Adrenocortical carcinoma : a study on epidemiology diagnostics and treatment of a rare endocrine malignancy

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ADRENOCORTICAL CARCINOMA

A STUDY ON EPIDEMIOLOGY, DIAGNOSTICS AND TREATMENT OF A RARE ENDOCRINE MALIGNANCY

THOMAS M.A. KERKHOF

ADRENOCORTICAL CARCINOMA

A STUDY ON EPIDEMIOLOGY, DIAGNOSTICS AND TREATMENT OF A RARE ENDOCRINE MALIGNANCY

COLOFON

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TABLE OF CONTENTS

Chapter 1	General Introduction	7
PART I: EPIDEMIOLOGY OF ADRENOCORTICAL CARCINOMA		17
Chapter 2	Adrenocortical carcinoma: A population-based study on incidence and survival in the Netherlands since 1993	19
	<i>Eur J Cancer. 2013 Jul;49(11):2579-86.</i>	
Chapter 3	Adrenocortical carcinoma in children: first population-based clinicopathological study with long-term follow-up.	35
	<i>Oncol Rep. 2014 Dec;32(6):2836-44.</i>	
PART II: DIAGNOSTICS OF ADRENAL TUMORS AND DIFFERENTIATION BETWEEN BENIGN AND MALIGNANT LESIONS		53
Chapter 4	Adrenal incidentaloma and adrenocortical carcinoma: a clinical guideline on treating the unexpected and a plea for specialized care	55
	<i>Contemporary Aspects of Endocrinology, 2011, ISBN: 978-953-307-357-6.</i>	
Chapter 5	Adrenal tumors with unexpected outcome: a review of the literature	75
	<i>Int J Endocrinol. 2015; 2015:710514.</i>	
Chapter 6	Diagnostic value of urinary steroid profiling in the evaluation of adrenal tumors	89
	<i>Horm Cancer. 2015 Aug;6(4):168-75.</i>	
PART III: TREATMENT OF ADRENOCORTICAL CARCINOMA		103
Chapter 7	Surgery for adrenocortical carcinoma in the Netherlands: analysis of national cancer registry data	105
	<i>Eur J Endocrinol. 2013 Jun 7;169(1):83-9.</i>	
Chapter 8	Comparison of two mitotane starting dose regimens in patients with advanced adrenocortical carcinoma	119
	<i>J Clin Endocrinol Metab. 2013 Dec;98(12):4759-67.</i>	
Chapter 9	Development of a pharmacokinetic model of mitotane: towards personalized dosing in adrenocortical carcinoma	137
	<i>Ther Drug Monit. 2015 Feb;37(1):58-65.</i>	
Chapter 10	Short-term variation in plasma mitotane levels confirms the importance of trough level monitoring	153
	<i>Eur J Endocrinol. 2014 Dec;171(6):677-83.</i>	
Chapter 11	General discussion and future perspectives	167
Summary		181
Samenvatting (summary in Dutch)		187
Valorization		193
Curriculum Vitae		199
List of publications		203
Dankwoord (acknowledgements in Dutch)		207

CHAPTER 1

GENERAL INTRODUCTION

GENERAL INTRODUCTION

Genetic alterations may occur in adrenocortical cells in a similar fashion as they may occur in virtually every other cell in the body. When these lead to uncontrolled proliferation, invasion of normal tissue and/or other organs, this is called cancer. The mechanism is extremely complex involving multiple intrinsic factors (genetic alterations, hormonal interference) and extrinsic factors, such as exposure to carcinogens like chemicals, radiation and viruses.

Incidence and clinical features

Adrenocortical carcinoma (ACC) is rare with an annual incidence of 0.5-2.0 patients per million population.¹⁻³ The disease occurs in men and women in a 45:55 ratio.⁴ ACC may arise in both adrenal glands, but the left adrenal is affected slightly more often. Patients may present with symptoms of an abdominal mass, symptoms related to autonomous hormonal production by the tumor, or both. In rare cases, ACC is discovered incidentally during diagnostic tests or medical imaging for reasons unrelated to the adrenal glands.

Autonomous and excessive production of glucocorticoids is the most common variant of hormonal overproduction in ACC, occurring in about 30% of patients.⁵ Excess cortisol causes Cushing's syndrome, which results in a typical phenotype characterized by centripetal fat deposition, moonface, buffalo hump, muscle atrophy and striae. In addition, patients may suffer from hypertension, easy bruising, high blood glucose (steroid-induced diabetes) and even psychiatric problems (depression, anxiety, confusion).

Second most common is overproduction of androgens ($\pm 11\%$ of patients), which causes virilization in women.⁵ Symptoms include hirsutism, deepened voice, acne, temporal balding, increased muscle mass and clitoromegaly. In men, excess androgens may cause aggressive behavior, and a decrease of testicle volume and sperm count. A combination of glucocorticoid and androgen overproduction is observed in approximately 8% of patients.⁵ Overproduction of estrogens, mineralocorticoids or other steroid combinations are rare, being diagnosed in $<5\%$ of patients.⁵

In about half of all ACC patients, hormonal overproduction is not clinically apparent upon presentation. These patients typically seek medical attention because of abdominal pain, fullness or a palpable mass. Non-specific symptoms related to malignancy such as weight loss, night sweats or fever may also be present.

Histological examination

The diagnosis of ACC needs to be established by histological examination of the tumor. In order to predict malignant behavior of (adult) adrenocortical tumors, histological criteria such as invasive growth, nuclear atypia and presence of atypical mitoses should be assessed. Several scoring systems have been established, from which the Weiss-score is the most widely used.⁶ It consists of nine criteria that each represent one point on a scale of nine. A cumulative score of 3 or higher is correlated with malignant behavior, whereas a score of 2 is associated with ambiguous behavior. In case there is doubt regarding the tumor's origin, immunohistochemical tests may be useful. Expression of steroidogenic factor 1

(SF1) is currently the most valid marker to determine adrenal origin.⁴ In addition, recent research indicates that increased expression of the cellular proliferation marker Ki-67 is associated with a higher risk of disease recurrence.^{7,8} Awaiting further proof in ongoing trials, a cut-off value of 10% is used in determining whether a patient is at low or high risk for recurrence. Problematic aspect of this technique is large interobserver variability of the Ki-67 measurement when performed by (expert) pathologists. Preliminary results suggest that automated software assessment of the Ki-67 index may result in more consistent results and better correlation with clinical outcome.⁹

Staging

Disease staging is done according to the European Network for Study of Adrenal Tumors (ENSAT) staging system.¹⁰ Stage I and II are defined as localized tumors ≤ 5 cm or >5 cm, respectively ($T_1N_0M_0$ and $T_2N_0M_0$); stage III consists of tumors that infiltrate surrounding tissue or display positive regional lymph nodes or tumor thrombus in the caval/renal vein ($T_{3-4}N_{0-1}M_0$ or $T_{1-2}N_1M_0$); stage IV consists of patients with distant metastases ($T_{1-4}N_{0-1}M_1$).

Genomic characterization

In recent years, the focus of research has been directed at unraveling the process of carcinogenesis, i.e. the cascade of genetic alterations that ultimately results in the occurrence of a carcinoma. Better understanding of these alterations could be very useful because it might identify new therapeutic targets and contribute to better prognostication of individual patients. Two main pathways that are frequently altered in ACC are the Wnt/ β -catenin pathway (39% of cases in a recent series of 122 ACCs) and the p53/Rb pathway (33% of cases).¹¹ Unfortunately interindividual differences are large, given that the most frequently altered gene (ZNF3, related to the β -catenin pathway) was altered in only 21% of cases. Furthermore, genomic analysis suggests the presence of two distinct ACC subgroups. The first group harbors tumors with multiple mutations and DNA methylation alterations and is associated with poor clinical outcome. The second group harbors patients with a less aggressive and a more indolent clinical course. Tumors in this group display dysregulation of two specific mRNA clusters.¹¹

Treatment

Complete surgical resection of the primary adrenal tumor is the sole curative option. Some surgeons advocate a laparoscopic approach in selected cases, mostly because of short-term benefits as lower morbidity and shorter hospital stay. Others are strongly in favor of laparotomy, mostly because of the supposedly lower risk of tumor rupture. It is difficult to design a trial that settles this discussion, mainly because individual preferences and skills are highly influential on the outcome. A recent literature review of 23 retrospective studies addressing this question concluded that open surgery should remain the default option in ACC.¹² However, in case of 'limited size tumors' (<10 cm) radical resection through a laparoscopic approach should be technically feasible if performed by an experienced surgeon. If there is evidence of invasive disease, laparotomy should be performed as extensive resection might be necessary. Completeness of resection can be difficult to determine due to

the usually close anatomical relationship between tumor capsule and adjacent organs such as liver or kidney. As a consequence, in some cases the largest possible margin consists of tumor capsule only, which may be only few cell layers thick.

