55-0.98]) in all stages (Figure 2C).

Figure 2. Kaplan Meier curve in all patients between 1993 and 2020.



Metastasized disease, stage IV

Between 2014 and 2019, 47 patients had stage IV disease upon diagnosis (34.6%). Of these patients, 24 received an open adrenalectomy and one a laparoscopic adrenalectomy. Indications for surgery such as debulking or hormone production were not collected. Of the 21 adrenalectomies with complete data, 13 resulted in

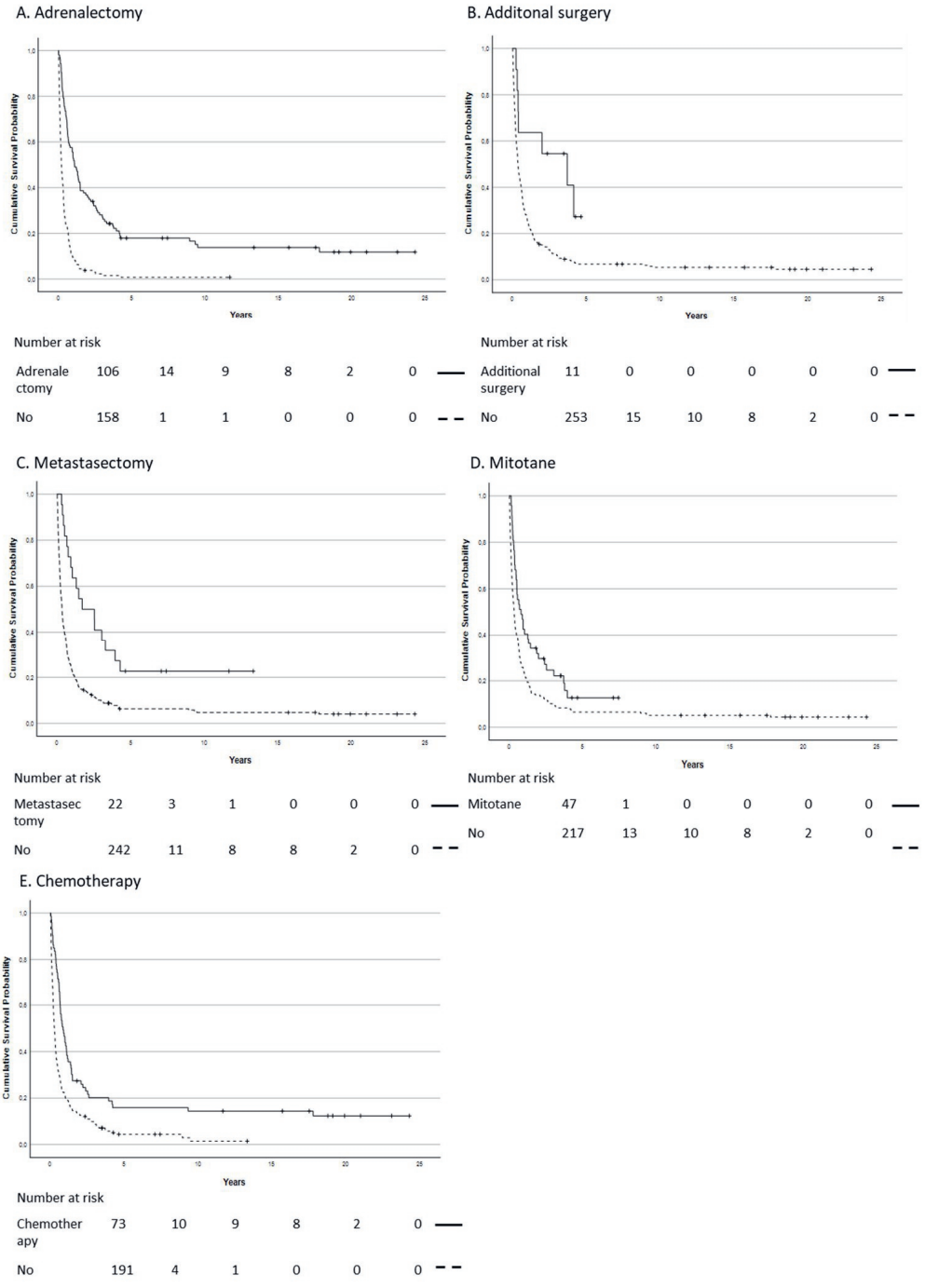
complete resection of the primary tumor (61.9%). Metastasis surgery included 5 lung (VATS or wig-resection), 7 liver (segmental or hemi-hepatectomy), 1 omentum, 2 peritoneum and 4 resections at an unknown location. Of the 13 metastasectomies with complete data, only one was registered as a complete resection of the metastasis (7.7%). For liver metastases, local therapies were used in 6 patients, including 3 microwave ablations, 1 radiofrequency ablation and 2 transarterial radio-embolisations. Multivariate cox-regression showed a survival benefit from adrenalectomy, additional surgery and metastasis surgery in stage IV disease (adjusted HR 0.39, 95%CI[0.29-0.54]; 0.40, 95%CI[0.18-0.91]; 0.37, 95%CI[0.22-0.63], respectively) (Figure 3A-C). No survival benefit has yet been found for local therapies because of the low number of patients.

Palliative mitotane was prescribed 65 times (2 times for irresectable stage III disease, 63 times for stage IV disease). Mitotane was mostly prescribed in DAN centers (96.0%) and only 4 times in a non-DAN center. Multivariate cox-regression showed a survival benefit from palliative mitotane therapy in stage IV disease (adjusted HR 0.69, 95%CI[0.47-0.99]) (Figure 3D).

In total, 26 patients received chemotherapy for stage IV disease. The FIRMACT scheme with etoposide-doxorubicin-cisplatin (EDP) with or without mitotane was prescribed in 18 patients (6). Other regimes were less common in the population (2 etoposide/carboplatin, 1 streptozocin, 2 temozolomide, 3 unknown). Only one patient received chemotherapy in a non-DAN center, however, this center is known to have ongoing online consultations with one of the DAN centers. Multivariate cox-regression showed a survival benefit from chemotherapy in stage IV disease (adjusted HR 0.64, 95%CI[0.45-0.91]) (Figure 3E).

Immunotherapy was prescribed in 5 patients (2 atezolizumab, 1 olaparib, 1 pembrolizumab, 1 unknown). In the Netherlands, immunotherapy for ACC is only available in clinical trials. Because the largest trial within the study period was in a non-DAN center, 4 of the patients were treated with immunotherapy outside a DAN center (7). No survival benefit has yet been found for immunotherapy because of the low number of patients.

Figure 3. Kaplan Meier curves in stage IV patients between 1993 and 2020.



Palliative radiotherapy was administered 14 times: 7 bone, 2 lung, 1 liver, 3 metastasis unknown, 1 internal radiation with Lutetium therapy. Radiotherapy, both adjuvant and palliative, was performed in a DAN center 12 times (60.0%) and 8 times in a non-DAN center. No survival benefit was found for radiotherapy in stage IV disease (adjusted HR 0.70, 95%CI[0.44-1.12]).

There were 21 patients with stage IV disease who did not receive any treatment. The reasons were high tumor load or progression (n=8), poor physical condition (n=3), dead before start of treatment (n=2), patient preference (n=1) or unknown (n=7). A third of these patients was never referred to a DAN center.

Mitotane therapy in practice

As demonstrated in the previous sections, both adjuvant and palliative mitotane treatment is associated with a survival benefit. In real-life however, mitotane therapy is often complicated due to difficult dosing and its (severe) side-effect profile resulting in impaired therapy adherence. Of all mitotane users between 2014 and 2019, both palliative and adjuvant, only 58% reached mitotane levels above 14 mg/L (range of maximum level reached: 1-36.9 mg/L) with a mean maximum dose of 6672 mg/day (range of maximum dosage used: 500-9000 mg/day). Of the 96 counts of mitotane treatment with known follow-up, 22% was discontinued before the predetermined treatment period (18% in adjuvant use and 24% in palliative use). In another 37.5% the dose was reduced or temporary discontinued, often with resumption at a lower dose, for reasons other than plasma level correction. Progression of disease accounted for 21% of adjustments and discontinuations (12% in adjuvant use and 27% in palliative use). However, 61% of adjustments and discontinuations was attributed to toxicity, including gastro-intestinal complaints, liver toxicity, skin problems, thrombotic events, infections, hematological, cardiac or renal toxicity, hypertension and general physical decline. In 8 patients, significant elevations in ALAT levels during or after mitotane therapy were registered. In 4 of the 8 patients, the ALAT levels normalized after mitotane discontinuation or dose reduction. The remaining 18% discontinued for unknown reasons. Notably, discontinuation was not associated with maximum dose (p-value 0.61) or maximum plasma levels (p-value 0.87).

Table 1. Detailed characteristics of the patients diagnosed between 2014 and 2019.

	N	%
Sex	136	
Female	86	63.2
Male	50	36.8
Primary tumor	136	
Right adrenal	75	55.1
Left adrenal	61	44.9
Hormone production	109	
Non-functional	37	34.0
Cortisol	24	22.0
Androgens	17	15.6
Cortisol + androgens	12	11.0
Estrogens	3	2.8
Aldosterone	1	0.9
Unknown	15	13.8
Cortisol 24-hour urine	108	
Elevated	38	35.2
Normal	32	29.6
Unknown	38	35.2
Dexamethasone suppression test	108	
Not-suppressed	16	14.8
Suppressed	15	13.9
Unknown	77	71.3
Pathology	108	
Weiss 2-3	9	8.3
Weiss >4	50	46.3
Weiss unknown	49	45.4
Vena cava invasion	110	
Yes	14	12.7
No	96	87.3
Ki-67	75	
≥10%	52	69.3
<10%	23	30.7
Mitosis per 50/HPF	84	
≥5	78	92.9
<5	6	7.1

Imaging	109	
CT-abdomen	78	71.6
CT-thorax	63	57.8
CT not otherwise specified	10	9.2
PET	37	33.9
MRI	14	12.8
Ultrasound	2	1.8
Unknown	10	9.2
ASA score	108	
No disease and medication, perfect health	5	4.6
Minor disease or medication, no influence on health	44	40.7
Major disease or medication, minor influence on health	25	23.1
Major disease or medication, major influence on health	0	0
Major disease or medication, dead expected <24 hours	0	0
Unknown	34	31.5
Creatinine (mean, range)	103	75.3 (40-200)
ALAT (mean, range)	90	47 (8-390)

Description of the new ACC-specific variables added to the registry after 2014. Data includes the 136 patients diagnosed with ACC between 2014 and 2019.

Discussion

This unique nation-wide registry study on ACC provides longitudinal real-life data on the incidence, survival over time according to tumor stage and the effects of various treatment modalities on survival. Every patient in the Netherlands diagnosed between 1993 and 2020 was included in this study, which provides the true incidence of 1.62 per million person-years for adult ACC. This incidence was stable over time. While the proportion of the different ENSAT stages upon diagnosis was generally in line with other larger ACC cohort, it was however not stable over time (8,9). We hypothesized that the percentage of stage I and II disease might increase at the expense of more advanced stages due the increase in use of abdominal imaging resulting in a higher rate of ACC presenting as incidentaloma. On the contrary, we found that the proportion of stage III disease increased from 13% to 25% at the expense of stage I-II disease. This finding is also different from the study of Daher et al., who found an increase in stage IV disease upon diagnosis between 1998 and 2019 (10). However, this was based on a single-center experience, therefore referral bias might have been present. Our study is a nation-wide registry including all diagnosed patients in the Netherlands in this period and

is therefore not influenced by referral bias. However, we have no data on whether the increase in stage III is caused by a recategorization of stage I-II due to better imaging techniques to correctly identify lymph node and adjacent organ involvement.

The current guidelines recommend adrenalectomy of stage I-III disease (1,2). The ADIUVO trial concluded that for low-grade localized disease adjuvant mitotane therapy does not increase 5-year recurrence-free and 5-year overall survival (11). For high-grade or locally advanced disease, when a high risk of recurrence exists, the guideline does recommend adjuvant mitotane therapy after adrenalectomy. The data in this study confirm that adrenalectomy and mitotane therapy are associated with increased survival in all stages of disease (adjusted HR 0.53, 95%CI[0.43-0.65], adjusted HR 0.73, 95%CI[0.55-0.98], respectively). These practices did not change over time, potentially explaining why survival for stage I-III disease remained stable over time with a one-year survival of 90% at stage I-II and 67% at stage III. There is however still room for improvement. The current ADIUVO-2 trial proposes the use of adjuvant etoposide-cisplatin (EP) chemotherapy in addition to adjuvant mitotane to increase the survival of patients with high risk of recurrence (NCT03583710). The results of this trial however, are not expected for another couple of years.

Another potential for improvement is the actual use of mitotane. As in our clinical experience, the ACC population data reflect the real-life problems with mitotane therapy adherence due to drug toxicity. The data showed that only 58% of patients ever reached therapeutic mitotane levels above the recommended 14mg/L and only two in every five patients completed mitotane therapy for the predetermined period (12). In 61% of patients, the reason for discontinuation or dose reduction was toxicity. A recent study on health-related quality of life in the Dutch ACC population found that patients experience a lower quality of life with mitotane therapy, which persists even after discontinuation (13). Careful monitoring and treatment of side-effects, for example by means of patient reported outcome measures, might help improve health-related quality of life, prevent early discontinuation and thereby improve survival.

While the one-year survival of stage IV disease also remained stable over time at 24%, the five-year survival increased from 3% to 11% in the most recent time

period. This is concurrent with the study of Daher, et al., who also found an increased survival for stage IV disease between 1998 and 2019 (10). For stage IV disease, the current guidelines recommend either chemotherapy in the FIRMACT scheme (etoposide, doxorubicin, cisplatin, mitotane) or local therapy with or without mitotane therapy (1,2). In our population, both chemotherapy and palliative mitotane were associated with increased survival in stage IV disease (adjusted HR 0.64, 95%CI[0.45-0.91], adjusted HR 0.69, 95%CI[0.47-0.99], respectively). The data on chemotherapy use in this population was analyzed previously in more detail in the study by Debets, et al. (14). Our data further confirm the results from recent studies on the benefit of local therapies in metastatic ACC, including the benefit of primary tumor resection in metastasized disease (15-19). In this population, adrenalectomy, additional surgery and metastasis surgery were all associated with a survival benefit in stage IV disease (adjusted HR 0.39, 95%CI[0.29-0.54]; 0.40, 95%CI[0.18-0.91]; 0.37, 95%CI[0.22-0.63], respectively). The number of patients (n=6) who received microwave ablation, radiofrequency ablation or trans-arterial radio-embolization for liver metastases was too small to determine survival benefit from other local therapies. However, the data confirms that for selected patients, surgical control of disease is possible and can indeed improve survival of stage IV disease. Radiotherapy has also been described as beneficial for local recurrence in adjuvant setting and local tumor control in palliative setting (20,21). However, only high dose radiotherapy in advanced ACC has been associated with increased overall survival (20). In our population, we also found no overall survival benefit from radiotherapy, both adjuvant and palliative. However, the registry only holds data on overall survival and no data on local tumor control or symptom relieve due to radiotherapy, both of which can be as important to patients as overall survival.

The finding that the five-year survival for stage IV disease did increase, while the one-year survival did not, might suggest that the survival benefit from the changes in chemotherapy and local therapies of the recent years only benefit patients with less aggressive stage IV disease who have survived the first year. This verifies the need for new or better tailored treatment options for ACC. Immunotherapy as monotherapy or added to other treatments is one of the newly proposed treatment options (22). This current study did not include sufficient number of patients treated with immunotherapy to draw any conclusions on its survival benefit and no data on treatment response other than survival was present.

The current ACC guidelines recommend patients to be referred to an expert center at least once during the course of the disease (1,2). We have confirmed that treatment in an expert center is indeed associated with an improved survival in all stages (adjusted HR 0.70, 95%CI[0.57-0.85]). In 1993, the majority of Dutch patients was treated in a general hospital, but this decreased slowly in the following years. The centralization rate increased considerably after 2004 when DAN was founded. From that moment, the majority of patients is treated in an expert center. Currently 85% of adrenalectomies are performed in an expert center and 96% of medical treatment. For medical treatment, especially in stage IV disease with poor prognosis, patients might prefer treatment closer to home over improved survival. There are non-expert centers in the Netherlands that provide medical therapy locally with electronic consultations from one of the expert centers. The true number of medical treatments with involvement of an expert center may therefore be closer to 100%. For surgery however, there is still room for improvement.

The current study is a nation-wide study on ACC without influence of the referral bias that occurs in a single-, or multicenter registry. Patient identification is performed through the national pathology data and hospital declaration data to ensure no missing cases and survival is based on national records. Since the study is observational, selection bias is always present. However, the aim of our study was to determine the real-life incidence, treatment strategies and centralization of care and its effects on survival in the total population of patients with ACC in the Netherlands. We aimed to correct for selection bias using a multivariate cox regression model. The quality of this more detailed data is dependent on the quality of the hospital record keeping and the ability of the data collectors to find the data. We trained the data collectors specifically on ACC from 2014 onwards which might introduce a slight difference in the data collection before and after 2014. However, even with this training, we saw a lot of missing data in the ACC-specific items and diagnostic characteristics recorded from 2014 onwards, suggesting it was poorly recorded in the hospital records. This did not influence the analysis of the 1993 to 2020 data on incidence, stages, treatment and survival. However, the survival models could not be adjusted of Ki-67, resection status and hormone production because of the lack of collected data so far. These factors are known to influence survival and would be interesting to include in a couple of years, when more patients in the registry have detailed data available (3).

Conclusion

This unique nation-wide registry study provides real-life longitudinal data on the incidence, treatment and survival of patients with ACC. The true incidence of adult ACC was 1.62 per million person-years and was stable between 1993 and 2020. The five-year survival for stage I-III remained stable, while the survival for stage IV increased in the most recent years. Centralization of care has improved since the founding of the Dutch Adrenal Network, which was associated with increased survival. Adrenalectomy and mitotane therapy are associated with increased survival in all stages of disease. Adrenalectomy, surgical control of disease, chemotherapy and mitotane therapy are associated with increased survival in stage IV disease. Even with this survival benefit, the one-year survival remained stable over time, verifying the need for new or better tailored treatment options. For example, currently only 58% of patients on mitotane therapy reach therapeutic drug levels and only 41,5% complete the treatment as planned. Better guidance during mitotane therapy might improve treatment adherence with to intent to reach a survival benefit in more patients.

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Chapter 7

The role of the IGF2 methylation score in diagnosing adrenocortical tumors with unclear malignant potential: feasibility of formalin-fixed paraffin embedded tissue

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Abstract

The differentiation between benign and malignant adrenocortical tumors based on pathological assessment can be difficult. We present a series of 17 patients with unclear malignant tumors, of whom six had recurrent or metastatic disease. The assessment of the methylation pattern of insulin-like growth factor 2 (IGF2) regulatory regions in fresh frozen material has shown to be valuable in determining the malignancy of adrenocortical tumors, although this has not been elaborately tested in unclear malignant tumors. Since fresh frozen tissue was only available in six of the patients, we determined the feasibility of using formalin-fixed paraffin-embedded (FFPE) tissue for this method. We isolated DNA from FFPE tissue and matched the fresh frozen tissue of three patients with adrenocortical carcinoma. Methylation patterns of IGF2 regulatory regions were determined by pyrosequencing using different amounts of bisulfite-converted DNA (5 ng, 20 ng, 40 ng). Compared to fresh frozen tissue, FFPE tissue had a higher failure rate (fresh frozen 0%; FFPE 18.5%) and poor-to-moderate replicability (fresh frozen rho = 0.89–0.99, median variation 1.6%; FFPE rho = −0.09–0.85, median variation 7.7%). There was only a poor-to-moderate correlation between results from fresh frozen and FFPE tissue (rho = −0.28–0.70, median variation 13.2%). In conclusion, FFPE tissue is not suitable for determining the IGF2 methylation score in patients with an unclear malignant adrenocortical tumor using the currently used method. We therefore recommend fresh frozen storage of resection material for diagnostic and biobank purposes.

Introduction

Adrenocortical tumors (ACT) can have various presentations, from small adrenocortical adenomas (ACA) needing no intervention to large adrenocortical carcinomas (ACC) with a high risk of recurrence or metastasis (1,2,3). The determination of malignancy in adrenocortical tumors based on imaging and pathology is not always clear. An unenhanced CT scan of a homogenous tumor with <10 Hounsfield Units rules out ACC, while a large, inhomogeneous tumor with Hounsfield Units > 21 suggests ACC (4,5,6). This leaves a group with an unclear diagnosis. The same problem occurs with pathological determination of tumor grade by means of the Weiss score. In patients with a Weiss score of 2–3 or with divergent characteristics, the diagnosis often remains uncertain. The Weiss score has the additional disadvantage of an imperfect inter-observer reliability (7). Proper diagnosis of ACT is important for selecting the best treatment and duration of follow-up for the patient. For example, patients with a high risk of recurrence can benefit from adjuvant mitotane therapy, while for other patients, watchful waiting with CT imaging might be sufficient (1,2). There is an unmet need for a more objective biomarker to determine malignant potential in ACT, especially for patients with unclear malignancy based on imaging and pathology alone (8).

Several molecular markers have been proposed as biomarkers for prognosis, such as steroid profile, differential gene expression, or methylation (9). Insulin-like growth factor 2 (IGF2) is one of the growth factors involved in adrenocortical cell function. In normal cells, the IGF2 gene is maternally imprinted and paternally expressed. Increased expression of IGF2 is associated with malignancy, but expression levels are highly variable and, therefore, less suitable for clinical use. IGF2 expression is regulated by the differential methylation of regulatory regions, including differentially methylated region 2 (DMR2), protein binding site CTCF3 in the imprinting control region, and nearby gene H19 (10). This differential methylation has more stable patterns and can be transformed into an IGF2 methylation score. The IGF2 methylation score has shown to be valuable in the distinction between ACA and ACC (11,12). In ACT with unclear malignant potential, the addition of the IGF2 methylation score might, therefore, improve the assessment of the tumor and may be useful for treatment decisions on adjuvant therapy and follow-up duration.

Before the IGF2 methylation score can be used as a diagnostic tool in clinical practice, further validation is needed in patients with ACT with unclear malignant potential. In this study, we will present a series of patients with unclear ACT and aim to explore the potential role of the IGF2 methylation score in their postoperative treatment and follow-up management. Thus far, the IGF2 methylation score has only been validated in fresh frozen tissue, which is not widely available in clinical setting. We therefore aimed to determine whether formalin-fixed paraffin-embedded (FFPE) tissue is suitable to measure the IGF2 methylation score.

Materials and Methods

We searched for patients with an unclear ACT in three different research databases. Inclusion criteria were adult patients with a localized ACT with unclear malignant potential as described in the patient record, diagnosed before 2016 to allow for sufficient follow-up. The unclear malignant potential was defined as a Weiss score of 2–3 or Weiss score of 0–1 with histopathological, biochemical, radiological, or clinical signs of malignancy. We selected six cases from the Dutch Adrenal Network retrospective cohort and 11 cases from two Dutch adrenal biobanks (Table 1) (13). Electronic patient records were consulted for the pathology report, treatment modalities, and case history up to December 2020. In six of the selected patients, the IGF2 methylation score had previously been determined in stored fresh frozen tissue (11). The IGF2 methylation score is calculated from the mean, standard deviation score (SDS) from the methylation percentages of CFTC3 CpGs 5–7, DMR2 CpGs 2–4, and H19 CpGs 1–3. A score below 1.28 indicates ACA; a score above 3.18 indicates ACC, and a score between 1.28 and 3.18 indicates an unknown ACT. The other patients had no available fresh frozen tissue. This study was conducted under the guidelines that had been approved by the Medical Ethics Committees of the Erasmus Medical Center and Máxima MC.

To explore whether FFPE tissue is suitable for determining the IGF2 methylation score, we selected three patients with ACC. Each patient had stored FFPE tissue and matched fresh frozen tissue. DNA from fresh frozen tissue (1 section of 10 µm) was isolated using the Wizard Genomic DNA Purification Kit (Promega, Madison, WI, USA). DNA from FFPE tissue (1–3 sections of 10 µm) was isolated using the QIAamp DNA FFPE Tissue procedure (Qiagen, Hilden, Germany) following the manufacturer's protocol. The isolated genomic DNA was sufficient for our

downstream applications with a median concentration of 149 ng/µL (range 80–418 ng/µL) and a median 260/280 nm ratio of 1.84 (range 1.7–1.9). The DNA yield was optimal in our sample when using 2 sections of 10 µm FFPE tissue.

The isolated genomic DNA (1 µg) was converted with sodium bisulfite using the EpiTect Plus DNA Bisulfite Kit (Qiagen) following the manufacturer's protocol. PCR amplification (FastStart High Fidelity PCR System kit, Roche, Basel, Switzerland) and pyrosequencing (PyroGold SQA reagent kit, Qiagen, on the Pyromark Q24 system) of the bisulfite converted DNA were performed as previously described using a predefined pyrosequencing assay of CpGs involved in expression of IGF2 (CTCF3 CpG 5–7, DMR2 CpG 2–4 and H19 CpG 1–3) (11). This step was repeated six times with three different amounts of bisulfite-converted DNA (5 ng, 20 ng, and 40 ng, measured in two independent assays) to evaluate the failure rate and replicability of the assay and the correlation between FFPE and fresh frozen tissues. Pyrosequencing was considered a failure (not evaluable) when peaks were below or equal to H2O control. Statistical analysis was performed in SPSS version 26 and GraphPad Prism 8.2.1. Replicability and correlation were assessed using Spearman's correlation coefficient (rho) because of non-parametric data.

Results

Case series of ACT with unclear malignant potential

We selected 17 patients with an unclear ACT, diagnosed between 2000 and 2016. All patients had localized disease with a Weiss score between 1 and 3 upon initial diagnosis (Table 1). The diagnosis was unclear for varying reasons, such as size, a positive PET-CT scan indicating high metabolic activity, hormone production, another classification system, and minor invasive behavior or necrosis.

During follow-up, six patients (cases 6, 7, 9, 12, 13, 14) developed recurrent disease or metastasis. In four patients, this occurred within 5 years. Two patients (cases 13, 14) had already been discharged from the follow-up and presented 7 and 17 years after diagnosis with physical complaints caused by pulmonary metastasis and local recurrence, respectively. Four patients (cases 6, 7, 9, 14) eventually died with metastases, and one patient (case 8) died from a cause unrelated to ACT. Five patients (cases 1, 7, 8, 10, 11) in our series received adjuvant mitotane therapy. Among these patients, one (case 7) developed metastatic disease within a year.

This patient died from metastatic disease 4 years after initial diagnosis. The other four patients remained disease-free, with the follow-up ranging from 4 to 10 years.

We aimed to validate the use of the IGF2 methylation score in our case series with unclear ACT. In six patients, the IGF2 methylation score had previously been determined in stored fresh frozen tissue (11,12). Five patients were classified as ACC and one as unclear according to the IGF2 methylation score. Based on the score, one patient (case 1) was treated with adjuvant mitotane therapy and has been free of disease for 4 years. One patient (case 6) died with metastases 2 years after the diagnosis without adjuvant mitotane therapy. In this case, the IGF2 methylation score was only determined after the development of metastasis. In hindsight, the addition of the IGF2 methylation score at the time of diagnosis might have been beneficial in the decision-making process for this patient. The other four patients are currently free from recurrence or metastasis (range of follow-up: 2–8 years).

Based on these six patients alone, we cannot conclude whether the addition of the IGF2 methylation score has a valuable role in the diagnosis of unclear ACT. Unfortunately, there was no fresh frozen tissue available from other patients. We therefore explored whether FFPE tissue is suitable for determining the IGF2 methylation score in these patients.

Table 1. Disease history of cases.