Even after complete resection, the recurrence rate is high with reported percentages of up to 60%.^{5,13-15} Adjuvant therapies are applied in order to reduce this recurrence rate. Adjuvant radiotherapy has not been tested yet in prospective series, but a reduction of local recurrences has been demonstrated in small retrospective patient series.^{16,17} In addition, retrospective studies described a prolongation of recurrence free survival using the adrenolytic drug mitotane as adjuvant treatment.¹⁸ At the moment, adjuvant mitotane is being evaluated prospectively (NCT00777244) among patients with low risk of recurrence (Ki-67 index $<10\%$).

Mitotane

Drug therapy with mitotane has since 1970 been registered for treatment of patients with irresectable or metastasized ACC.¹⁹⁻²¹ Mitotane is derived from the insecticide dichlorodiphenyltrichloroethane (DDT) and is administered orally in tablets in two to three daily doses. The drug exerts an anti-neoplastic effect on ACC tissue and in addition inhibits cortisol synthesis, which is beneficial in patients with Cushing's syndrome.²²⁻²⁵ The antineoplastic effect is correlated with the plasma level of mitotane. A therapeutic response was observed in patients with plasma levels >14 mg/L, which is therefore considered as the lower limit of the therapeutic window.^{26,27} Mitotane is well known for its toxicity, in particular for the gastro-intestinal tract (nausea, diarrhea, vomiting) and the nervous system (ataxia, forgetfulness, confusion).²⁸ Toxicity of mitotane appears to be correlated with its plasma concentration, which is why a plasma level of 20mg/L is considered the upper limit of the therapeutic window.²⁷ This may complicate clinical management, especially since little is known about mitotane pharmacokinetics. The drug's half-life is quite variable between patients but is in general extremely long (13-159 days).²⁹ Therefore, it takes a very long time (on average 3 months) to reach the therapeutic level between 14 and 20 mg/L. Patients on mitotane should receive supportive care to treat side-effects, for example anti-emetic and anti-diarrheal drugs. Also, co-administration of hydrocortisone in supraphysiological doses is necessary because of strong induction of CYP3A4 by mitotane, which increases glucocorticoid metabolism.³⁰ The strong CYP3A4 induction necessitates careful selection of supportive drugs.^{31,32} Furthermore, mitotane causes strongly decreased activity of 5 α -reductase, resulting in decreased levels of 5 α -reduced steroids.³⁰

Chemotherapy

In case of progressive disease despite mitotane monotherapy or presentation with extensively metastasized disease, chemotherapy should be considered. The regimen of choice consists of etoposide, doxorubicin, cisplatin in combination with mitotane.³³ In a recent randomized controlled trial, an objective response was achieved in 23% of patients.³⁴ Disease control (objective response or no disease progression for at least 8 weeks) was achieved in 58% of patients. A comparative study to establish the second-best chemotherapy

has not yet been performed. Based on retrospective studies, reasonable alternative regimens seem gemcitabine/capacitabine³⁵, thalidomide³⁶ or metronomic therapy.³⁷

Targeted therapy

Genomic analysis studies in ACC have identified several candidate genes for targeted therapy. Among the targets examined with receptor antagonists or –inhibitors were the epidermal growth factor receptor (EGFR), vascular endothelial growth factor (VEGF), tyrosine kinase pathway and mechanistic target of rapamycin (mTOR) pathway.³⁸⁻⁴⁵ In general, these studies demonstrated disappointing results without any objective response and with only few patients experiencing a short-lived stabilization of their disease. Interaction with mitotane due to the latter's strong CYP3A4 inducing effect may have influenced the therapeutic response in these studies through enhanced elimination of the drugs at study. One of the most frequently dysregulated pathways in ACC is the IGF signaling pathway, which is therefore a promising therapeutic target.¹¹ So far, therapies targeting the IGF system have not yet proven to be clinically beneficial in small trials. Results of the GALACTIC-trial with the monoclonal antibody and IGF1R antagonist linsitinib are expected in 2015 (NCT00924989).

Prognosis

Disease stage at diagnosis is the most important marker of survival. In a prospective cohort study including 492 patients, overall 5-year survival in patients presenting with localized disease (ENSAT stage I or II) was 82% and 61%, respectively.¹⁰ In patients presenting with locally advanced disease (stage III) overall 5-year survival was 50%. Within this group, survival is mainly influenced by the presence of positive lymph nodes, a venous tumor thrombus and infiltration of surrounding tissue. Patients with distant metastases (stage IV) form a heterogeneous group. In some patients disease progresses aggressively and hardly responds to treatment. As a result, survival is only a few weeks or months. In other patients, the disease follows a more indolent course and survival of up to several years has been observed.⁴⁶ As discussed earlier, this difference appears to be based on genetic differences between tumors.⁴⁷ Overall 5-year survival in patients with stage IV disease was 13% in the cohort study mentioned before.¹⁰ In another study, patients' age and number of affected organs were found to be important prognostic determinants in patients with stage IV disease.⁸ Furthermore, histological factors like a Weiss-score >6 and a ki67 index ≥20% were also associated with negative outcome.⁸

AIMS AND OUTLINE OF THIS THESIS

The present thesis was designed to investigate adrenocortical carcinoma from a clinical perspective with a focus on three major topics:

Part I: Epidemiology of adrenocortical carcinoma.

Part II: Diagnostic work-up of adrenal tumors and differentiation between benign and malignant lesions.

Part III: Treatment of adrenocortical carcinoma.

Part I: Epidemiology

As stated earlier, ACC is a rare disease. However, updated population-based studies on its incidence were lacking. In **chapter 2**, incidence and mortality of ACC from 1993 to 2010 in the Netherlands are described. In addition, trends in surgery and survival rates are evaluated. Among children, ACC occurs even less frequently than in adults. Again, updated population-based studies were lacking. In **chapter 3**, incidence, presentation, pathological characteristics, treatment and survival of pediatric patients with ACC in the Netherlands is described. Moreover, this chapter contains a comprehensive overview of the literature on pediatric ACC.

Part II: Diagnostic work-up

Due to increasing use of advanced medical imaging techniques, the incidence of serendipitously found adrenal tumors has risen as well. These so-called incidentalomas are mostly benign adenomas without autonomous hormone production. However, important diagnoses to exclude are (subclinical) Cushing's syndrome, primary aldosteronism, sex hormone overproduction, pheochromocytoma or malignancy. **Chapter 4** contains a clinical guideline on the diagnostic work-up of adrenal incidentalomas. After exclusion of most common diagnoses, there remains a category of adrenal lesions that are hormonally inactive and display non-specific imaging characteristics. **Chapter 5** provides a succinct literature review on the underlying pathology in this last category. Imaging and histological characteristics are discussed, as well as clinical management. Current diagnostic algorithms for differentiating between benign and malignant adrenal lesions largely depend on imaging studies, including follow-up studies. This strategy, however, has several disadvantages such as limited diagnostic value, health hazards and costs. In **chapter 6**, it is investigated whether urinary steroid profiling by gas-chromatography/tandem mass-spectrometry (GC/MS/MS) could be helpful in the characterization of an adrenal incidentaloma.

Part III: Treatment

Surgery is the only curative treatment option in ACC. Previous research has suggested that surgery of adrenal tumors with a high clinical suspicion for malignancy should be performed in an expert center. **Chapter 7** contains a study aimed at determining whether there are differences in survival between patients operated on in specialized Dutch Adrenal Network (DAN) hospitals or in non-DAN hospitals.

Mitotane is the only approved drug for treatment of ACC. Its pharmacokinetic properties are not fully elucidated and different dosing regimens have never been compared head-to-head. In **chapter 8**, the relationship between mitotane dose and its plasma concentration is examined by prospectively comparing two dosing regimens. This trial was nested within the international FIRM-ACT study. **Chapter 9** further elaborates on mitotane pharmacokinetics. The aim of this study was to develop a pharmacokinetic model of mitotane that enables clinicians to adjust the mitotane dose based upon a target drug exposure, which facilitates personalized therapy. In **chapter 10**, short-term variation of mitotane plasma levels are investigated prospectively. The aim was to study whether random sampling of mitotane plasma levels yields similar results as trough sampling.

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PART I: EPIDEMIOLOGY OF ADRENOCORTICAL CARCINOMA

The background features a dark gray field. On the left, a stylized DNA double helix is composed of numerous small, light gray dots. On the right, there is a large, low-poly geometric illustration of an animal's head, possibly a dog or wolf, rendered in various shades of gray.

CHAPTER 2

ADRENOCORTICAL CARCINOMA: A POPULATION-BASED STUDY ON INCIDENCE AND SURVIVAL IN THE NETHERLANDS SINCE 1993

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ABSTRACT

Background

The reported annual incidence of adrenocortical carcinoma (ACC) is 0.5-2.0 cases per million individuals. Updated population-based studies on incidence are lacking. The aim of this nationwide survey was to describe the incidence and survival rates of ACC in the Netherlands. Secondary objectives were to evaluate changes in both survival rates and the number of patients undergoing surgery.

Methods

All ACC patients registered in the Netherlands Cancer Registry (NCR) between 1993 and 2010 were included. Data on demographics, stage of disease, primary treatment modality and survival were evaluated.

Results

Included were 359 patients, 196 of whom were female (55%). Median age at diagnosis was 56 years (range 1-91). The 5-year age-standardised incidence rate decreased from 1.3 to 1.0 per one million person-years. Median survival for patients with stage I-II, stage III and stage IV disease was 159 months (95% CI 93-225 months), 26 months (95% CI 4-48 months) and 5 months (95% CI 2-7 months), respectively ($P < 0.001$). Improvement in survival was not observed, as reflected by the lack of association between survival and time of diagnosis. The percentage of patients receiving treatment within six months after diagnosis increased significantly from 76% in 1993-1998 to 88% in 2005-2010 ($P = 0.047$), mainly due to an increase in surgery for stage III-IV patients.

Conclusion

These nationwide data provide an up-to-date survey of the epidemiology of ACC in the Netherlands. A trend towards a decreasing overall incidence rate was observed. Survival rates did not change during this period despite an increased number of surgical procedures.

INTRODUCTION

Adrenocortical carcinoma (ACC) is an aggressive neoplasm with a reported annual incidence of 0.5-2.0 cases per million persons.¹⁻³ Data on the incidence of ACC are scarce, being based mainly on the United States National Cancer Institute (NCI) survey from the 1970s and a study of US Surveillance, Epidemiology and End Results (SEER) database published in 2006.^{2,3} The only European study on ACC incidence thus far has been a Norwegian study published in 1992.⁴ These latter two studies show an ACC incidence rate of 0.7 per million and 1.5 per million, respectively.^{2,4}

ACC can be part of rare hereditary syndromes (e.g. Beckwith-Wiedemann syndrome, Li-Fraumeni syndrome), but most ACCs occur as sporadic tumors, of which the molecular pathogenesis is poorly understood.^{5,6} Most patients present in the sixth or seventh decade of life and a female predominance has been reported.

Patients present with symptoms related either to a mass effect of the tumor or hormonal overproduction. Serendipitous discovery of ACC occurs in up to 16% of cases.⁷ In recent reports, overall survival remains low with a stage-dependent 5-year survival of 84% for stage I and 15% for stage IV.^{1,2}

Radical resection of the primary tumor is the only curative option for patients with local or locally advanced disease.^{1,5} Surgical treatment should also be considered in selected patients with distant metastases or recurrent disease, since this could yield a survival benefit.^{8,9} Treatment with the adrenolytic drug mitotane is the mainstay of therapy for metastasized disease.¹⁰ It is also used increasingly as adjuvant therapy: retrospective evidence suggests a significant increase in recurrence-free survival of 17 to 32 months.¹¹⁻¹³ A prospective trial to confirm these results (ADIUVO-trial) is currently recruiting. In advanced stages cytotoxic chemotherapy can be added to the treatment with mitotane. A regimen of etoposide, doxorubicin and cisplatin (EDP) is the most effective first-line therapy, as was recently demonstrated in a large multicenter trial.¹⁴ However, the objective response rate of EDP was only 23% and the median duration of progression-free survival was five months. New developments in treatment focus on targeted therapies, but major breakthroughs have not yet been reported.

The aim of this study was to present recent data on population-based incidence and survival of patients with ACC in the Netherlands. In addition, we examined whether treatment or survival of patients with ACC changed during the study period.

PATIENTS AND METHODS

Patients

Data were obtained from the Netherlands Cancer Registry (NCR), a nation-wide, population-based registry containing clinical data on cancer patients diagnosed since 1989. Completeness of case ascertainment is estimated to be at least 95%.¹⁵ Registration and coding is conducted

according to the guidelines of the World Health Organization and the International Association of Cancer Registries.¹⁶ The NCR contains data on all patients with histopathologically proven disease, as well as most patients with cancer diagnosed otherwise. In the Netherlands, hospital pathology departments all participate in a nationwide network (PALGA), thereby supplying NCR with data on patients and their corresponding diagnoses. The NCR also obtains data from the offices of hospital medical records, which provide lists of the diagnoses of both outpatients and hospitalized cancer patients. Trained registrars from the NCR extract patient and tumor characteristics from the medical records. Topography and histology are coded according to the International Classification of Diseases for Oncology, third edition (ICD-O-3).¹⁷ All tumors with ICD-O-3 topography code C74.0 (adrenal cortex) and classification 'malignant' were selected. The following data were used: age at time of diagnosis, diagnostic modality, sex, tumor laterality, stage of disease, treatment modality employed within the first six months after diagnosis (surgery, chemotherapy, radiation therapy, other) and overall survival. Notably, the use of mitotane is not registered in the NCR. Vital statistics in the NCR are updated on a yearly basis through a link with the Municipal Personal Records Database, which contains personal files for everyone who lives or has lived in the Netherlands. Malignant adrenocortical tumors have been registered in the NCR since January 1, 1993. In order to have at least one year of follow-up, the cut-off date for inclusion was December 31, 2010 and the end of the observation period was December 31, 2011.

In order to facilitate comparison of our data with other studies, disease staging was converted to the system proposed by the European Network for the Study of Adrenal Tumors (ENS@T-staging, Table 1).^{18,19} Because the NCR does not register tumor size for ACC, it was not possible to differentiate between ENS@T-stage I and II.

Table 1: Staging systems in adrenocortical carcinoma: conversion from Extent of Disease-code to ENS@T-stage of disease.

EoD-code	Explanation	ENS@T-stage
1	Carcinoma in situ	I-II
2	Localized in tissue of origin	I-II
3	Tumor infiltration into surrounding tissue	III
4	At least one positive lymph node	III
5	Tumor infiltration into surrounding tissue and at least one positive lymph node	III
6	Presence of distant metastasis	IV

EoD: Extent of disease. ENS@T: European Network for the Study of Adrenal Tumors.

Incidence analysis

The five-year age-standardised incidence rate per one million person-years was calculated. The European standard population was used for standardisation (European Standardised Rate, ESR).²⁰ For patients with multiple primary tumors (n=2) only information about the first primary tumor was used. Evaluation of the trend in incidence was performed by calculating the estimated

annual percentage of change (EAPC). Patients were clustered into three study periods of six years according to the time of diagnosis: 1993-1998, 1999-2004, 2005-2010.

Differences in age at diagnosis between these groups were tested for significance using the Kruskal-Wallis test. Distribution of sex, tumor stage at diagnosis, tumor laterality and treatment modalities were classified according to group and compared using the Chi-squared test. A *P*-value <0.05 was considered significant. The age-standardised incidence rates and the relative survival estimates were calculated with SAS V9.3, all other statistical analyses were performed using SPSS Statistics 19.0.

Survival analysis

Patients with a survival of zero days and a diagnosis established by autopsy (*n*=11) were excluded from survival analyses. Median survival was calculated using the Kaplan-Meier method and subgroups were compared using the log-rank test. One and 5-year relative survival were calculated according to group, age group and stage of disease at presentation. Relative survival is an estimation of disease-specific survival, being the absolute survival among patients with ACC divided by the expected survival for the general population adjusted for sex and age. For the 5-year survival estimates of the last period (2005-2010) only the patients diagnosed in 2005 and 2006 had 5-year follow-up. Recent changes in survival might therefore not be represented by standard survival estimates.²¹ Cox-proportional hazards regression analysis was used for multivariable analysis of risk factors for mortality. This analysis was based on absolute survival. The results of the Cox model are presented as a hazard ratio (HR) with a 95% confidence interval (CI).