Case	Diagnosis pathologist	Diagnosis IGF2 methylation score	Treatment	History
1	Weiss 3, unclear malignancy, 3.8cm, PET positive	3.51, ACC	Adjuvant mitotane	4 years disease free
2	Weiss 3, adenoma, 10cm, PET positive	3.34, ACC	Watchful waiting	4 years disease free
3	Weiss 3, oncocytairy adenoma, 5.5 cm, PET positive high uptake	3.08, unclear	Watchful waiting	4 years disease free
4	Weiss 1, 1 minor LWB criteria oncocytairy adenoma, 9.5 cm	4.29, ACC	Watchful waiting	2 years disease free, discharged from follow-up

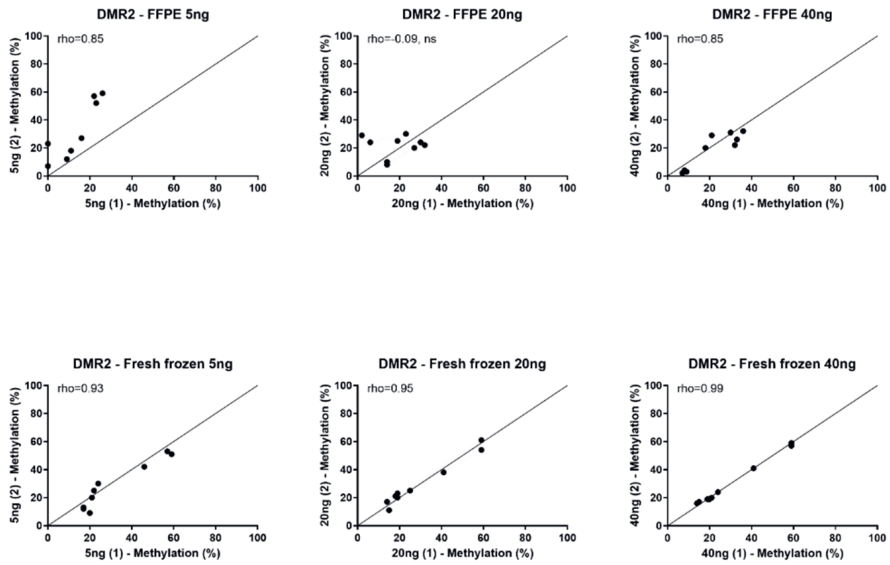
5	Weiss 1, partially invasive adenoma, 6 cm	4.20, ACC	Watchful waiting	8 years disease free
6	Weiss 3	4.68, ACC	Watchful waiting	2 years death with positive lymph nodes and bone metastases
7	Weiss 3	No fresh frozen material	Adjuvant mitotane	1 year abdominal wall/omental metastases (resection) 2 years bone metastasis (RT/resection) 3 years lung metastasis (mitotane) 4 years death with disease
8	Weiss 3	No fresh frozen material	Adjuvant mitotane	10 years death from other cause, no disease recurrence
9	Weiss 3	No fresh frozen material	Watchful waiting	4 years death with peritonitis carcinomatosis
10	Weiss 2, cortisol and androgen production	No fresh frozen material	Adjuvant mitotane	12 years disease free, discharged from follow-up
11	Weiss 3	No fresh frozen material	Adjuvant mitotane	9 years disease free
12	Weiss 3	No fresh frozen material	Watchful waiting	3 years liver metastases (RFA/mitotane) 7 years recurrent liver metastases (resection/mitotane) 9 years disease free
13	Adenoma with necrosis, 10cm	No fresh frozen material	Watchful waiting	17 years recurrence 30cm (resection) 20 years disease free
14	Adenoma (upon revision after recurrence Weiss 4)	No fresh frozen material	Watchful waiting	7 years lung metastases (mitotane discontinued for liver toxicity, resection, RT) 10 years abdominal metastases (EDP) 12 years death with lung and abdominal disease
15	Weiss 3, 7.8cm	No fresh frozen material	Watchful waiting	4 years disease free
16	Weiss 2, 2.9cm, cortisol production	No fresh frozen material	Watchful waiting	4 years disease free, discharged from follow-up
17	Weiss 3, cortisol production	No fresh frozen material	Watchful waiting	4 years disease free

ACC: adrenocortical carcinoma. EDP: etoposide, doxorubicin, cisplatin chemotherapy. PET: positron-emission tomography. RFA: radiofrequency ablation. RT: radiotherapy. IGF2 methylation score = mean SDS (CFTC3, DMR2 and H19); <1.28 adrenocortical adenoma (ACA); >3.18 adrenocortical carcinoma (ACC); 1.28-3.18 unknown.

Feasibility of using FFPE tissue

We measured the methylation of IGF2 regulatory regions CTCF3, DMR2, and H19 in matched fresh frozen and FFPE tissues of three patients with ACC (Table S1). We discovered that the pyrosequencing failure rate was high in the FFPE tissues (10/54 FFPE assays; 0/54 fresh frozen assays). Nine of these failures occurred when using 5 ng and 20 ng DNA extracted from FFPE tissue, while the 40 ng assays only had one failure. None of the assays using fresh frozen tissue, including 5 ng DNA, showed any problems.

Figure 1. Replication of methylation percentage in FFPE and fresh frozen tissue.



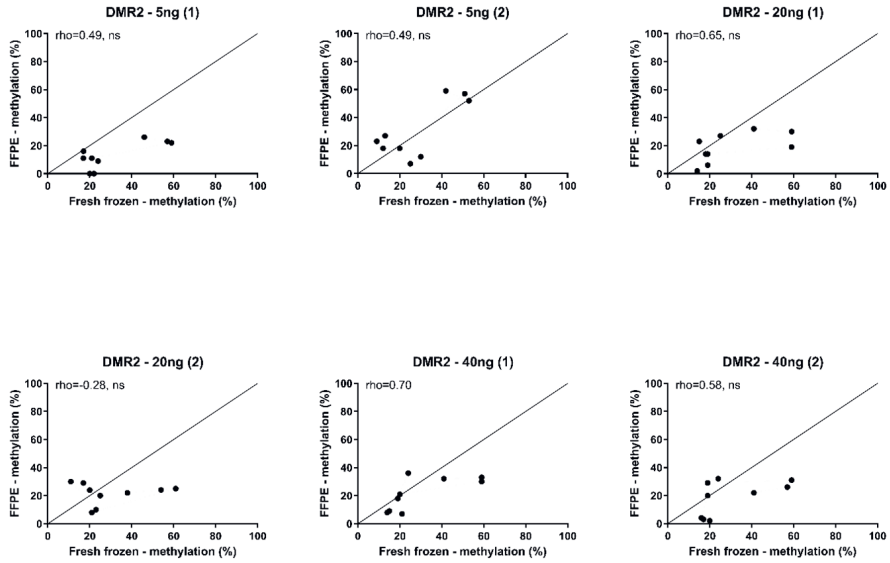
Spearmans's correlation (rho) of methylation percentages within formalin-fixed paraffin embedded (FFPE) tissue replications (upper row) and fresh frozen tissue replications (bottom row). Pyrosequencing was performed using primers for DMR2 CpG 2-4 in 5ng, 20ng and 40ng bisulfite converted DNA, measured in two independent assays with same concentrations of DNA. (1) first assay, (2) second assay. ns: not significant, p>0.05. Reference line represents perfect replication.

Because of the high failure rate in FFPE-extracted DNA, replication of methylation percentages using the same amount of DNA could not be achieved for most assays. Only the DMR2 assays were completed in FFPE and fresh frozen tissues of all three patients without failures. In these assays, the replication in FFPE was poor-to-moderate (Spearman's rho = −0.09–0.85) with a median variation of 7.7% in both directions (range 1–35%) (Figure 1). In contrast, replication of the methylation percentages from fresh frozen extracted DNA was very good across all assays

(Spearman's rho = 0.89–0.99) with low variation (median variation 1.6%, range 0–11%) (Figure 1 and Figure S1).

We then compared the methylation percentages between the FFPE-extracted DNA to the matched fresh frozen tissues in the completed DMR2 assays. We found the DNA methylation percentages from FFPE-extracted DNA to be poorly to moderately correlated to the percentages from fresh frozen tissues (Spearman's rho = −0.28–0.70). The median variation between the methylation percentages found in FFPE and fresh frozen tissues was 13.2% in both directions (range 1–40%) (Figure 2).

Figure 2. Correlation of methylation percentage in FFPE and fresh frozen tissue.



Spearmans's correlation (rho) of methylation percentages between formalin-fixed paraffin embedded (FFPE) tissue and fresh frozen tissue. Pyrosequencing was performed using primers for DMR2 CpG 2-4 in 5ng, 20ng and 40ng bisulfite converted DNA, measured in two independent with same concentrations of DNA assays. (1) first assay, (2) second assay. ns: not significant, p>0.05. Reference line represents perfect replication.

Discussion

The determination of malignancy in ACT is not always clear, which can lead to difficult follow-up and treatment decisions. The current guidelines lack recommendations for the follow-up duration of patients with unclear malignant ACT (1,3). Patients with ACA are discharged from follow-up after surgery, while patients with ACC are followed for at least 5 years, with a median time to

recurrence of 11 months (14). Many patients with unclear ACT will remain disease-free, and a long follow-up might result in unnecessary anxiety and costs (15,16). However, our case series of 17 patients with unclear malignant ACT included four patients with recurrence within 5 years and two with late recurrence, suggesting that early discharge might result in underdiagnosis. Four patients eventually died from metastasis. In patients with proven ACC, adjuvant mitotane treatment is recommended in patients after radical surgery with a perceived high risk of recurrence (ENSAT stage III, R1 resection or Ki67-index > 10%). Adjuvant mitotane is, however, not recommended for unclear ACT (1). Our case series included five patients with adjuvant mitotane therapy, of whom only one developed metastasis. Even though the series is small, these cases do suggest that watchful waiting might not always be the best option for patients with an unclear ACT. Some patients might benefit from adjuvant mitotane therapy; however, this decision is solely based on clinical judgment and patient preference. There is an unmet need for a more objective measure of malignancy and prognosis in patients with an unclear ACT to better guide the follow-up and treatment decisions.

The IGF2 methylation score can help predict malignancy (11,12). However, this has not yet been confirmed in a larger series of patients with unclear malignant ACT. We aimed to validate the use of the IGF2 methylation score in our case series. Since only six patients had available fresh frozen tissue, and no conclusion could be drawn from their data alone, we determined the feasibility of using FFPE tissue in the other 11 patients. Unfortunately, we found a high pyrosequencing failure rate when using FFPE tissue (18.5%) and no failures when using fresh frozen tissue. Replication and correlation could, therefore, not be determined in most assays. Even in the completed DMR2 assays, replicability in FFPE tissue was poor-to-moderate, while replicability in fresh frozen tissue was very good. These assays only had a poor-to-moderate correlation between IGF2 methylation patterns in FFPE tissue and fresh frozen tissue of the same patients, and variations between the measurements were high in both directions. FFPE tissue is, therefore, not suitable for the determination of the IGF2 methylation score in patients with unclear malignant ACT using the currently used laboratory method.

There have been several studies comparing the results of pyrosequencing methylation assays between FFPE and frozen tissue with varying results. Some studies show a promising correlation (17,18,19,20,21,22,23,24). However, upon

closer examination of the results, many studies experience high failure rates when using FFPE tissue (23,24,25,26,27), and both random and non-random deviations from the methylation percentage were found in frozen tissue (19,20,21,22,25,26, 27,28,29). Similar articles using other methods for methylation determination, such as Infinium Beadchip, Methylight, or methylation-specific PCR, show the same problems and limitations. Some studies find a good correlation between FFPE and frozen tissues (28,29,30,31,32,33), while others experience problems with failure rates (27,28,34,35,36) and poor correlation (28,29,36,37).

A possible explanation for the higher failure rate in FFPE tissue and the lack of replicability and correlation can be found in the degradation of DNA during storage (38). DNA isolated from FFPE tissue can be of poor quality with high rates of fragmentation related to storage time and fixation protocol (39). Compared to other methods for DNA isolation from FFPE tissues, the QIAmp method used in our study has a higher DNA yield in terms of quantity, quality, and PCR performance (40,41,42). However, DNA in FFPE tissue already shows significant fragmentation before isolation. This fragmentation can lead to less successful PCR amplification and consecutive pyrosequencing failure, which we observed in our analysis. The use of short amplicons of less than 500 base pairs has, therefore, been recommended for analyzing DNA from FFPE tissue. Even though the amplicons in our study were less than 150 base pairs, we still experienced 18.5% pyrosequencing failure in the FFPE samples and no failures in the fresh frozen samples.

A second hypothesis is that the fresh frozen and FFPE tissue came from different parts of the same tumors. ACT have shown heterogeneity within one tumor (43). We would expect this to impact the correlation between the results from FFPE tissue and fresh frozen tissue. However, we also found poor-to-moderate replicability when retesting the FFPE tissue, while the fresh frozen tissue resulted in near-perfect replication. This suggests another mechanism also causing random error, not only a systematic error due to heterogeneity.

A possible explanation for this random error is the introduction of DNA modifications during formalin fixation. These formaldehyde modifications can cause random errors during the bisulfite conversion (25). Firstly, formalin fixation causes interstrand cross-linking resulting in incomplete conversion of unmethylated cytosines (22). Secondly, mutational artifacts are introduced by hydrolytic deamination of methylated cytosines to thymines and unmethylated

cytosines to uracil (44,45). Methods for the removal of formalin modifications have been proposed over the years and applied in the QIAmp method for DNA isolation (22,45). However, we still found more variation in the methylation levels measured in the FFPE tissue than in the fresh frozen tissue, suggesting random errors introduced in the analysis. The current method did not include a DNA-restauration step before Pyrosequencing. Further research should determine whether including this step in the process eliminates errors caused by formalin-fixation.

The IGF2 methylation score is assessed in two independent cohorts to distinct ACA from ACC in fresh frozen tissue (11,12). This can help to determine the duration of follow-up and whether or not to start adjuvant mitotane treatment. Ideally, we would like to validate this result for diagnostic use in patients with unclear malignant ACT. However, since stored FFPE tissues are not suitable, a prospective study should be performed with the collection of fresh frozen biobank material. The current guideline recommends operating on patients with suspected ACC in specialized adrenal centers (1). Besides the higher chance of survival, these centers also have the capacity to handle and store fresh frozen tumor tissue (46). We therefore recommend also operating patients with unclear malignancy based on imaging in these specialized centers. In case of an unclear pathology result, a section of the fresh frozen tissue can then be sent to a central laboratory to perform the IGF2 methylation analyses and determine the likelihood of malignancy. As an added advantage, these tissues can be stored in biobanks to facilitate future validation of the IGF2 methylation score in unclear ACT and other research initiatives.