RESULTS

Demographics

We included 359 patients diagnosed with ACC between January 1, 1993 and December 31, 2010. Among them were 163 male patients (45%, Table 2). Median age at diagnosis was 56 years (range 1-91), age distribution shows a peak in the sixth decade of life (Figure 1). Left-sided tumors were present in 186 patients (52%), four patients had bilateral tumors and laterality was unknown for eight patients (2%). Diagnosis of ACC was histologically confirmed for 99.7% (*n*=358) of patients.

At presentation, 117 patients (33%) had disease stage I or II. Stage III was diagnosed in 37 patients (10%) and stage IV in 125 patients (35%). For 80 patients (22%), stage was unknown. No significant changes were seen in stage at presentation during the study period (*P*=0.137). The number of patients with an unknown stage at presentation decreased from 41 (35%) in 1993-1998 to seven (6%) in 2005-2010 (*P*<0.001).

The 5-year moving-average age-standardised incidence rate in the Netherlands exhibits a decreasing trend: from 1.3 per one million person-years in 1993 to 1.0 per one million person-years in 2010 (Figure 2). The EAPC was -1.8% (95% CI: -3.8% to 0.2%, *P*=0.067). In females, the EAPC was -0.7% (95% CI: -4.0% to 2.7%, *P*=0.680), in males this was -2.9% (95% CI: -5.3% to

-0.4%, *P*=0.025). At the end of the observation period (December 31, 2011), 96 patients (27%) who had been diagnosed with ACC since 1993 were still alive in the Netherlands.

Table 2: Overview of demographic characteristics, disease staging and treatment modalities in patients with adrenocortical carcinoma according to period of diagnosis.

		Overall (n=359)	1993-1998 (n=119)	1999-2004 (n=130)	2005-2010 (n=110)	<i>P</i> -value
Sex						
[n (%)]	Male	163 (45)	64 (54)	48 (37)	51 (46)	0.028
	Female	196 (55)	55 (46)	82 (63)	59 (54)	
Age (yr)						
[median (range)]		56 (1-91)	58 (1-91)	54 (1-84)	58 (1-82)	0.519
Laterality						
[n (%)]	Left	186 (52)	62 (52)	64 (49)	60 (55)	0.797
	Right	161 (45)	51 (43)	61 (47)	49 (45)	
	Bilateral	4 (1)	3 (3)	1 (1)	0	
	Unknown	8 (2)	3 (3)	4 (3)	1 (1)	
Stage of Disease						
[n (%)]	Stage I-II	117 (33)	36 (30)	47 (36)	34 (31)	0.137
	Stage III	37 (10)	8 (7)	10 (8)	19 (17)	
	Stage IV	125 (35)	34 (29)	41 (32)	50 (46)	
	Unknown	80 (22)	41 (35)	32 (25)	7 (6)	
Treatment modality						
[n (%)]	Surgery alone	219 (61)	68 (57)	77 (59)	74 (67)	0.185
	Chemotherapy alone	16 (5)	6 (5)	5 (4)	5 (5)	
	Surgery + Chemotherapy	41 (11)	13 (11)	15 (12)	13 (12)	
	Other treatments	14 (4)	2 (2)	7 (5)	5 (5)	
	No treatment	67 (19)	29 (24)	25 (19)	13 (12)	
	Unknown	2 (1)	1 (1)	1 (1)	0	

P-value for comparison between groups (Chi-squared test). * No statistics computed due to small sample size.

Figure 1: Age distribution of patients with adrenocortical carcinoma diagnosed between 1993 and 2010.

Treatment

The majority of patients (n=219, 61%) underwent surgery as primary treatment. The percentage of patients who were not treated with surgery, chemotherapy or other treatments within six months after diagnosis decreased significantly from 24% in the first study period (1993-1999) to 12% in the last study period (2005-2010, $P=0.047$). The percentage of patients with stage III or IV disease receiving surgery as part of their initial treatment increased significantly from 52% (n=22) in 1993-1999 to 71% (n=49) in the last study period ($P=0.025$, not shown in table). There was no significant change in the use of chemotherapy or radiotherapy within six months after diagnosis between the different time periods.

All patients who presented with localised disease received surgery as initial treatment, some (12%) also underwent chemotherapy (Table 3).

Figure 2: Five-year moving average European standardised adrenocortical carcinoma incidence rates per 1,000,000 person-years.
ESR: European Standardised Rate

Table 3: Employed treatment modalities according to stage of disease in patients diagnosed with adrenocortical carcinoma between 1993 and 2010.

	Stage I-II (n=117)	Stage III (n=37)	Stage IV (n=125)	Unknown (n=80)
Treatment modality				
[n (%)]				
Surgery alone	103 (88)	31 (84)	41 (33)	44 (55)
Chemotherapy alone	-	-	14 (11)	2 (3)
Surgery + Chemotherapy	14 (12)	6 (16)	17 (14)	4 (5)
Other treatments	-	-	11 (9)	3 (4)
No treatment	-	-	41 (33)	26 (33)
Unknown	-	-	1 (1)	1 (1)

Survival

Overall survival

Survival analyses were based on 348 patients, since eleven patients with survival of zero days and diagnosis at autopsy were excluded. The overall median survival was 17 months (95% CI: 11-23 months). Figure 3 shows overall survival according to stage of disease at diagnosis. Median survival was 15 months (95% CI 0-33 months) for patients with an unknown stage of disease (not shown in figure).

Figure 3: Overall survival according to ENS@T stage of disease among 278 patients diagnosed with adrenocortical carcinoma between 1993 and 2010.
ENS@T: European Network for the Study of Adrenal Tumors.

Relative survival

No significant changes in relative 1-year survival were observed between the three periods, ranging from 61% (95% CI: 51-70%) in 1993-1998 to 61% (95% CI: 53-70%) in 1999-2004 and 56% (95% CI: 46-66%) in 2005-2010 (Table 4).

Relative survival was significantly lower among patients aged 60-74 years at diagnosis; 1-year survival was 46% (95% CI: 36-55%) and 5-year survival was 23% (95% CI: 13-32%) compared to 72% (95% CI: 63-80%) and 36% (95% CI: 27-46%), respectively, for patients aged 45-59 years at diagnosis.

Relative survival of patients with stage I-II disease was significantly longer compared to patients with stage III (90% versus 68% after one year and 62% versus 34% after five years). Furthermore, relative survival was significantly lower for stage IV patients compared to stage III patients (29% versus 68% after one year).

Multivariable Cox regression analysis showed that age >60 years at diagnosis and disease stage III or IV at diagnosis were significantly associated with mortality: age 60-74 years, HR 1.7 (1.2-2.4; $P=0.002$); age >75 years, HR 3.3 (2.1-5.2; $P<0.001$); stage III, HR 2.3 (1.4-3.6 $P=0.001$); stage IV, HR 7.0 (4.9-9.9; $P<0.001$).

Table 4: Relative survival in patients diagnosed with adrenocortical carcinoma according to period of diagnosis, age group and stage of disease and hazard ratios for mortality.

		Relative survival		Multivariable analysis	
		1-year [% (95% CI)]	5-year [% (95% CI)]	HR (95% CI)	P-value
Overall		(n=348)	60% (54-65)	32% (27-38)	-
Period of diagnosis					
1993-1998	(n=109)	61% (51-70)	35% (25-44)	1	
1999-2004	(n=129)	61% (53-70)	36% (27-44)	0.9 (0.9-1.2)	0.406
2005-2010	(n=110)	56% (46-66)	24% (14-34)	0.9 (0.7-1.3)	0.654
Age at diagnosis (years)					
0-44	(n=93)	68% (58-77)	42% (32-52)	1	
45-59	(n=115)	72% (63-80)	36% (27-46)	1.3 (0.9-1.8)	0.129
60-74	(n=105)	46% (36-55)	23% (13-32)	1.7 (1.2-2.4)	0.002
>75	(n=35)	37% (20-53)	-	3.3 (2.1-5.2)	<0.001
Stage of disease					
Stage I-II	(n=117)	90% (84-96)	62% (52-72)	1	
Stage III	(n=37)	68% (53-84)	34% (17-51)	2.3 (1.4-3.6)	0.001
Stage IV	(n=124)	29% (20-37)	-	7.0 (4.9-9.9)	<0.001
Unknown	(n=70)	58% (46-70)	33% (21-45)	2.2 (1.5-3.3)	<0.001

95% CI: 95% confidence interval. HR: Hazard ratio. P -value for Cox regression analysis based on absolute survival.