Conclusions

A subset of patients with unclear malignant ACT develops a recurrent adrenal tumor or metastases during (long-term) follow-up. Currently, there are no guidelines on how to monitor these patients and when to add adjuvant mitotane treatment. The IGF2 methylation score is a candidate biomarker for prognosis in patients with ACT with unclear malignant potential and might aid follow-up and treatment decisions. Unfortunately, FFPE tissue is not suitable for determining the IGF2 methylation score using the currently used method. We therefore recommend performing surgical resection of unclear ACT in specialized centers with the capacity for fresh frozen storage of pathological material for diagnostic and biobank purposes.

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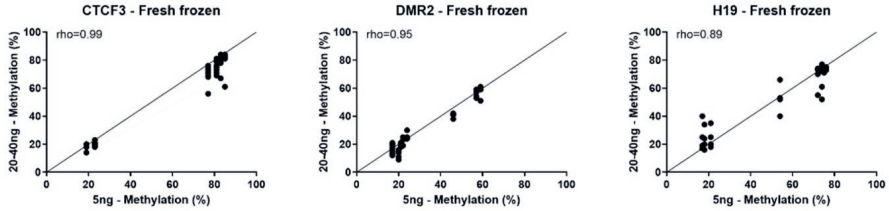
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Supplemental materials

Figure S1. Replication of methylation percentage in fresh frozen tissue CTCF3, DMR2, and H19.



Spearmans's correlation (rho) of methylation percentages within fresh frozen tissue replications. Pyrosequencing was performed using primers for CTCF3 CpG 5-7, DMR2 CpG 2-4 and H19 CpG 1-3 in 5ng, 20ng and 40ng bisulfite converted DNA, measured in two independent assays with same concentrations of DNA. ns: not significant, p>0.05. Reference line represents perfect replication.

Table S1. Methylation percentage and IGF2 methylation score of FFPE and fresh frozen tissues.

Fresh frozen 5ng (1)						**FFPE 5ng (1)**					
CTCF3	**7**	**6**	**5**	**Mean**	**|SDS|**	**CTCF3**	**7**	**6**	**5**	**Mean**	**|SDS|**
Tissue 1	23	23	19	22	5	Tissue 1	3	0	0	1	9
Tissue 2	83	85	77	82	6	Tissue 2	77	75	26	59	2
Tissue 3	81	81	77	80	6	Tissue 3	58	57	55	57	2
DMR2	**2**	**3**	**4**	**Mean**	**|SDS|**	**DMR2**	**2**	**3**	**4**	**Mean**	**|SDS|**
Tissue 1	17	17	20	18	0,95	Tissue 1	16	11	0	9	3,15
Tissue 2	22	24	21	22	0,10	Tissue 2	0	9	11	7	3,72
Tissue 3	59	57	46	54	7,82	Tissue 3	22	23	26	24	0,43
H19	**1**	**2**	**3**	**Mean**	**|SDS|**	**H19**	**1**	**2**	**3**	**Mean**	**|SDS|**
Tissue 1	21	18	17	19	3	Tissue 1	#	#	#		
Tissue 2	72	74	54	67	3	Tissue 2	95	86	96	92	5
Tissue 3	76	74	75	75	4	Tissue 3	#	#	#		

IGF2 MS	**CTCF3**	**DMR2**	**H19**	**Mean |SDS|**	**Diagnosis**
Tissue 1	5	0,95	3	2,78	Unclear
Tissue 2	6	0,10	3	2,99	Unclear
Tissue 3	6	7,82	4	5,74	ACC

Fresh frozen 5ng (2)						**FFPE 5ng (2)**					
CTCF3	**7**	**6**	**5**	**Mean**	**|SDS|**	**CTCF3**	**7**	**6**	**5**	**Mean**	**|SDS|**
Tissue 1	20	18	14	17	6	Tissue 1	15	13	13	14	6
Tissue 2	67	61	56	61	3	Tissue 2	#	#	#		
Tissue 3	69	75	68	71	4	Tissue 3	100	47	54	67	4
DMR2	**2**	**3**	**4**	**Mean**	**|SDS|**	**DMR2**	**2**	**3**	**4**	**Mean**	**|SDS|**
Tissue 1	13	12	9	11	2,58	Tissue 1	27	18	23	23	0,18
Tissue 2	25	30	20	25	0,75	Tissue 2	7	12	18	12	2,33

Tissue 3	51	53	42	49	6,52	Tissue 3	57	52	59	56	8,31
H19	**1**	**2**	**3**	**Mean**	**|SDS|**	**H19**	**1**	**2**	**3**	**Mean**	**|SDS|**
Tissue 1	35	34	40	36	1	Tissue 1	#	#	#		
Tissue 2	55	61	40	52	1	Tissue 2	#	#	#		
Tissue 3	74	73	71	73	3	Tissue 3	#	#	#		
IGF2 MS	**CTCF3**	**DMR2**	**H19**	**Mean**	**|SDS|**	**Diagnosis**					
Tissue 1	6	2,58	1	2,95		Unclear					
Tissue 2	3	0,75	1	1,43		Unclear					
Tissue 3	4	6,52	3	4,67		ACC					

Fresh frozen 20ng (1)						**FFPE 20ng (1)**					
CTCF3	**7**	**6**	**5**	**Mean**	**|SDS|**	**CTCF3**	**7**	**6**	**5**	**Mean**	**|SDS|**
Tissue 1	20	20	18	19	5	Tissue 1	9	2	16	9	7
Tissue 2	78	82	71	77	5	Tissue 2	90	81	68	80	6
Tissue 3	73	79	73	75	5	Tissue 3	35	38	30	34	2
DMR2	**2**	**3**	**4**	**Mean**	**|SDS|**	**DMR2**	**2**	**3**	**4**	**Mean**	**|SDS|**
Tissue 1	14	19	15	16	1,44	Tissue 1	2	6	23	10	2,82
Tissue 2	19	25	18	21	0,30	Tissue 2	14	27	14	18	0,87
Tissue 3	59	59	41	53	7,58	Tissue 3	19	30	32	27	1,24
H19	**1**	**2**	**3**	**Mean**	**|SDS|**	**H19**	**1**	**2**	**3**	**Mean**	**|SDS|**
Tissue 1	20	20	19	20	2	Tissue 1	#	#	#		
Tissue 2	70	72	52	65	2	Tissue 2	92	95	98	95	6
Tissue 3	73	73	73	73	3	Tissue 3	54	67	81	67	3
IGF2 MS	**CTCF3**	**DMR2**	**H19**	**Mean**	**|SDS|**	**Diagnosis**					
Tissue 1	5	1,44	2	3,05		Unclear					
Tissue 2	5	0,30	2	2,70		Unclear					
Tissue 3	5	7,58	3	5,30		ACC					

Fresh frozen 20ng (2)						**FFPE 20ng (2)**					
CTCF3	**7**	**6**	**5**	**Mean**	**|SDS|**	**CTCF3**	**7**	**6**	**5**	**Mean**	**|SDS|**
Tissue 1	19	19	20	19	5	Tissue 1	#	#	#		
Tissue 2	80	81	69	77	5	Tissue 2	89	63	63	72	4
Tissue 3	71	76	71	73	5	Tissue 3	72	73	52	66	3
DMR2	**2**	**3**	**4**	**Mean**	**|SDS|**	**DMR2**	**2**	**3**	**4**	**Mean**	**|SDS|**
Tissue 1	17	20	11	16	1,44	Tissue 1	29	24	30	28	1,40
Tissue 2	23	25	21	23	0,27	Tissue 2	10	20	8	13	2,25
Tissue 3	61	54	38	51	7,09	Tissue 3	25	24	22	24	0,43
H19	**1**	**2**	**3**	**Mean**	**|SDS|**	**H19**	**1**	**2**	**3**	**Mean**	**|SDS|**
Tissue 1	25	24	25	25	2	Tissue 1	#	#	#		
Tissue 2	73	77	53	68	3	Tissue 2	100	80	89	90	5
Tissue 3	75	76	74	75	4	Tissue 3	67	64	70	67	3
IGF2 MS	**CTCF3**	**DMR2**	**H19**	**Mean**	**|SDS|**	**Diagnosis**					
Tissue 1	5	1,44	2	2,87		Unclear					
Tissue 2	5	0,27	3	2,77		Unclear					
Tissue 3	5	7,09	4	5,07		ACC					

Fresh frozen 40ng (1)						**FFPE 40ng (1)**					
CTCF3	**7**	**6**	**5**	**Mean**	**|SDS|**	**CTCF3**	**7**	**6**	**5**	**Mean**	**|SDS|**
Tissue 1	23	21	20	21	5	Tissue 1	16	19	14	16	6
Tissue 2	84	84	74	81	6	Tissue 2	94	92	64	83	7
Tissue 3	81	81	76	79	6	Tissue 3	65	66	51	61	2
DMR2	**2**	**3**	**4**	**Mean**	**|SDS|**	**DMR2**	**2**	**3**	**4**	**Mean**	**|SDS|**
Tissue 1	15	21	14	17	1,278	Tissue 1	9	7	8	8	3,39
Tissue 2	19	24	20	21	0,222	Tissue 2	18	36	21	25	0,75
Tissue 3	59	59	41	53	7,580	Tissue 3	30	33	32	32	2,38
H19	**1**	**2**	**3**	**Mean**	**|SDS|**	**H19**	**1**	**2**	**3**	**Mean**	**|SDS|**
Tissue 1	18	18	16	17	3	Tissue 1	6	7	20	11	3
Tissue 2	71	74	52	66	2	Tissue 2	80	82	66	76	4
Tissue 3	74	73	74	74	3	Tissue 3	65	73	82	73	3
IGF2 MS	**CTCF3**	**DMR2**	**H19**	**Mean**	**|SDS|**	**Diagnosis**					
Tissue 1	5	1,278	3	2,954		Unclear					
Tissue 2	6	0,222	2	2,934		Unclear					
Tissue 3	6	7,580	3	5,592		ACC					
Fresh frozen 40ng (2)						**FFPE 40ng (2)**					
CTCF3	**7**	**6**	**5**	**Mean**	**|SDS|**	**CTCF3**	**7**	**6**	**5**	**Mean**	**|SDS|**
Tissue 1	21	20	18	20	5	Tissue 1	#	#	#		
Tissue 2	82	83	73	79	6	Tissue 2	100	93	67	87	7
Tissue 3	75	79	74	76	5	Tissue 3	59	54	57	57	2
DMR2	**2**	**3**	**4**	**Mean**	**|SDS|**	**DMR2**	**2**	**3**	**4**	**Mean**	**|SDS|**
Tissue 1	17	20	16	18	1,03	Tissue 1	3	2	4	3	4,61
Tissue 2	19	24	19	21	0,30	Tissue 2	20	32	29	27	1,24
Tissue 3	59	57	41	52	7,42	Tissue 3	31	26	22	26	1,08
H19	**1**	**2**	**3**	**Mean**	**|SDS|**	**H19**	**1**	**2**	**3**	**Mean**	**|SDS|**
Tissue 1	18	17	15	17	3	Tissue 1	48	21	30	33	1
Tissue 2	73	73	53	66	3	Tissue 2	68	70	28	55	1
Tissue 3	77	73	73	74	3	Tissue 3	88	47	64	66	3
IGF2 MS	**CTCF3**	**DMR2**	**H19**	**Mean**	**|SDS|**	**Diagnosis**					
Tissue 1	5	1,03	3	3,00		Unclear					
Tissue 2	6	0,30	3	2,90		Unclear					
Tissue 3	5	7,42	3	5,36		ACC					

IGF2 methylation score (MS) = mean SDS (CFTC3, DMR2 and H19); <1.28 adrenocortical adenoma (ACA); >3.18 adrenocortical carcinoma (ACC); 1.28-3.18 unknown. # Pyrosequencing failure (FFPE 10/54 assays, fresh frozen 0/54 assays). FFPE: formalin-fixed paraffin embedded.

Chapter 8

How close are we to personalized mitotane dosing in the treatment of adrenocortical carcinoma? State of the art and future perspectives

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Abstract

Introduction: Mitotane is the only drug registered specifically for adrenocortical carcinoma. Finding the optimal dose for a patient is difficult due to large differences in bioavailability, toxicity and effect. We therefore look to improve personalized dosing of mitotane.

Areas covered: We searched PubMed for studies related to mitotane dosing, pharmacokinetics, pharmacogenetics and combination therapy. Comparison of different dosing strategies have not resulted in an optimal advice. Several computerized pharmacokinetic models have been proposed to predict plasma levels. The current pharmacokinetic models do not explain the full variance in plasma levels. Pharmacogenetics have been proposed to find the unexplained variance. Studies on combination therapy have not yet led to a potential dose adjustment for mitotane.

Expert opinion: Computerized pharmacokinetics models are promising tools to predict plasma levels, further validation is needed. Pharmacogenetics are introduced in these models, but more research is required before clinical application. We believe that in the near future, personalized mitotane dosage will be aided by a validated web-based pharmacokinetic model with good predictive ability based primarily on clinical characteristics, adjustable for actual plasma levels and dosage.

Introduction

Adrenocortical carcinoma (ACC) is a rare cancer with an incidence of 1 per million person-years (1). Surgery is the main treatment modality. However, due to the often aggressive nature of ACC, risk of recurrence or metastasis is high and half of patients already have advanced disease upon presentation. Mitotane is the only drug registered specifically for ACC. It is indicated as monotherapy or in combination with classical chemotherapy in patients with irresectable or metastatic disease and increasingly used as adjuvant therapy after complete resection in patients at high risk for recurrent disease (2,3). Many studies have been performed to find an alternative for mitotane, for example immunotherapy, but no therapy has successfully completed phase III clinical testing thus far (4).

Mitotane is derived from DDT, an insecticide with adrenotoxic properties (5). Its first successful application in adrenocortical carcinoma treatment dates back to 1959 (6). It is a potent inhibitor of adrenal steroidogenesis. Its clinical benefit in adjuvant setting for patients with low risk of recurrence is currently under study in a randomized trial (ADIUVO clinicaltrials.gov), and results are expected this year. Although not undisputed, current guidelines recommend adjuvant mitotane for two years in patients with high risk of recurrence based on several retrospective studies (2,3). Studies on mitotane therapy for advanced disease are all observational with response to mitotane monotherapy ranging from 10 to 30% and response to combination therapy ranging from 10 to 50% (2,3).

Treatment with mitotane comes with many clinical challenges. Mitotane causes adrenal insufficiency requiring glucocorticoid and incidentally mineralocorticoid supplementation. Dosages required for supplementation are often twice as high as in patients with Addison's disease, due to mitotane induced steroid clearance and cortisol binding (2). In addition, mitotane causes many side-effects including gastro-intestinal, neurological and psychological effects, hypercholesterolemia and hepatotoxicity (2). These side-effects often require the use of supportive drugs (7). However, the choice of these supportive drugs is challenged by drug interactions, mostly due to CYP3A4 induction by mitotane (8,9).

Adequate mitotane dosing might be the most challenging aspect. A clinician must find the narrow balance between efficacy and toxicity. The optimal dose is different for each patient and can even differ over time in the same patient. With this review

we aim to find the answer to the question how close we are to personalized dosing in adrenocortical carcinoma. To answer this, we have searched PubMed up till 1-1-2021 for references related to mitotane dosing, pharmacokinetics of mitotane, pharmacogenetics of mitotane and combination therapy with mitotane in adrenocortical carcinoma (Table 1). We will conclude this review with an expert opinion from our research group and our view on future developments on this topic.

Personalized mitotane dosing

Dosing strategies

Traditionally, dosing of mitotane is based on expert opinion and guided by toxicity and plasma mitotane levels. The first assay for mitotane measurement was described in 1977 (10). Mitotane plasma levels are currently measured by high performance liquid chromatography or gas liquid chromatography. Both measurements have a good agreement and the same cutoff values for therapeutic window (11). Recently a home-based measurement was proposed using dried capillary blood (12). However, since there is no specific target range available yet for this assay, this method is currently not suitable for clinical practice.

The therapeutic window of mitotane was first established by Van Slooten, et al. in 1982 (13). They proposed 14 mg/l as the cutoff for low or high plasma levels, based on the correlation with tumor response. Various studies confirmed this result and found that patients with plasma levels above 14 mg/l have improved progression-free survival (14–20). The effect on overall survival was only evident in some of these studies. Most patients experienced side-effects, already starting at plasma levels of 5 mg/l. Patients with plasma levels above 20 mg/l have a significantly increased risk of experiencing severe side-effects, mostly neurological (13). Therefore, the therapeutic window between 14 mg/l and 20 mg/l was proposed (2).

The challenge for physicians is to propose a dosing scheme to reach this therapeutic window while keeping side-effects tolerable for the patient. And even in patients with steady-state dosing, mitotane plasma levels have shown to be variable (21,22). Physicians and their patients have to choose between a high-dose strategy which builds the dose up to 6 g/day in 1 week and a low-dose strategy which builds up to 4 g/day in 2 weeks. In both strategies the dose is adjusted based on the first plasma levels. A study comparing 20 patients on high-dose to 12 patients on low-dose

mitotane showed no significant difference in reaching therapeutic window within 3 months or side-effects between the two regimens (23). However, there was a trend toward more patients reaching the desired plasma level within 3 months using a high start dose.

A study on high-dose mitotane (>4 g/day, median 6 g/day in 2 weeks) showed 10 out of 22 patients reaching the therapeutic window within 3 months and an additional 4 patients after 3 months (24). Three of these patients had severe side-effects. These findings on the high-dose strategy were already demonstrated in a previous study that showed four patients out of six reached therapeutic levels within 4 weeks and toxic level within 6 weeks, on a rapidly progressive dose of 6–9 g/day in 2 weeks (25). Three of these patients experienced reversible side-effects, of which one severe (nausea grade 3). In contrast, a series of eight patients on low-dose (2–3 g/day) mitotane treatment all reached the therapeutic window after 3–5 months, with acceptable toxicity in seven patients (26). This suggests a more conservative approach to dosing can also lead to successful treatment with a more gradual increase in side-effects. Current guidelines recommend the high strategy dose for patients with good performance status as long as gastro-intestinal side-effects remain manageable. For other patients, the low-dose strategy is recommended (2,3).

Pharmacokinetics

For many years it has been known that mitotane is a lipophilic drug with a low absorption rate and an extremely long half-life (27–29). These characteristics illustrate why it takes weeks or months to reach steady-state concentrations. Several efforts have been made to construct pharmacokinetic models of mitotane. The aim of these studies is to improve clinical management of mitotane by helping the physician to predict the plasma level buildup in individual patients.

The first pharmacokinetic model was constructed using iterative 2-stage Bayesian fitting based on a population of 20 patients including 302 plasma level measurements (30). This was a 3-compartment model with an estimated mitotane clearance of 0.94 L/h and volume of distribution of 161 L/kg. Later, several models were constructed by using non-linear mixed-effects modeling, another computerized technique that never before had been employed on this old drug.

Table 1. Overview of pharmacokinetic and pharmacogenetics models of mitotane.

Study	Method	Pharmacokinetic parameters	Pharmacogenetic parameters
Kerkhofs 2015 (30)	Iterative 2-stage Bayesian fit 3-compartment model 20 patients, 302 plasma levels	Distribution volume: 161L/kg Clearance: 0.94L/h Covariates: lean body mass influences distribution	
Arshad 2018 (31)	Non-linear mixed-effect 1-compartment model 76 patients, 1137 plasma levels	Distribution volume: 6086L Clearance: 1L/day+3.97L/day/mg/L Covariates: mitotane increases enzyme synthesis which causes increased clearance	
Cazaubon 2019 (32)	Non-linear mixed-effect 1-compartment model 38 patients, 503 plasma levels	Distribution volume: 8900L Clearance: 70L/day Covariates: triglyceride and high density lipoprotein decrease mitotane clearance	
Yin 2020 (33)	Non-linear mixed-effect 2-compartment model 48 patients, 914 plasma levels, 1936 genetic variants	Distribution volume: 6210L central compartment and 18100L peripheral compartment Clearance: 43L/day Covariates: lean body weight and fat amount influence distribution	Reduced clearance rate: CYP2C19*2A (rs4244285) 44.9% SLCO1B1 571T>C (rs4149057) 40.2% (heterozygote) and 30.2% (homozygote) SLCO1B3 699A>G (rs7311358) 39.9%
Mornar 2012 (36)	Case report with high mitotane plasma level RT-PCR of CYP2C9 and CYP2C19		The patient was a CYP2C9 intermediate metabolizer and had a high plasma level of mitotane without its metabolites
D'Avolio 2013 (37)	27 patients Genotype of CYP2B6 and ABCB1 in relation to mitotane plasma level after 3, 6, 9 and 12 months of therapy		CYP2B6*6 (rs3745274) correlated with high plasma level
Ronchi 2014 (38)	239 patients		CYP2W1 immunoreactivity

	qRT-PCR and immunohistochemistry of CYP2W1	associated with better response to mitotane
Altieri 2020 (39)	182 patients CYP2W1 and CYP2B6 SNPs in germline DNA in relation to mitotane plasma levels	CYP2W1*6 lower plasma levels and response CYP2B6*6 higher plasma levels

Arshad, et al. composed a one-compartment model based on data of 76 patients including 1137 plasma level measurements (31). This model includes a mechanism of enzyme autoinduction, which assumes that mitotane increases enzyme synthesis and by doing so causes increased clearance of mitotane over time. Estimated clearance increase was 3.97 L/day per mg/L mitotane plasma level with fixed baseline clearance of 1 L/day. This model estimates the volume of distribution at 6086 L.

Cazaubon, et al. also constructed a one-compartment model, which was based on 38 patients and 503 plasma level measurements (32). Clearance was estimated at 70 L/ day and volume of distribution at 8900 L. This model does not include a mechanism of enzyme induction, but does identify a covariate effect of triglyceride (Tg) level and highdensity lipoprotein (HDL) level, i.e. higher Tg and HDL levels result in decreased mitotane clearance.

Yin, et al. developed a two-compartment model based on data of 48 patients and 914 plasma level measurements (33). A novel feature in this model is that it includes pharmacogenetic variables which were added by genotyping 1936 genetic variants of enzymes and transporters involved in absorption, distribution metabolism and excretion of drugs. According to this model, single nucleotide polymorphisms in genes coding for the CYP2C19 enzyme and transporters SLCO1B3 and SLCO1B1 are associated with mitotane clearance. This model's estimates of clearance was 43 L/day, volume of distribution was estimated at 6210 L for the central compartment and 18,100 L for the peripheral compartment.

Pharmacogenetics

The current pharmacokinetic models only explain 40% of the variance in mitotane plasma levels in individual patients. Genetic variation is expected to account for a

considerable amount of the remaining variance. Even though the exact mechanism of mitotane metabolism is unknown, we do know that mitotane activation occurs by beta-, or alphahydroxylation into two active components, o,p-DDA and o, p-DDE (17,34). Several variants of cytochrome p450 enzymes have been linked to this process and turned out to correlate with mitotane pharmacokinetics (35). An individual with high mitotane plasma levels was found to be a CYP2C9 intermediate metabolizer (36). The genotypic variant CYP2B6*6 (rs3745274), was found to correlate with mitotane higher plasma levels 3 and 6 months after start of treatment (37). High CYP2W1 activity was associated with better response to mitotane therapy in terms of overall survival and progression-free survival, both in adjuvant and palliative setting (38).

A recent European multicenter study in 182 ACC patients confirmed the association of CYP2W1 and CYP2B6*6 with mitotane plasma levels (39). Patients with a CYP2W1 wildtype had a better chance to reach therapeutic mitotane plasma levels, longer time to progression and higher disease control rate than patients with a CYP2W1*6 variation. Patients with CYP2B6*6 had a better chance to reach the therapeutic range, especially when a CYP2W1 wild-type was also present. However, these associations were only significant in patients with advanced ACC (N = 79), not in patients after radical resection who received mitotane as adjuvant treatment (N = 103). The authors suggest that local (i.e. tumoral) expression of CYP2W1 and CYP2B6 could be responsible for this remarkable observation.

A recent publication by Yin, et al. was the first to propose a model including pharmacogenetic variables to aid mitotane dosing (33). They identified three genetic variants to have a significant effect on mitotane clearance in 48 patients. Clearance was reduced by 44.9% in CYP2C19*2A carriers (rs4244285) and by 39.9% in SLCO1B3 699A>G carriers (rs7311358). Heterozygotic SLCO1B1 571 T > C carriers (rs4149057) had 40.2% of the clearance levels of wild-type patients, and homozygotic carriers 30.2%. Together with lean body weight at the start of treatment and fat amount as a measure of mitotane distribution, this model explained almost 60% of total plasma level variance. Interestingly, the previous finding on CYP2B6, CYP2C9, and CYP2W1 were not replicated in this study.

The model was used to simulate individual reactions to different dosing regimens (33). The optimal regimen in terms of time to reach therapeutic window, risk of

toxicity and time within therapeutic window, was a fixed individual starting dose and adjustment after 100 days based on plasma levels. The fixed dose was dependent on the model parameters of the individual. This proposed model has not yet been validated in an independent cohort.

Combination therapy

There have been several research efforts to increase mitotane bioavailability or function through co-medication. In theory, this might lead to a lower dose requirement of mitotane. Patients are already advised to take mitotane with a fatty product such as milk. This is found to increase mitotane absorption due to its lipophilic character (28). Building on this lipophilic character, many different vehicles have been proposed and tested to increase bioavailability, such as nanocarriers, micellar formulation, micro-emulsions and liposomal mitotane (40–43). These studies have thus far not let to a clinically usable solution.

For advanced ACC, mitotane can be combined with chemotherapy. The most frequently used combination is etoposide, doxorubine, cisplatin and mitotane, based on the results of the FIRMACT trial (44). In vivo studies have shown that mitotane works synergistically with doxorubicine and cisplatin, but surprisingly not with etoposide (45–47). Many other chemotherapeutic and some immunotherapeutic drugs have also shown a synergistic effect with mitotane in vitro (48–54). Even certain food and vitamin supplements have demonstrated an additive effect when administered with mitotane in ACC cell lines (55–57). However, many of these findings have not been replicated and have not been tested in clinical studies or even ‘in vivo’. The compounds that have been studied in patients have not led to potential dose adjustment for mitotane (44,58). Furthermore, caution is warranted in combination therapy since mitotane is known for its many drug interactions and antagonistic effects have been seen in vitro (59).

The role of lipids metabolism in mitotane function has been studied extensively. Mitotane induces increased LDL, HDL and Tg levels (60). Importantly, the resulting high lipid levels in turn reduce mitotane efficacy by binding mitotane (61). Thus, lowering of lipid levels by use of statins, might increase the effect of mitotane. This hypothesis was confirmed in a retrospective study where patients on mitotane and rosuvastatin had better tumor control (62). One of the cytotoxic effects of mitotane is caused by inducing endoplasmic reticulum (ER) stress by increasing intracellular

free cholesterol levels (63,64). ACC cell lines treated with rosuvastatin and mitotane showed increased intracellular free cholesterol levels inducing apoptosis (65). Apart from statins, new therapeutic compounds have been proposed to increase mitotane activity through increasing ER stress (66,67), by increasing intracellular free cholesterol levels (68) or by lowering mitotane-lipid binding (69). Not all tested compounds have resulted in a synergistic effect (70) and none of the findings have resulted in clinical trials or potential lowering of mitotane dosing.

Conclusion

Personalized dosing of mitotane is currently based on expert opinion guided by toxicity and plasma levels. Pharmacokinetic models based on clinical parameters with or without genetics variants are promising but not yet validated for clinical use. There are no supportive drugs yet that can lower the required mitotane dose while maintaining its adrenolytic effect.

Expert opinion

Mitotane is the only adjuvant treatment option for ACC and the cornerstone of palliative treatment. Finding the optimal dose is difficult due to large individual differences in bioavailability, toxicity and effect. Research groups are searching for alternatives, but thus far, no promising options exist. We therefore look to improve personalized dosing of mitotane.