DISCUSSION

This study focused on the epidemiology of ACC in the Netherlands from 1993 to 2010 shows a trend towards a decreasing overall incidence rate of ACC, from 1.3 per million person-years in 1993 to 1.0 per million person-years in 2010. Over the last decade, surgical treatment was used increasingly as initial treatment for patients presenting with advanced disease (stage III and IV), but an increase in overall survival was not observed.

The observed incidence rate was higher than the rate of 0.72 per million reported in the study based on the SEER-database but comparable to a Norwegian study from 1992.^{2,4} The observed incidence rate in the latter study was 1.5 per million in the years 1970-1984 (n=99). Of note, this rate was not standardised according to the European Standardised Rate. Similar to the Norwegian study, our data were based on a national cancer registry. The SEER-study covered the years 1973-2000 and approximately 26% of the United States population (n=725).² The age-adjusted incidence rate reported in that study was determined using the 2000 US standard population. The etiology of the decreasing trend in overall incidence remains speculative. A possible explanation for this decrease is that patients with suspect and/or 'pre-malignant' adenomas increasingly undergo surgery, resulting in a lower number of true ACCs. However, we cannot underpin this hypothesis with clinical data. The influence of environmental factors on the pathogenesis of ACC is unknown. It is possible that changes in these factors, however unidentified, are associated with a decreasing incidence. Also, differentiating between adrenal adenomas and carcinomas can be difficult. It is possible that a shift towards more stringent classification of these tumors throughout the study period caused an apparent decrease in ACC incidence.

The observed female to male ratio of 1.2:1 is in agreement with the ratio found in the SEER-study (1.17:1), but lower than the sex ratio of 1.5:1 usually reported.^{22, 23} The incidence rate according to sex in the present study shows an even stronger female predominance in the time period 2000 to 2004. This finding, however, might be the result of variations due to chance.

The age distribution of ACC is usually characterized by a peak in childhood and in the fourth and fifth decades of life, with a median age at diagnosis around 51 years.^{2, 7, 24} In the current study, we observed a peak in the sixth decade with a median age at diagnosis of 56 years. Notably, we did not find a peak incidence in early childhood.²⁵ In agreement with previous reports, there was a small predominance of left sided tumors in our study population. The explanation for this difference in lateralisation remains elusive.⁴

In our cohort, the proportion of patients presenting with ENS@T stage IV was 35%. This is comparable to some previous studies^{7,8} but is at variance with other reports.^{26,27} Differences are likely to be explained by heterogeneity between the populations studied. For instance, in some studies only surgical patients were described^{26, 28} whereas other series also included patients who did not undergo surgery. In addition, studies differ in the extent of the diagnostic work-

up performed at presentation. It seems likely that all studies underestimate the percentage of patients with stage IV disease at presentation.

It has been suggested that more ACCs will be discovered at an earlier stage of disease as a result of increased application of medical imaging techniques.²⁵ In the present study, however, we did not observe such a trend. Due to limitations in the specificity of current diagnostic tools, it is possible that small ACCs are initially misclassified as benign and that they are only clinically recognized as ACC when growth has been demonstrated at repeat imaging. Currently, new and more specific diagnostic tools such as urinary steroid profiling are being evaluated for application in clinical practice.²⁹ We did observe a decreasing percentage of patients classified with disease stage 'unknown', which probably reflects improved registration and/or better diagnostics in recent years.

The percentage of patients in our study not receiving treatment within six months after diagnosis decreased significantly from 24% in 1993-1998 to 12% in 2005-2010. This is mostly accounted for by an increase in surgery as initial treatment for stage III and IV patients. It is remarkable that the use of chemotherapy did not increase over the years. This is probably related to scarce data on the efficacy of chemotherapy, the increasing number of patients with advanced disease who undergo surgery and the system of registration. The NCR only registers primary treatment, i.e. treatment administered within the first six months after diagnosis. We observed that more patients who present with metastasized disease undergo surgery within the first six months after diagnosis. Previous research indicated that surgical treatment in patients with stage IV disease could yield a survival benefit.⁸ On theoretical grounds, we expect a radical resection in stage IV patients to be beneficial. Also, debulking surgery might be associated with a survival benefit in selected patients with slowly progressive disease.

The present study reconfirms the value of the ENS@T staging system in discriminating between localized, infiltrative and metastasized disease.^{18,19} Overall survival rates observed in the present study are comparable to those reported in other large studies. A recently published overview on endocrine carcinomas in Europe reports a 5-year relative survival rate of 36% among 1464 patients with an adrenal carcinoma.³⁰ However, this study includes patients with a malignant pheochromocytoma, which impairs a fair comparison of the survival rates. Nonetheless, the results are in agreement with our findings: overall 5-year survival was 32% in the present study. The prognosis for metastasized disease is very grim, with only 5% surviving after three years. The relatively low survival rate for patients with localised disease (62% after 5 years) illustrates the aggressiveness of this malignancy. Improvements in survival over recent years have not been observed. This is in agreement with other studies and is mainly due to the lack of new, effective therapies.³¹

Our study has some limitations. Information on presenting symptoms, tumor size, histological characteristics, recurrences and mitotane treatment were not available in the NCR. Also, the lack of a central pathology review makes the NCR dependent on accurate registration of the true diagnosis by the local pathologist and physician. For this reason, we acknowledge that

some tumors could have been wrongfully classified as ACC. Also, true ACCs could be missing in our study if they have been wrongfully classified as another malignancy. It should be noted, however, that the present study represents the most complete population-based registration available which is not influenced by selection bias as is often a problem in institution- or network based studies.

In conclusion, these nationwide data provide an up-to-date assessment of ACC epidemiology in the Netherlands. A trend towards a decreasing overall incidence rate was observed. We found neither an increase in patients presenting with stage I-II disease nor an improvement of overall survival in recent years. The results underline the need for new and more effective treatments for adrenocortical carcinoma.

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CHAPTER 3

ADRENOCORTICAL CARCINOMA IN CHILDREN: FIRST POPULATION-BASED CLINICOPATHOLOGICAL STUDY WITH LONG-TERM FOLLOW-UP

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ABSTRACT

Adrenocortical carcinoma (ACC) is rare in both adult and pediatric populations. Literature suggests significant differences between children and adults in presentation, histological properties and outcome. Aim of this first nationwide study on pediatric ACC was to describe incidence, presentation, pathological characteristics, treatment and survival in the Netherlands.

All ACC patients aged <20 years at diagnosis and registered in the population-based Netherlands Cancer Registry between 1993 and 2010 were included. Clinical data were extracted from medical records. Archival histological slides were collected via the Dutch Pathology Registry (PALGA). We compared our findings to all clinical studies on pediatric ACC that were found on PubMed.

Twelve patients were identified: 8 females and 4 males. Median age was 4.1 years (range 1.1-18.6). Population-based age-standardized incidence rate for patients <20 years was 0.18 per million person-years. Autonomous hormonal secretion was present in ten patients. Seven patients were aged ≤ 4 years at diagnosis, five presented with localized disease and two with locally advanced disease. Five patients were aged ≥ 5 years, three presented with distant metastases and one with locally advanced disease. For all patients histological examination displayed malignant characteristics. All patients aged ≤ 4 years at diagnosis survived, median follow-up was 97 months (57-179 months). All patients aged ≥ 5 years died, median survival was 6 months (0-38 months).

In conclusion, pediatric ACC is extremely rare in the western world. Clinical outcome was remarkably better in patients aged ≤ 4 years. This is in accordance with less advanced stage of disease at presentation, but contrasts with the presence of adverse histological characteristics. Clinical management in advanced disease is adapted from adult practice in absence of evidence regarding pediatric ACC.