Comparison of different dosing strategies have not resulted in a preferential regimen. The choice of starting dose is based on a subjective estimate of the patient's performance status and personal customs of the physician. We are looking for a model that ideally would be based on individual patient characteristics beyond performance alone. Several computerized pharmacokinetic models have been proposed based on measurable characteristics predicting absorption, distribution, metabolism and elimination. Computerized tools like these are theoretically usable in clinical practice, but only by clinicians experienced with this kind of methods and only if the necessary software and data are available. None of these models are tested in real-world clinical practice against the current standard, which is dosing based on clinician's experience.

The current pharmacokinetic models do not explain the full variance in plasma levels. Pharmacogenetics have shown a great promise to find this unexplained

variance. We expect future research efforts can lead to the discovery of a genetic panel to assist mitotane dosing. However, discovery and validation will take several more years. In addition, there are logistical and financial limitations to using genetic testing in clinical practice. We therefore do not see an added value for pharmacogenetics in personalized mitotane dosing at this moment.

For the near future, we do see possibilities in further development of the existing pharmacokinetic models using clinical characteristics. Self-learning computer models using clinical data, dosing data and plasma levels of large numbers of patients can be used to improve the current models. An example of a dataset suitable for this 'machine learning' is the Lysosafe Online® database. This database contains data on dosing and plasma levels of a large proportion of the European ACC population. When combined with clinical data, a usable model can be produced.

Any model would need to be tested in a trial compared to the current 'expert opinion dosing'. The control group should be a comparable center using either local customs or ideally one of the regimens used in the FIRM-ACT trial (low-dose or high-dose) (44). The primary outcome should be time to reach adequate mitotane plasma level. The secondary outcomes should include time with plasma levels higher than the therapeutic window, experienced toxicity and progression-free survival time.

Before clinical implementation, any validated model has to fulfill two additional criteria. Firstly, the model needs to be widely available and easy to use for clinicians. A web-based tool would meet that requirement. Secondly, the model should be self-learning based on actual plasma levels and should provide both a starting dose advice as well as dose corrections during treatment. We believe that such a model could and should be available for clinical practice in the near future.

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Part III

Elaboration

Chapter 9

General discussion and future perspectives

This thesis focused on the quality of life and care in patients with the rare cancer adrenocortical carcinoma (ACC). There are many challenges that clinicians and patients with this disease face. The 2018 ENSAT guideline ends with the following statement:

> *"Due to the fact that the evidence for most of the recommendations provided in these guidelines is weak or even very weak, there are no doubts that major efforts are needed to improve diagnosis, treatment, and quality of life for patients with ACC. Among many important research questions, we selected ten topics as particularly important."* (1)

This thesis provides stronger evidence to support the recommendations and addresses some of these important topics, starting with the final topic:

> *"No studies so far have revealed the wishes and experiences of patients. Given the poor prognosis and the toxic therapies, there is a definite need for 'Patient Related Outcomes'. PRO's should be measured (PROM's) and incorporated in our strategy for value-based cure and care."* (1)

Development of an ACC HRQoL questionnaire

Part I of the thesis focused on the health-related quality of life (HRQoL) of patients with ACC. We aimed to determine the currently perceived HRQoL in patients with ACC and specific issues that can be addressed to increase HRQoL. At the time of the guideline publication, no tool existed to measure HRQoL specifically in patients with ACC. ACC research up to that point used the European organization for research and treatment of cancer (EORTC) QLQ-C30 questionnaire, which is validated in patients with cancer (2). The upside is that this questionnaire is widely used in cancer research and can therefore be used to compare the HRQoL of patients with ACC to patients with other cancers. The downside is that the questionnaire does not include specific issues that only patients with ACC face. It is therefore less useful in monitoring individual patients with ACC or identifying specific issues that need to be improved. We therefore developed the ACC-QoL questionnaire with ACC specific issues, to be used alongside the EORTC-QLQ-C30 questionnaire. This was done according the EORTC methodology for questionnaire development, which is divided in four phases (3):

I. Gather all known HRQoL issues by literature search (**Chapter 2**).
II. Identify HRQoL issues that were previously unknown and determine which HRQoL issues are most important by interviews with patients (**Chapter 3**) and interviews with experts in the field (**Chapter 4**).
III. Transform the most important HRQoL issues into questions and validate the questionnaire in the target population (**Chapter 4**).
IV. Perform an international, cross-cultural validation of the questionnaire (future perspective).

For phase I, a systematic review was conducted in **Chapter 2** to determine what was already known on HRQoL in patients with ACC. This identified only six studies up till then that measured HRQoL in 323 patients with ACC, all indicating a reduced HRQoL compared to the general population. The FIRMACT trial found that HRQoL of patients with ACC was reduced compared to the general population, and that chemotherapy-mitotane further reduced HRQoL even though recurrence-free survival improved (4). Clinical aspects of the disease, including cortisol and aldosterone production, and adrenal insufficiency have shown great impact on HRQoL in benign disease, even after recovery of hormonal status (5). However, the impact of malignant adrenal disease and treatment options on HRQoL including adrenalectomy, radiotherapy, mitotane therapy, and chemotherapy had not been sufficiently studied in patients with ACC. Although the number of HRQoL studies in patients with ACC was limited, existing literature did indicate that ACC has a large impact on patients' HRQoL, with disease specific aspects such as specific side-effects of mitotane therapy.

These disease specific HRQoL aspects were further investigated in Phase II by conducting focus group interviews, as discussed in **Chapter 3**. Ten patients and their partner or other family member were asked which HRQoL issues they faced since being diagnosed with ACC. Six of the patients were treated with mitotane and four had surgery only. From the interviews it was clear that ACC had a large impact on the HRQoL of all participants. The interviews identified four domains related to HRQoL in patients with ACC, namely physical complaints, mental consequences, social consequences, and functional limitations. For example, physical complaints included symptoms of the disease and side-effects of mitotane therapy; mental consequences included feeling insecure and living from scan to scan; and functional limitations included daily activities and mobility. They further indicated that

positive or negative experiences with the health care system and health care professionals influenced their HRQoL, as did the perspectives of their partner or family member.

The literature search, focus group interview and interviews with healthcare professionals who are experts in the field of ACC identified relevant HRQoL issues that patients with ACC face and were not already included in the general EORTC-QLQ-C30 questionnaire. In phase III, these HRQoL issues specific to patients with ACC were transformed into a questionnaire using the style of the EORTC question bank, as discussed in **Chapter 4** (6). The project was conducted only in the Netherlands to increase the feasibility, it was therefore not conducted as an official EORTC project, so the ACC-QoL questionnaire is not an official EORTC product. The ACC-QoL was validated in Dutch patients who were currently or formally treated for ACC. This research was performed with help of the Netherlands Cancer Registry PROFILES study, a HRQoL registry in Dutch patients with cancer. The proposed version of the ACC-QOL consists of 26 questions and includes 3 scales (mitotane side-effects, hypercortisolism/hydrocortisone effects and emotional effects) and three single items (physical limitations due to surgery, virilization, need for peer support). The questionnaire has good psychometric properties in terms of face and construct validity, reliability, and responsiveness. This means it is a valid instrument to measure HRQoL in Dutch patients with ACC.

Adequate HRQoL assessments are important for both research and clinical care of patients with ACC. In trials, HRQoL is often used as a secondary outcome. A questionnaire should be sensitive to all HRQoL issues that can be caused by the disease and treatment under study. HRQoL questionnaires thus far did not include effects of mitotane side-effects, hormone production or hydrocortisone substitution and therefore lacked sensitivity to detect all HRQoL issues relevant for patients with ACC. HRQoL questionnaires can also be used to monitor side-effects during treatment, to improve patient–doctor communication, and to aid in treatment decisions (7,8). A disease-specific questionnaire is useful for detecting all issues relevant for the patient. The ACC-QOL is designed to be used alongside the EORTC QLQ-C30 and together the two questionnaires provide a more precise evaluation of HRQoL in patients with ACC, especially during active treatment. It can therefore be used in research setting and individual patient follow-up, without the added use of a general HRQoL questionnaire to reduce patient burden.

Before it can be enrolled internationally, the questionnaire needs to be translated and cross-culturally validated in a larger international study, in line with phase IV of the EORTC methodology. Afterwards, the ACC-QOL can be used in international clinical trials to determine the HRQoL effects of new treatments, in international cohort studies to compare and improve the HRQoL in patients with ACC across the World and in clinical setting to monitor and guide individual patients.

Identifying HRQoL issues that can be improved

Now that HRQoL can be measured adequately in patients with ACC, the EORTC-QLQ-C30 and the ACC-QoL questionnaires were used in **Chapter 4** to determine the currently perceived HRQoL in patients with ACC in the Netherlands. This is the first time HRQoL was assessed in a population-based cohort of patients with ACC. ACC survivors reported a good HRQoL, but slightly lower compared to other cancer survivors in a Dutch reference population (9). They reported to experience residual fatigue, sleep problems, emotional problems and problems with their sex life potentially resulting in this slightly lower perceived HRQoL. These patients might benefit from early referral to rehabilitation, psychology or sexology services for extra support (10). Patients who underwent recent treatment for ACC reported a lower HRQoL and experienced problems in many domains, including fatigue, specific mitotane side-effects, physical limitations due to surgery, problems with role functioning, cognitive functioning, emotional problems and the lack of peer support options. Especially patients on mitotane therapy or recent chemotherapy experienced a lower HRQoL. Notably, patients who received mitotane in the past, report a persistently reduced HRQoL compared to patients that were never treated with mitotane. Most of these patients reported persistent fatigue as a reason for this reduced HRQoL, but also persistent emotional and sexual problems. The ACC-QoL questionnaire can assist with early recognition of side-effects during mitotane treatment when administered regularly during follow-up visits. Most electronic patient systems already have the ability to send patients a questionnaire before an outpatient follow-up visit. The ACC-QoL would be a good tool for this application to monitor patients more closely during treatment and identify side-effects. Managing these side-effects earlier in the treatment, for example by dose adjustment, comedication or referral to a supportive specialist, might result in a higher HRQoL, better treatment adherence and improved survival. A prospective trial using the ACC-QoL during mitotane or chemotherapy treatment could assist in the further development of the ACC-QoL.

As an illustration of how much ACC can affect HRQoL, **Chapter 5** describes the case of a young girl with ACC. ACC affects one in five million children each year Worldwide. For the Netherlands that means approximately one case every other year. Most children present before four years of age with virilization, usually as part of a genetic tumor predisposition syndrome (11). They have a better prognosis than adults, with a five-year survival of 90%. Children older than four years however, usually present with more advanced disease and have a five-year survival of only 30% (12,13). Clinicians therefore often choose aggressive adjuvant treatment with etoposide, doxorubicin, cisplatin (EDP) chemotherapy and mitotane. This recommendation is only based on expert consensus (14,15). And while the effects of EDP are known from experience with other childhood cancers, little is known how mitotane treatment impacts the life of children with ACC.

Our patient was a ten-year-old girl with advanced ACC treated with chemotherapy and two years of mitotane after irradical adrenalectomy. She developed an impactful decrease in cognitive development measured by a standardized neuropsychological assessment before and during mitotane therapy. This decrease was mostly measurable in terms of decreased processing speed and concentration and a significant drop in school performance. Combined with fatigue and insecurity, this caused problems in short-term memory and the need to change her school type. Luckily, upon cessation of mitotane, her cognitive development returned back to normal and at the time of printing she was free from disease and happily went to school. Fourteen more studies were identified describing a range of gastro-intestinal, endocrine, developmental and neuropsychological side-effects in young children treated with mitotane with or without additional chemotherapy. Neurotoxicity included motor- and speech delay, decreased concentration and lower school performance. These side-effects appear to be reversible after mitotane discontinuation. This should provide reassurance to parents, patients and physicians in case EDP-mitotane treatment is indicated and result in better counseling before start of treatment.

Providing stronger evidence to support the recommendations

Part II of the thesis focused on quality of care for patients with ACC. As the authors of the guideline stated, most of the recommendations in the guideline are based on weak or very weak evidence (1). **Chapter 6** includes data from the Netherlands Cancer Registry, which is a nation-wide prospective cohort study including all patients diagnosed with ACC in the Netherlands between 1993 and 2020. Compared to other observational studies, this registry does not suffer from referral bias and therefore provides reliable data on incidence and survival. Off course it is not comparable to a randomized controlled trial when researching treatment, but it does give insight into real-life treatment selection and real-life treatment effects. The true incidence of adult ACC was 1.62 per million person-years and was stable between 1993 and 2020. The five-year survival remained stable over time for stage I-II at 65% and stage III at 35%, while the survival for stage IV increased from 3% to 11% in the last time-period.

The current guidelines recommend adrenalectomy for stage I-III disease (1,16). Even after complete resection, the risk of recurrence is still high for a large group of patients. The ADIUVO trial concluded that for low-grade localized disease (stage I/II, complete resection, Ki-67 index <10%) the recurrence rate is low and adjuvant mitotane therapy does not increase 5-year recurrence-free and 5-year overall survival (17). For high-grade or locally advanced disease, when a high risk of recurrence exists, the guideline does recommend adjuvant mitotane therapy after adrenalectomy. The data in this thesis confirmed that adrenalectomy and mitotane therapy are associated with increased survival in all stages of disease. Ki-67 index was added to the registry from 2014 onwards. For the registration of this item, data managers are dependent on hospital record keeping. Surprisingly, Ki-67 index was very poorly recorded. The results from the ADIUVO study could therefore not yet be verified in the Netherlands Cancer Registry. A few years from now, when more patients have been included with detailed data, the analyses can be repeated and provide this answer. In the meantime, it is recommended that the Ki-67 be added to all pathology reports on ACC, as has already been established in neuro-endocrine tumor pathology reports.

Mitotane is beneficial for a large group of patients with ACC. However, it is difficult to dose, it is associated with high toxicity, and it has a large impact on HRQoL, as was concluded in **part I** of this thesis. As in our clinical experience, the ACC registry

reflects the real-life problems with mitotane dosing and adherence due to toxicity. The data showed that only 58% of patients ever reached therapeutic mitotane levels above the recommended 14mg/L (18). In addition, only two in every five patients completed mitotane therapy for the predetermined period. In 61% of patients, the reason for discontinuation or dose reduction was toxicity. Careful monitoring and treatment of side-effects might help improve HRQoL, prevent early discontinuation and thereby improve survival. The ACC-QoL questionnaire is suitable for this purpose and can be useful in clinical practice.

For stage IV disease, the current guidelines recommend either chemotherapy in the FIRMACT regimen (etoposide, doxorubicin, cisplatin, mitotane) or local therapy with or without mitotane therapy (1,16,19). The registry confirms that both chemotherapy and palliative mitotane are associated with increased survival in stage IV disease. Our data further confirm the results from recent studies on the benefit of local therapies in metastatic ACC, including the benefit of primary tumor resection in metastasized disease (20-24). In this population, adrenalectomy, additional surgery and metastasis surgery were all associated with a survival benefit in stage IV disease. So, for selected patients with metastatic disease, surgical control is possible and can indeed improve survival. However, the decision for debulking surgery in patients with metastatic disease should be made on an individual basis by a multidisciplinary team.

The guidelines do not have a clear recommendation on the use of adjuvant or palliative radiotherapy (1,16). It is stated that radiotherapy can be considered for individual patients to improve local tumor control or symptom relieve. This recommendation is supported by some recent publications (25,26). Unfortunately, the registry holds no data on local tumor control or symptom relieve. However, both adjuvant and palliative radiotherapy provided no additional survival benefit in the Dutch ACC population, which is in line with the current literature (25,26).

Even with the above-mentioned survival benefit from existing therapies in a select group of patients, the overall survival of stage IV disease is still very poor. Immunotherapy has been proposed as a new therapy modality in ACC (27). The registry did not include sufficient number of patients treated with immunotherapy to draw any conclusions on its survival benefit. And thus far, all immunotherapy trials have only been beneficial for a small number of patients. ACC tumors appear

to have a molecular resistance mechanism to immunotherapy, which is being studied intensively at the present (27). One of the studied hypotheses is the lack of tumor infiltrating lymphocytes due to the immunosuppressive function cortisol in ACC tumor cells, even without clinical Cushing syndrome. The future of immunotherapy could therefore probably be sought in combination therapies to eliminate resistance mechanisms, for example adding mitotane or another cortisol reducing drug.

The current ACC guidelines recommend patients to be referred to an expert center at least once during the course of the disease (1,16). This thesis confirms that treatment in an expert center is indeed associated with an improved survival in all stages. In 1993, the majority of Dutch patients was treated in a general hospital, but this number decreased slowly in the following years. The centralization rate increased considerably after 2004, when the Dutch Adrenal Network was founded to unite all clinical and research expert centers on adrenal diseases in the Netherlands. From that moment, the majority of patients was treated in an expert center. Currently 85% of adrenalectomies are performed in an expert center and 96% of medical treatment. So, for surgery there is still room for improvement. For medical treatment, especially in stage IV disease with poor prognosis, treatment closer to home might sometimes be preferred to preserve HRQoL, as long as an expert center is consulted for treatment advise. In this light, the next guideline should state that treatment should be performed in or be indicated by an expert center, not just one visit during the course of the disease.

Improving diagnostics

A potential issue for improvement, is correct identification of patients who can benefit from adjuvant mitotane therapy and patients who can be spared its toxicity. This starts with a correct diagnosis, which is topic eight of the ten important topics listed in the guideline (1). The differentiation between benign and malignant adrenocortical tumors based on imaging and pathological assessment can sometimes be difficult (28,29). In **Chapter 7**, seventeen patients with unclear malignant tumors were presented, of whom six had recurrent or metastatic disease. Correctly identifying those six patients upon diagnosis could have resulted in treatment with adjuvant mitotane and potentially prevented some of the recurrences. Several molecular markers have been proposed as diagnostic biomarkers for ACC. Insulin-like growth factor 2 (IGF2) is one of the growth factors

involved in adrenocortical cell function. Methylation of the IGF2 gene region differs between ACC cells and normal adrenal cells. This differential methylation can be measured in pathology material and converted to a IGF2 methylation score. This score can help to distinct patients with ACC from patients with a benign adrenal tumor (30,31).

The IGF2 methylation score measured in fresh frozen pathology material has been validated as a diagnostic tool (30,31). The current practice however is to place a resected tumor directly in formaldehyde during surgery before transport to the pathology department. The current study therefore determined the feasibility of using formalin-fixed paraffin-embedded (FFPE) tissue for this method. Unfortunately, FFPE tissue had a higher failure rate compared to fresh frozen tissue and only a poor-to-moderate correlation and replicability. This is probably due to DNA modifications caused by formalin fixation and DNA degradation caused by storage. FFPE tissue is therefore not suitable for determining the IGF2 methylation score using the current laboratory techniques. These techniques are being developed further, so in the future, FFPE might be usable for this diagnostic tool. But as it currently stands, the diagnostic tool is depended on fresh frozen tissue, which is not routinely collected after adrenalectomy. Adrenal expert centers have the capacity to handle and store fresh frozen tumor tissue. This further supports the recommendation to perform adrenalectomy for all suspected adrenal tumors only in an adrenal expert center, not just for the associated survival benefit (1,16). As an added advantage, these fresh frozen tissues can be stored in biobanks to facilitate future validation of the IGF2 methylation score in unclear adrenal tumors and facilitate other research initiatives such as immunotherapy resistance mechanisms (27).

Improving mitotane dosing

As discussed previously, mitotane treatment is associated with increased survival in adjuvant and palliative setting. In reality however, it is difficult to find the correct dose to reach adequate plasma levels, due to large individual differences in bioavailability. Furthermore, mitotane is associated with high toxicity even at sub-optimal plasma levels. This causes only two in five patients to complete their mitotane therapy at adequate plasma levels and for the prescribed period. Dosing of mitotane is currently based on expert experience, guided by toxicity and plasma levels. Improving this process is part of topic six of the ten important topics listed

in the guideline (1). **Chapter 8** discusses methods to aid clinicians with individual dosing with the aim to improve mitotane adherence at adequate plasma levels with manageable toxicity to maximize the survival benefit.

Direct comparison of different dosing strategies has not resulted in an optimal dosing advice (18). Several computerized pharmacokinetic models based on clinical parameters have been developed to predict plasma levels. The current models, however, do not explain the full variance in plasma levels. Pharmacogenetics have been proposed to find the unexplained variance (32). These are promising tools, but none of these models are tested in real-world clinical practice against the current standard, which is dosing based on expert experience. Most of these models are only available in experienced centers where the necessary software and data is available. Genetic testing will take several more years to validate and comes with logistical and financial limitations. Ideally, personalized mitotane dosage will be aided by a validated web-based pharmacokinetic model with good predictive ability based on clinical characteristics. It should provide a starting dose advice as well as advise on dose corrections during treatment based on plasma levels and toxicity, preferably with a self-learning algorithm.

Conclusions

Each year 1.6 in every million adults are diagnosed with ACC. Five years after the diagnosis, only 65% of patients with a local tumor, 35% with an extensive tumor and 11% of patients with metastasis are still alive. This survival probability has only improved slightly for patients with metastasis, and not for patients with local disease only. This thesis shows that treatment in an expert center is associated with improved survival and that there is room for further centralization of treatment of ACC. This is especially true for surgical treatment. Extensive surgery is associated with increased survival also for selected cases with advanced or even metastatic disease. More patients should therefore be referred to an adrenal expert center where local treatment options are considered even in patients with advanced disease.

In any disease, especially cancer, quality of life-years gained by therapy is just as important as survival itself. This thesis is the first to address not just survival but also HRQoL in patients with ACC. The first disease specific HRQoL questionnaire ACC-QoL was developed and validated in a cohort of Dutch patients with ACC. After

international cross-cultural validation, this questionnaire can be used in future research into ACC treatment effects. With help of this questionnaire, patients reported an impact of especially mitotane therapy on HRQoL with residual problems, even after discontinuation. The Netherlands Cancer Registry study in this thesis further illustrates problems with mitotane dosing and therapy adherence, while mitotane treatment is associated with increased survival. Personalized mitotane dosing with a web-based pharmacokinetic model might help in the future, but the current models are not validated for clinical use yet. The IGF2 methylation score as a diagnostic tool to determine whether a patient with an unclear malignant adrenal tumor has an ACC and might therefore benefit (or be spared) from mitotane therapy, is not yet available in FFPE tissue. The ACC-QoL questionnaire however, can already be used in Dutch clinical practice to monitor and react to individual treatment side-effects, with the aim to increase both HRQoL and survival.

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Chapter 10

Summary/Samenvatting

Summary

This thesis focuses on the quality of life and care in patients with the rare cancer adrenocortical carcinoma (ACC). There are still many challenges that clinicians and patients with this disease face.

Part I of this thesis focused on the health-related quality of life (HRQoL) of patients with ACC. To adequately measure HRQoL, you need a questionnaire validated for use in the specific patient group. The disease specific questionnaire ACC-QoL was therefore developed to measure HRQoL in patients with ACC. This was done using a preset methodology consisting of four phases. For phase I, a systematic review was conducted in **Chapter 2** to determine what was already known on HRQoL in patients with ACC. The studies on HRQoL in patients with ACC were limited, but did indicate that ACC has a large impact on HRQoL. The HRQoL issues were further investigated in Phase II by conduction focus group interviews, as discussed in **Chapter 3**. From these interviews it was clear that ACC had a large impact on the HRQoL of all participants. They experiences HRQoL issues in many aspects of their life. In phase III, the relevant HRQoL issues, along with issues identified by healthcare professionals, were transformed into a questionnaire and validated in **Chapter 4**. The questionnaire needs to be validated in an international cross-cultural validation study in phase IV before it can be used in non-Dutch speaking patients with ACC.

The questionnaire was used in **Chapter 4** to determine HRQoL in patients with ACC in the Netherlands. ACC survivors reported a good HRQoL, but slightly lower compared to other cancer survivors. They reported to experience residual fatigue, emotional problems and problems with their sex life. Patients who were recently treated, reported a lower HRQoL and experienced many problems, including fatigue, mitotane side-effects, physical limitations due to surgery, cognitive problems, emotional problems and the lack of peer support. Especially patients on mitotane therapy or recent chemotherapy experienced a lower HRQoL. Notably, patients who received mitotane in the past, report a persistent reduction of HRQoL.

As an illustration of how much ACC can affect HRQoL, **Chapter 5** describes the case of a ten-year-old girl with ACC. She was treated with chemotherapy and two years of mitotane after irradical adrenalectomy. She developed an impactful decrease in cognitive development resulting in a significant drop in school performance.

Luckily, upon cessation of mitotane, her cognitive development returned back to normal, she could happily go to school and was free from disease. Other studies also described a range of side-effects in young children treated with mitotane with or without chemotherapy. These side-effects appear to be reversible after mitotane discontinuation. This should provide reassurance to parents, patients and physicians in case EDP-mitotane treatment is indicated.

Part II of this thesis focuses on quality of care for patients with ACC. **Chapter 6** includes data from the Netherlands Cancer Registry. The incidence of ACC was 1.62 per million person-years and was stable between 1993 and 2020. The five-year survival remained stable over time for stage I-II at 65% and stage III at 35%, while the survival for stage IV increased from 3% to 11% in the last years. Centralization of care has improved since the founding of the Dutch Adrenal Network, which was associated with increased survival. Adrenalectomy and mitotane therapy are associated with increased survival in all stages of disease. Adrenalectomy, surgical control of disease, chemotherapy and mitotane therapy are associated with increased survival in stage IV disease. However, the data showed that only two in every five patients completed mitotane therapy for the predetermined period at an adequate plasma level. The majority of discontinuations and dose reductions was toxicity related. Careful monitoring and treatment of side-effects might help improve HRQoL, prevent early discontinuation and thereby improve survival. The ACC-QoL questionnaire is suitable for this purpose and can be useful in clinical practice.

Another potential issue for improvement is correctly identifying who can benefit from adjuvant mitotane therapy and who can be spared its toxicity. The IGF2 methylation score is a diagnostic tool used to distinct an unclear malignant adrenal tumor from ACC. **Chapter 7** shows that this diagnostic tool is unfortunately not valid in formalin-fixed tissue, and therefore not yet widely available in clinical practice.

Chapter 8 discusses that personalized mitotane dosing with a web-based pharmacokinetic model might help to improve mitotane adherence at adequate plasma levels with manageable toxicity. Unfortunately, the current models are not validated for clinical use yet.

Samenvatting

Dit proefschrift beschrijft de kwaliteit van leven en kwaliteit van zorg voor patiënten met de zeldzame kankersoort bijnierschorcarcinoom (adrenocortical carcinoma - ACC). Er zijn nog veel uitdagingen waar patiënten en artsen mee te maken krijgen bij de behandeling van deze ernstige ziekte.

Deel I van dit proefschrift gaat over kwaliteit van leven (QoL) van patiënten met ACC. Om de kwaliteit van leven te meten, heb je een vragenlijst nodig die is getest (gevalideerd) bij patiënten met de ziekte die je wilt onderzoeken. Hiervoor is de ACC-QoL vragenlijst in dit proefschrift ontwikkeld volgens een methode met vier verschillende fasen. Voor fase I is in **hoofdstuk 2** een systematische literatuur zoektocht gedaan om vast te stellen wat er al bekend is over kwaliteit van leven van patiënten met ACC. Er waren maar een paar studies over dit onderwerp, maar die lieten wel zien dat ACC een grote impact heeft op het leven. Deze impact is verder onderzocht in **hoofdstuk 3** (fase II) tijdens focusgroep interviews met patiënten en hun naasten. Zij vertelden dat de ziekte veel invloed had op hun leven. Vooral de behandeling met mitotaan (tabletten die schadelijk zijn voor de bijnier) gaf veel bijwerkingen. De specifieke problemen die zij vertelden, zijn samen met interviews van artsen verwerkt tot de ACC-QoL vragenlijst. De ACC-QoL is in fase III gevalideerd. Dit is te lezen in **hoofdstuk 4**. Voordat de ACC-QoL gebruikt kan worden in andere talen, moet hij nog internationaal worden gevalideerd in fase IV.