INTRODUCTION

Adrenocortical carcinoma (ACC) is a rare disease in both adult and pediatric patients. A recent population-based study estimated incidence between 1.0 and 1.3 patients per million person-years.¹ According to a study from the United States Surveillance, Epidemiology and End Results (SEER) database, incidence is even lower among patients under 20 years of age: 0.2-0.3 patients per million.² Several case series demonstrated that pediatric patients with adrenocortical tumors more often present with symptoms of hormonal overproduction than adult patients. Virilization and precocious puberty are the most common symptoms, reported in 80 to 100% of patients.³⁻⁶

Apart from differences in incidence and clinical presentation, there appear to be differences in biological behavior as well. Several authors reported significantly better outcomes in pediatric ACC patients compared to adult patients, even when tumors display similar malignant characteristics upon histological examination.^{7, 8} Overall 5-year survival in the SEER-study was 57% which is significantly higher than typical rates in adult populations which vary around 30% to 40%.² Moreover, survival in pediatric populations appears to be strongly correlated to age: survival rates >80% are reported in subgroups where age at presentation is <4 years.^{2, 4} Following the observed discrepancy between clinical outcome and histological characteristics, there are no clear-cut pathological criteria for malignancy in pediatric adrenocortical tumors, whereas adult tumors can be adequately classified based on the Weiss or Van Slooten score.⁸⁻¹⁰ This uncertainty poses a problem for clinicians that are confronted with suspect adrenal tumors in pediatric patients. The need for surgical resection in case of localized disease is evident, but the necessity of adjuvant therapy remains elusive. Also, in metastasized disease evidence on treatment options is scarce. Recently published results from the German GPOH-MET 97 trial emphasize the importance of radical surgery in localized disease and suggest that chemotherapy (Vincristine/Ifosfamide/Adriamycin or Carboplatin/VP16) in combination with mitotane could yield a survival benefit in metastasized disease.^{3, 11}

Aim of the present study was to investigate incidence, clinical presentation, pathological characteristics, treatment and clinical outcome of pediatric ACC in the Netherlands. Our primary objective is to assess the magnitude of the problem by describing the population and current clinical management. Our secondary objective is to identify possible prognostic factors by investigating whether there are clinical or histological characteristics correlated with clinical outcome.

METHODS

The Netherlands Cancer Registry (NCR) is a nation-wide, population-based registry containing data on cancer patients diagnosed since 1989. The NCR contains data on all patients with histopathologically proven disease, as well as most patients with cancer diagnosed otherwise. Completeness of case ascertainment is estimated to be at least 95%. Topography and histology are coded according to the International Classification of Diseases for Oncology. All tumors with ICD-O-2/ICD-O-3 topography code C74.0 (adrenal cortex), classification 'malignant' and age at

diagnosis <20 years were selected. Malignant adrenocortical tumors have been registered in the NCR since 1st January 1993, the cut-off date for inclusion was 31st December 2010 and follow-up was available for at least two years. Trained registrars from the NCR extracted clinical data from the medical records. Information on survival was included since vital statistics in the NCR are updated on a yearly basis through a link with the Municipal Personal Records Database, which contains personal files for everyone who lives or has lived in the Netherlands. Overall survival was calculated using the Kaplan-Meier estimator. The age-specific and age-standardized incidence rate for patients aged 0 to 20 years was calculated. The European standard population was used for standardization (European Standardized Rate, ESR).¹² In the Netherlands, hospital pathology departments all participate in a nationwide network and registry of histo- and cytopathology (PALGA), thereby supplying NCR with data on patients and their corresponding diagnoses.¹³ Archival tumor slides were collected through PALGA. All available slides were reviewed by an expert pathologist (RdK) and the Weiss-score, Van Slooten-index and Wienenke-index were determined. The Weiss-score and Van Slooten-index are widely used scoring systems to assess malignancy in (adult) adrenocortical tumors. We direct the reader to the literature for further details.^{9, 10, 14} The Wienenke-index is used to estimate malignancy in pediatric adrenocortical tumors.⁸ It consists of nine macroscopical and microscopical criteria that are scored as present or absent. The presence of up to two criteria is supposed to be associated with benign clinical outcome, three criteria suggests uncertain/indeterminate malignant potential and the presence of four or more criteria is associated with poor clinical outcome. Disease staging is defined according to the ENSAT classification, i.e. stage I and II are defined as localized tumors ≤5cm or >5cm, respectively (T1NoMo and T2NoMo); stage III consists of tumors that infiltrate surrounding tissue or display positive regional lymph nodes or tumor thrombus in the caval/renal vein (T3-4No-1Mo or T1-2N1Mo); stage IV consists of patients with distant metastases (T1-4No-1M1). In the Netherlands, anonymous use of clinical data and histological slides is permitted without explicit informed consent from the patient or legal representative. For the present research, this was confirmed by the medical research ethics committee of Máxima Medical Centre. Consent to review clinical data was obtained from the (former) local physician or his/her representative. The NCR's privacy committee and the board of PALGA agreed with the protocol.

RESULTS

Demographics

Twelve patients were identified in the NCR. There were 8 females and 4 males, median age at diagnosis was 4.1 years (range 1.1 to 18.6, Table 1). Seven patients had a left-sided tumor, five patients had a right-sided tumor. The diagnosis was histologically confirmed in all patients: nine patients underwent therapeutic resection of the tumor, in two patients biopsy of metastatic tumor-tissue was performed and in one patient the diagnosis was histologically confirmed after autopsy.

The age-specific and age-standardized incidence rate for patients <20 years of age was 0.18 per million person-years between 1993 and 2010.

Clinical signs and symptoms

Data on clinical presentation were collected for eleven patients (medical records of the twelfth patient could not be traced back). Ten patients presented with clinical signs of hormonal overproduction, which included moonface, acne, hirsutism, enlargement of the genitalia and progressively increased height (Table 1). Abdominal complaints were reported in five patients. In three patients changes in behavior were reported by the parents. One patient showed hyperactive behavior, one patient presented with insomnia and excessive crying and one patient presented with an increased need for sleep.

Laboratory investigations revealed increased testosterone serum levels in eleven patients. Androgen precursors androstenedione and dehydro-epiandrosterone-sulfate (DHEAS) were increased in ten and nine patients, respectively. Hypercortisolism was observed in four patients and estradiol was increased in two female patients.

Staging

Six patients presented with localized disease (stage I-II), five of whom were ≤4 years of age. Three patients presented with locally advanced disease (stage III), two of whom were ≤4 years of age. Distant metastases at presentation were reported in three patients, all aged ≥10. One patient had both lung and liver metastases, one patient had liver metastases only and one patient had lung metastases only. Of note, there were no patients aged ≥5 and <10 years in the present population.

Table 1: Overview of clinical signs and laboratory findings in twelve pediatric patients with adrenocortical carcinoma.

	Age ≤ 4 years							Age ≥ 5 years				
Patient case no.	1	2	3	4	5	6	7	8	9	10	11	12
Gender	F	F	F	F	F	M	M	F	F	F	M	F
Clinical signs												
Moonface	-	+	-	-	-	-	-	+	-	+	0	+
Acne	-	+	+	0	-	-	-	+	-	-	0	-
Hirsutism	+	0	+	+	+	+	0	+	0	0	0	0
Enlarged genitalia	+	+	+	+	+	+	+	+	-	-	0	0
Increased height	-	+	+	-	+	+	+	0	-	-	0	0
Laboratory findings												
Cortisol	-	-		+	-	=	=	+	+	=	0	+
Testosterone	+	+	+	+	+	+	+	+	+	+	0	+
DHEAS	+	+	+	+	+	+	=	+	0	+	0	+
Androstenedione	+	+	+	+	+	+	+	+	=	+	0	+
Estradiol	0	0		=	+	0	0	-	0	=	0	+

DHEAS: Dehydroepiandrosterone-sulphate; F: female; M: male
Clinical signs: + present; - absent; 0 unknown; Laboratory findings: + increased; - decreased; = within reference interval; 0 unknown.

Therapy

Nine patients underwent surgical resection of the primary tumor. A microscopically radical resection was achieved in all patients except one in whom tumor cells were observed in the resection margin of the suprarenal vein. In another patient, resection was attempted but the tumor turned out to be irresectable due to extensive growth into the vena cava (T4N0M0, stage III). In two patients resection was not performed due to irresectable tumor combined with the presence of distant metastases (stage IV). None of the patients received adjuvant radiation therapy.

Mitotane therapy was administered to three patients who were diagnosed with metastasized disease. Two patients received in addition multi-agent chemotherapy, i.e. cisplatin/etoposide/doxorubicine (EDP) schedule according to the FIRM-ACT protocol.¹⁵ Mitotane dosing ranged from 4 to 12 gram per day (per 1.7m² to 1.5m², respectively) and therapeutic plasma levels (>14mg/L) were reached in one patient. The best response of the two patients on combination therapy was partial response and stable disease, respectively. One of these patients was also treated with streptozotocin and etoposide/thalidomide/cyclophosphamide in a later stage. The third patient, who was on mitotane only, had progressive disease.

Pathology

Revision of pathology slides was possible in nine patients. In the remaining three patients representative pathology slides were not available: in two patients only a biopsy from a metastatic lesion was acquired and in one patient archival tissue acquired during autopsy showed necrosis only, while initially obtained material was not available anymore. Regarding the Weiss-score, the criteria mitotic rate (≥5/50 high power fields [HPF]) and diffuse architecture were present in all evaluated specimens. Invasion of tumor capsule was not observed. Presence of other criteria is summarized in Table 2A. Regarding the Van Slooten-index, the criteria ‘loss of normal structure’ and mitotic rate (>2/10 HPF) were present in all tumors examined. Other criteria are summarized in Table 2B.

Determination of the Wieneke-index resulted in a median score of 2 (range 1-6) across nine patients (Table 2C). It should be noted that tumor weight was registered in five patients only. Among patients aged ≤4 years at diagnosis (n=7), six had a Wieneke-index of 1 (n=3) or 2 (n=3) and one patient had a Wieneke-index of 6. Among patients aged ≥5 years at diagnosis (n=5), two had a Wieneke-index of 5. The remaining three patients had been diagnosed with metastatic and/or invasive disease upon presentation, also representative pathology slides of these patients were not available.

Table 2 A-C: Summary of individual elements from Weiss-score, Van Slooten-index en Wieneke-index in nine pediatric patients with adrenocortical carcinoma

A, Weiss-score		Total count
High nuclear grade		6
Mitotic rate ≥5/50 HPF		9
Atypical mitoses		5
Clear cells comprising <25% of tumor		8
Diffuse architecture		9
Confluent necrosis		6
Invasion of venous structures		4
Invasion of sinusoidal structures		5
Invasion of capsule		0
Total Weiss-score [median (range)]		6 (4-8)
B, Van Slooten-index (points per item)		
Regressive changes (5.7)		6
Loss of normal structure (1.6)		9
Nuclear atypia (2.1)		6
Nuclear hyperchromasia (2.6)		5
Abnormal nucleoli (4.1)		5
Mitotic rate >2/10 HPF (9.0)		9
Capsular and/or vascular invasion (3.3)		6
Total Van Slooten-score [median (range)]		21,7 (15,3-28,4)
C, Wieneke-index		
Tumor weight >400g		2 ^a
Tumor size >10.5cm		3
Extension into periadrenal tissue/organs		1
Invasion into vena cava		0
Venous invasion		4
Capsular Invasion		0
Presence of necrosis		6
Mitotic rate >15/20 HPF		4
Presence of atypical mitotic figures		5
Total Wieneke-score [median (range)]		2 (1-6)

^a Data available from five patients

Survival

Survival of all twelve patients was described. All patients aged ≤4 years at diagnosis survived (n=7), median follow-up was 97 months (range 57-179 months). In Table 3 individual pathology findings and survival data are summarized. All patients aged ≥5 years at diagnosis died from their disease (n=5). Median survival in this subgroup was 6 months (range 0-38 months). One patient had a disease-free interval of 22 months after resection of the primary tumor followed by treatment with mitotane, EDP and eventually surgical resection of lung metastases.

However, lung metastases recurred and progressed under treatment with second and third line chemotherapy. The patient died 38 months after primary diagnosis.

Table 3: Summary of individual disease staging, pathology findings and survival data in twelve pediatric patients with adrenocortical carcinoma

Patient case no.	Age ≤ 4 years							Age ≥ 5 years				
	1	2	3	4	5	6	7	8	9	10	11	12
Stage of disease (ENSAT)	I	III	II	I	I	III	I	IV	IV	III	II	IV
Tumor size (cm)	3.5	15.0	6.0	5.0	4.0	10.0	2.0	12.0	-	-	16.0	-
Weiss score	5	7	5	6	4	5	6	8	-	-	6	-
Van Slooten score	15.3	21.7	19.4	23.7	20.4	22.2	22.7	21.7	-	-	28.4	-
Wieneke index	1	6	2	2	1	2	1	5	-	-	5	-
Deceased	No	No	No	No	No	No	No	Yes	Yes	Yes	Yes	Yes
Survival (months)	57	179	92	178	97	159	91	38	6	1	25	3

ENSAT: European Network for the Study of Adrenal Tumors. Stage I: T₁N₀M₀; Stage II: T₂N₀M₀; Stage III: T₁₋₂N₁M₀ or T₃₋₄N₀₋₁M₀; Stage IV T₁₋₄N₀₋₁M₁.

DISCUSSION

This study provides a population-based description of epidemiology, clinical presentation, treatment and survival of all pediatric ACC patients diagnosed in the Netherlands between 1993 and 2010. Patients ≤4 years of age presented with clinically less advanced disease, even when histology showed supposedly malignant characteristics. Accordingly, overall survival in this subgroup was markedly better compared to the older patients. Clinical management in advanced ACC is adapted from adult practice in absence of specific evidence regarding the pediatric population.

The incidence rate in the present study is comparable to the rate reported in a recent study with data of the SEER-database, which presented an incidence rate of 0.21 per million person years.² To our knowledge, there are no other population-based reports of incidence apart from studies on a population in southern Brazil, where the incidence is up to 15 times greater due to a prevalent germline mutation of the TP53 gene (R337H TP53).¹⁶ The incidence rate of 0.18 per million person-years in a population aged <20 years corresponds to less than one diagnosis per year in a western country with 15.2 to 16.6 million inhabitants during the study period. Thus, pediatric ACC appears to be extremely rare in the western world. Table 4 provides a summary of all clinical studies and case reports regarding pediatric adrenocortical carcinoma that were found on PubMed. A total number of 910 patients (present report included) was found. However, overlap between several reports from the same region could not be excluded. When available, the presence of clinical signs of hormonal overproduction, stage of disease, age and survival of the included patients are displayed.

Table 4: Overview of clinical studies and case reports on pediatric adrenocortical carcinoma.

Reported	Inclusion	No. of patients ⁱ	Clinical hormonal syndrome	Stage at diagnosis ⁱⁱ				Age	5-year survival	Region	
				Local (I-II)	Loc. Adv. (III)	Meta (IV)	Unkn.				
Malign. Indet.											
Michalkiewicz (2004) ⁴	1990-2001	254 ACT	90%	192	25	37	0	3-2y (0-19y)	54.7% (48.7%-60.7%)	Brazil	
McAteer (2013) ²	1973-2008	85	-	41	10	28	6	- (0-19y)	57%	USA	
Wieneke (2003) ⁸	1965-1997	74	-	59	15	0	0	7.1y (mean) (-)	69%	USA	
Sandrini (1997) ²⁵	1966-1992	58 ACT	91%	41	4	8	5	4-3y (3d-15.7y)	-	Brazil	
Sabbaga (1993) ⁵	1969-1991	55	-	96%	45	10	0	<2: n=17 >2: n=38	< 2y: 83% > 2y: 36%	Brazil	
Redlich (2012) ³	1997-2011	50	10	80%	31	4	25	5-9y (0.2-17.8y)	<4y: 83% ≥4y: 50%	Germany	
Borges (2013) ²⁶	1991-2009	46	1	-	32	8	7	0	41m (5m-187m)	-	Brazil
Sbragia (2005) ²⁷	1980-2004	33 ACT	94%	27	3	3	0	0	27m (2m-96m)	-	Brazil
Ribeiro (1990) ²⁸	1966-1987	36	-	≥93%	31	5	0	0	- (0-15.7y)	45% ⁱⁱⁱ	Brazil
Klein (2011) ²⁹	1918-2009	29 ACT	66%	-	-	-	-	-	3-7y (-)	74% ^{iv} (60%-88%)	USA
Ribeiro (2001) ³⁰	1996-1999	27 ⁵	-	≥96%	21	6	0	0	3y (4m-13.5y)	-	Brazil