De vragenlijst is in **hoofdstuk 4** gebruikt om de kwaliteit van leven te meten van Nederlandse patiënten met ACC. Patiënten die de ziekte langer geleden hebben gehad, hebben een goede kwaliteit van leven. Maar deze kwaliteit was wel iets lager dan bij patiënten die een ander soort kanker hebben gehad, bijvoorbeeld borstkanker of darmkanker. De patiënten vertelden nog last te hebben van vermoeidheid, emotionele problemen en problemen met hun seksleven. Patiënten die nog werden behandeld, hadden een lagere kwaliteit van leven. Zij hadden last van onder andere vermoeidheid, mitotaan bijwerkingen, klachten na een operatie, geheugen en emotionele problemen. Verder vertelden ze lotgenotencontact te missen, omdat de ziekte zeldzaam is. Zeker patiënten met mitotaan en/of chemotherapie-behandeling hadden een verminderde kwaliteit van leven. Bijzonder is dat na het stoppen van mitotaan, de kwaliteit van leven blijvend lager was dan van patiënten die nooit met mitotaan waren behandeld.

In **hoofdstuk 5** wordt het verhaal verteld van een tienjarig meisje met ACC, als voorbeeld van hoeveel invloed de ziekte heeft op het leven. Na een operatie, waarbij helaas niet de hele tumor verwijderd kon worden, werd ze behandeld met chemotherapie en twee jaar mitotaan. Tijdens de behandeling bleef haar ontwikkeling stil staan en moest ze na de brugklas naar een lager middelbare schoolniveau gaan. Dit had een grote impact op haar leven, omdat ze, naast alle problemen met de kanker, ook niet meer de steun had van haar klasgenootjes. Na het afronden van de mitotaan-behandeling, ging haar ontwikkeling weer door en op het moment dat dit boekje is gedrukt, gaat ze met veel plezier naar school en is de ziekte helemaal weg. Ook andere studies in dit hoofdstuk laten ernstige bijwerkingen zien van mitotaan met of zonder chemotherapie bij jonge kinderen. Maar deze bijwerkingen lijken volledig te herstellen nadat de behandeling is afgerond. Dit is geruststellend voor ouders, kinderen en artsen als de behandeling gestart moet worden.

Deel II van dit proefschrift gaat over de kwaliteit van zorg voor patiënten met ACC. **Hoofdstuk 6** laat cijfers zien uit de Nederlandse Kankerregistratie. ACC komt voor bij 1,6 op de miljoen volwassen Nederlanders per jaar. Dat zijn iets meer dan 20 nieuwe patiënten per jaar. Dit aantal veranderde niet tussen 1993 en 2020. Van de patiënten met een enkele tumor (stadium I en II) is na 5 jaar nog 65% in leven en van de patiënten met een uitgebreide tumor (stadium III) nog 35%. Deze overlevingscijfers veranderde niet in de tijd. In patiënten met uitzaaiingen (stadium IV) verbeterde de vijfjaarsoverleving wel van 3% naar 11% in de laatste paar jaar. Steeds meer patiënten worden behandeld in een expertisecentrum aangesloten bij het Bijniernetwerk DAN (Dutch Adrenal Network). Deze patiënten hebben een betere overleving dan patiënten die niet hier zijn behandeld. Verwijderen van de tumor met een operatie (adrenalectomie) en mitotaan-behandeling zorgen voor een betere overleving bij alle stadia van ziekte. Patiënten met uitzaaiingen hebben een betere overleving na operatief verwijderen van de bijniertumor en alle uitzaaiingen (als dat mogelijk is), chemotherapie en mitotaan-behandeling. Helaas laten de cijfers wel zien dat maar twee op de vijf patiënten hun mitotaan behandeling helemaal afmaakt. Patiënten stoppen hun behandeling vaak vanwege de bijwerkingen. De ACC-QoL vragenlijst kan gebruikt worden om de bijwerkingen bij te houden tijdens behandeling, zodat deze bijwerkingen op tijd bestreden kunnen worden. Dit zou de kwaliteit van leven tijdens de behandeling kunnen

verbeteren, waardoor minder patiënten voortijdig stoppen en hopelijk meer patiënten overleven.

Een ander verbeterpunt in de behandeling is om vooraf beter te bepalen wie behandeld moet worden met mitotaan en wie deze behandeling bespaard kan blijven. De IGF2 methylatie score is een test die een patholoog kan gebruiken om een goedaardige bijniertumor van een kwaadaardige te onderscheiden. **Hoofdstuk 7** laat zien dat deze test helaas nog niet kan worden gedaan op tumoren die op formaldehyde bewaard worden. Daarom kan nog niet elk ziekenhuis deze test uitvoeren, omdat formaldehyde de meest gebruikte manier is om een tumor te bewaren voor de patholoog.

Het doseren van mitotaan is niet makkelijk. Elke patiënt reageert anders en heeft een andere hoeveelheid tabletten nodig om de juiste spiegel te halen. Daarnaast beginnen de bijwerkingen vaak al bij een lage spiegel. In **hoofdstuk 8** worden online modellen besproken die artsen kunnen helpen met de dosering van mitotaan. Helaas zijn deze modellen nog niet gevalideerd om in de praktijk gebruikt te worden.

Chapter 11

Impact paragraph

Adrenocortical carcinoma (ACC) is a cancer originating from the outer layer of the adrenal gland, the cortex, and occurs in 1.6 per million adults in the Netherlands each year, which makes it a rare cancer. The chances of survival are dependent on the extent of the disease. After five years, 65% of patients with a single tumor is still alive and 35% of patients with an extensive tumor. Only 3-11% of patients with metastasized disease survive longer than five years. All of these patients experience a large impact of the disease on their life. The current guidelines are mostly based on expert experience because a rare disease makes it more difficult to perform good quality research. The objective of this thesis was therefore to measure and improve both the quality of life and the quality of care for this small group of patients.

Quality of life is something a person perceives. To make it measurable for use in research and clinical care, we use health-related quality of life (HRQoL) questionnaires. In trials testing a new treatment, the effect on HRQoL is usually a secondary outcome, but for patients it is just as important as the effect on survival and recurrence. HRQoL questionnaires can also be used in clinical care to monitor side-effects during treatment, to improve patient–doctor communication, and to assist treatment decisions. A questionnaire should be sensitive to all HRQoL issues that can be caused by the disease and the treatment options. No HRQoL questionnaires existed for ACC. An often used existing questionnaire only measured a part of the issues that patients with ACC face and therefore underestimated the impact of ACC on HRQoL. There was an unmet need for a questionnaire measuring all aspects of HRQoL in ACC. In this thesis, the first disease specific HRQoL questionnaire ACC-QoL was therefore developed and validated in a cohort of Dutch patients with ACC. The development was done with the help of patients, their family members and healthcare professionals who are experts in the field of ACC.

With this questionnaire, Dutch ACC survivors reported a good HRQoL but with residual fatigue, emotional problems and problems with their sex life. These patients might have benefited from early referral to rehabilitation, psychology or sexology services for extra support. Based on these results, multidisciplinary teams involved in the care of patients with ACC should add health-care professionals from supporting specialties to improve both quality of life and quality of care. To achieve this, this thesis will be shared with healthcare professionals involved in adrenal expert centers. Furthermore, a patient participation day was organized together

with the patient organizations to inform patients on the possibilities of non-medical support services.

In the same study using the ACC-QoL, patients reported an impact of especially mitotane therapy on HRQoL, with residual problems even after discontinuation. Mitotane has a broad spectrum of side-effects that impact HRQoL. The data from the Netherlands Cancer Registry used in this thesis, further illustrated problems with mitotane dosing and therapy adherence, while mitotane is an important part of the treatment to increase survival. The ACC-QoL is ready to be implemented in Dutch adrenal centers to monitor patients during the course of mitotane treatment. The questionnaire can be used to monitor and react to treatment side-effects, with the aim of improving treatment adherence and thereby improving both HRQoL and survival. Before the questionnaire can be used internationally in future ACC research and international adrenal centers, it needs further testing in an international cross-cultural validation study. The Dutch ACC-QoL was presented at an annual meeting of the European Network for the Study of Adrenal Tumors (ENSAT) to initiate collaboration on this future project.

This thesis discusses two other options for improvement of mitotane therapy. Personalized mitotane dosing with a web-based pharmacokinetic model might help in the future with adequate dose finding with manageable toxicity. But the current models are not validated for clinical use yet. Ideally, personalized mitotane dosage will be aided by a validated web-based pharmacokinetic model with good predictive ability based on clinical characteristics. It should provide a starting dose advice as well as advise on dose corrections during treatment based on plasma levels and toxicity, preferably with a self-learning algorithm. When such a web-based tool is validated, it should be made available to all adrenal expert centers to aid a successful implementation. The IGF2 methylation score is a diagnostic tool to determine whether a patient with an unclear malignant adrenal tumor has ACC and patients might therefore benefit (or be spared) from mitotane therapy. This thesis shows that the test is not yet feasible in formalin-fixed paraffin-embedded tissue. It is available in fresh frozen tissue, but a patient then needs to be operated in an adrenal expert center. This is not a disadvantage, because this thesis shows a survival benefit when patients are treated at an adrenal expert center. Currently 85% of adrenal surgery is performed in an expert center. Ideally, this would become 100% in the next couple of years. This can be achieved by educating both healthcare

professionals and patients on the outcomes of this thesis and the benefits of adequate referral. A summary of this thesis was therefore presented at both national and international expert meetings, and a written summary will be presented to patient organizations.

The current guidelines on the management of ACC are based on expert experience and mostly weak or even very weak evidence. This thesis provides data from the Netherlands Cancer Registry to support the next guideline update, which will improve quality of care for patients with ACC. The nation-wide prospective cohort study included all patients diagnosed with ACC in the Netherlands between 1993 and 2020. Compared to other observational studies, this registry does not suffer from referral bias and therefore provides more reliable data on incidence and survival. Off course it is not comparable to a randomized controlled trial when comparing treatments, but it does give insight into real-life treatment selection and real-life treatment effects. This is especially important for a rare disease like ACC, since the low number of new patients makes it difficult to conduct randomized controlled trials.

The guidelines recommend complete surgical removal of the tumor (adrenalectomy) for localized disease, followed by mitotane therapy when a high risk of recurrence exists. The data in this thesis confirm an improved survival in adrenalectomy and mitotane therapy for all stages. The recent trial result that mitotane therapy has no added benefit in patients with a low risk of recurrence could not yet be confirmed but probably can be in a few years. For metastatic disease the guidelines recommend chemotherapy and mitotane therapy, which both showed an improved survival in this thesis. It is further suggested to consider local therapy of metastasis along with mitotane therapy in some cases. This suggestion was not yet supported by evidence at the time the guidelines were published, but this thesis shows a survival benefit from surgical removal of the primary tumor and surgical removal of the metastasis when possible. Other recent studies also support this claim. The next guideline update can therefore include a recommendation for surgical control of metastatic disease for patients where it is technically feasible, although this always should be decided on an individual basis. Radiotherapy did not result in improved survival. But other studies have shown improved tumor control and symptom management with radiotherapy, microwave ablation, radiofrequency ablation and other local metastasis therapies. The

Netherlands Cancer Registry does not include data on local tumor control or local symptoms to confirm these results.

Even with the above-mentioned survival benefit from existing therapies in a select group of patients, the overall survival of stage IV disease is still very poor. Immunotherapy has been proposed as a new therapy modality in ACC. The registry did not include sufficient number of patients treated with immunotherapy to draw any conclusions on its survival benefit. And thus far, all immunotherapy trials have only been beneficial for a small number of patients. ACC tumors appear to have a molecular resistance mechanism to immunotherapy, which is currently studied intensively. The future of immunotherapy should therefore probably be sought in combination therapy to eliminate the resistance mechanisms. More translational studies are needed, followed by clinical trials to make this a reality. To get the number of patients needed for a study like this can only be achieved from international cooperation between adrenal expert centers such as through the collaborative efforts of the ENSAT.

The registration of patients with ACC in the Netherlands Cancer Registry will continue after the completion of this thesis, including the newly added data items specific to ACC. It can be used to answer future questions, such as the role of immunotherapy, the effect of personal mitotane dosing regimens and the real-life effects of newly developed treatments.

In conclusion, this thesis is the first to address the quality of life in patients with ACC along with the quality of care. Both are important to patients faced with this rare and impactful disease, their family members, the clinicians that treat them and the researchers that are searching for further improvements.



Appendix

Curriculum vitae

List of publications

Acknowledgement/Dankwoord

Curriculum vitae

Rebecca Steenaard was born in Schiedam in 1991. In high school her teachers encouraged her to participate in the Junior Med School program at the Erasmus Medical Center Rotterdam, where she was introduced to the world of medical research. She completed a research internship at Capri cardiac rehabilitation Rotterdam. She happily continued to work there as a research assistant during medical training at the Erasmus University Rotterdam. She further combined her studies with the Erasmus MC Honours Class and the NIHES Health-Sciences Research Master. During the research master she switched from cardiac rehabilitation research to epigenetics in cardiovascular diseases and diabetes mellitus. During clinical rotations (coschappen) she realized that endocrinology and internal medicine fit her better than cardiology. After graduation she started her PhD in adrenocortical carcinoma at Máxima MC Veldhoven/Eindhoven. During her PhD she worked parttime as resident not in training (ANIOS) at the internal medicine department; the evening, night and weekend shifts for urgent care (escalatiedienst); the covid unit; the pulmonary medicine department and the intensive care department as covid buddy. For her PhD she worked as a guest researcher for the Integral Cancer Center the Netherlands (IKNL) to set up the newly registered adrenal specific variables. She continues this work as the chair of the IKNL adrenal scientific committee. During her PhD she started internal medicine training (AIOS) at Máxima MC. Currently she is continuing to specialize in endocrinology at the Erasmus Medical Center.

List of publications

This thesis:

1. Steenaard RV, de Wit S, Rutjens M, van der Bruggen B, Blijdorp K, Wouters-van Poppel P, Feelders R, Haak HR, on behalf of the Dutch Adrenal Network. Time trends in incidence, treatment and survival of patients with adrenocortical carcinoma, a nationwide study. JCEM. 2026. Doi.org/10.1210/clinem/dgag088.
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