Ciftci (2001) ⁶	1970-1999	20	-	80%	4	13	3	0	6-7y (mean) (2.5-13y)	Turkey
Orhan (2006) ³¹	-	19	-	-	-	-	-	-	-	Turkey
West (2007) ³²	2007	18	1	100%	10	5	3	1	4-5y (<1-15y)	Brazil
Martins (2005) ³³	1979-1999	18	-	94%	-	-	-	-	3-5y (1-17y)	Brazil
Hanna (2008) ³⁴	1976-2005	16	-	61%	5	1	10	0	- (34d-19y)	USA
Chen (2011) ³⁵	1991-2010	15	-	90%	9	6	0	0	4-3y (0-16y)	China
Cagle (1986) ³⁶	1953-1983	21 ACT	≥95%	-	-	-	-	-	- (3m-16y)	USA
Zancanella (2006) ³⁷	2003-2004	11	-	100%	3	3	5	0	5y (2-11y)	Brazil
Narasimhan (2003) ³⁸	1989-2000	9 ACT	89%	-	-	-	-	-	2-5y (1.5m-11y)	India
Stewart (2004) ³⁹	1974-2003	8	-	88%	8	0	0	0	- (0.6-11y)	Canada
Magro (2012) ⁴⁰	2000-2007	7	1	≥60%	4	1	3	0	- (2m-210m)	Italy
McDonnell (2003) ⁴¹	1976-2001	5	7	≥67%	9	3	3	0	2-4y (0.5-15.6y)	Australia
Ahmed (2009) ⁴²	-	-	5	100%	5	5	0	0	21m (13m-28m)	USA
Cho (2012) ⁴³	1996-2010	3	-	67%	2	1	0	0	7y (7m-15y)	Korea

Table 4 continues on the next page

Continuation of table 4

Reported	Inclusion	No. of patients ⁱ		Clinical hormonal syndrome	Stage at diagnosis ⁱⁱ			Age	5-year survival	Region
		Malign.	Indet.		Local (I-II)	Loc. Adv. (III)	Meta (IV)	Unkn.	median (range)	(95% conf. int.)
23 case reports ⁴⁴⁻⁶⁶	1988-2013	24	-	≥67%	8	7	5	4	3y (0-12y)	2y ^{viii} (0.2-15y)
This report	1993-2010	12	-	83%	6	3	3	0	4.1y (1.1-18.6y)	50%

The Netherlands

Malign: Malignant; Indet: Indeterminate; Loc.Adv: Locally Advanced; Meta: Metastasized disease; Unkn: Unknown; y: year; m: months; d: days;
ⁱ In case no differentiation between adrenocortical carcinoma or other diagnosis was made, patients are registered as adrenocortical tumor (ACT)

ⁱⁱ Numbers in between columns indicate cumulative counts of both categories

ⁱⁱⁱ Event-free survival, estimated by interpolating in graph (exact value not given)

^{iv} 4-year disease-free survival

^v All patients had a p53 germline mutation

^{vi} Follow-up ranges from 6m-11y

^{vii} Follow-up ranges from 5m-9.5y

^{viii} In individual case reports, survival is specified in years

In the majority of our patients clinical signs of hormonal overproduction such as virilization or Cushing's syndrome triggered the diagnostic work-up that led to the discovery of ACC. Similar findings were reported by other investigators. In the adult population this is typically the case in only 60% of patients, although autonomous hormone secretion is estimated to be present in at least 80% of adult patients.^{17,18} Likely, symptomatic hormonal changes are more remarkable and thus sooner detected in children, whereas in adults such conditions may persist longer allowing tumors to grow and cause abdominal pain or discomfort.

We observed a striking correlation between clinical outcome and age at presentation. All patients ≤4 years at diagnosis were alive without evidence of disease after at least 5 years follow-up, whereas all patients aged 10 years or older died from their disease. The strong association between age and outcome was also reported in other series.^{3-5, 8} We acknowledge that in the present study disease stage was lower in the younger subgroup which might explain the better outcome, but perhaps age and stage of disease are directly correlated. It has been suggested that ACC in patients under 4 years of age originates from fetal adrenal tissue and that it is essentially another type of cancer.^{2, 7} A histological substrate to substantiate this theory has not yet been found. Certain biochemical features such as increased expression of IGF-II and placental alkaline phosphatase (PLAP) that are characteristic of fetal adrenal tissue have been demonstrated in pediatric ACC as well, suggesting a relation between the two.¹⁹ However, these features do not seem to be unique to patients under 4 years of age.²⁰ The age-related survival benefit does also seem to be present in patients from the Brazilian region where the p53 germline mutation is prevalent.^{5, 21} This could be compatible with extra-tumoral factors beneficial to survival in the youngest patients.

In our population, the Weiss-score and Van Slooten-index did not appear to be related to clinical outcome as they are in adults. It should be noted that low Weiss-scores were not expected in our population since we only included tumors initially determined as carcinoma by local clinicians and pathologists. Nonetheless, a correlation between high Weiss-scores (>6) and adverse outcome as seen in adults was not observed.²² When the Wieneke-index is attributed to our population, it appears this correlates better to clinical outcome than the Weiss-score and Van Slooten-index. Unfortunately, the limited sample size prevents a strong conclusion.

It was obvious (and expected) that complete surgical resection is the primary treatment of choice. We did not encounter patients in whom a diagnostic biopsy was performed before surgery was attempted. We expect that this is related to the high percentage of patients presenting with clinical symptoms, which suggests an indication for surgical resection anyway. We recommend to exercise restraint in performing biopsy of an adrenal tumor, especially when there are signs of virilization or precocious puberty. In the adult population, biopsy is contraindicated in patients without evidence of metastatic disease due to the high false-negative rate and the risk of complications.¹⁷

Adjuvant treatment with mitotane and/or radiotherapy were not administered to any patient. Expectative management after successful surgery seems to be adequate in patients with localized

disease and/or a low Wieneke-index, which applies to all patients aged ≤ 4 years at diagnosis in our study. In adult patients, adjuvant treatment with mitotane is currently being evaluated prospectively, while retrospective studies suggest it is indicated in patients with a ki-67 index $>10\%$.^{23, 24} Based on our data, it is not possible to make recommendations for patients with a high Wieneke-index and age ≥ 5 years. However, it is tempting to speculate that in the latter category the disease follows the 'adult' course and that similar clinical management should apply. Accordingly, adult clinical management was applied to three patients who presented with metastatic disease. Evidence on the efficacy of mitotane and cytotoxic chemotherapy in pediatric patients is scarce. In the GPOH-MET 97 trial, the duration of mitotane therapy and the achievement of therapeutic plasma levels were associated with increased overall survival.³ However, despite the commendable effort and outstanding organization of that study, the results were based on mitotane administration in 34 patients only. Interpretation is also complicated due to co-treatment with cytotoxic chemotherapy. Given the rarity of the disease, a prospective trial comparing different kinds of chemotherapy in pediatric ACC should be set up internationally in the same fashion as the FIRM-ACT-trial.¹⁵

Our population-based study identified twelve pediatric ACC patients who were diagnosed in a time span of eighteen years and who were treated in five different hospitals. It is intuitively logical to strive to concentration of care in a single centre for so few patients with a rare and complex disease.

In conclusion, these nationwide data provide an assessment of pediatric ACC epidemiology, clinical management and survival in the Netherlands. Population-based incidence in a western country was estimated at 0.18 per million person-years. The clinical outcome is remarkably better in patients ≤ 4 years of age. This is in accordance with a less advanced stage of disease at presentation, but contrasts with the presence of adverse histological characteristics in many of our young patients. In absence of adequate evidence regarding pediatric ACC, clinical management in advanced disease is adapted from the adult practice. Due to the rarity of this disease, clinical trials are likely to succeed only in international setting.

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PART II: DIAGNOSTICS OF ADRENAL TUMORS AND DIFFERENTIATION BETWEEN BENIGN AND MALIGNANT · LESIONS

The background features a dark gray field. On the left, a white, pixelated or noisy representation of a DNA double helix curves upwards. On the right, a low-poly, geometric illustration of an animal's head, possibly a dog or wolf, is rendered in shades of gray, looking towards the left.

CHAPTER 4

ADRENAL INCIDENTALOMA AND ADRENOCORTICAL CARCINOMA: A CLINICAL GUIDELINE ON TREATING THE UNEXPECTED AND A PLEA FOR SPECIALIZED CARE

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INTRODUCTION

An adrenal incidentaloma is an important clinical finding that is often considered harmless, but can be the tip of the iceberg. The term incidentaloma indicates an adrenal mass larger than 1 cm, incidentally discovered during imaging studies performed for reasons other than suspicion of adrenal pathology. Lesions identified during staging procedure or work-up for patients with a known extra-adrenal malignancy are not considered to be an incidentaloma.¹⁻⁵

The entity incidentaloma is not a new finding and has been reported for many years. Because of the increased use of imaging techniques and improvement in abdominal imaging, the frequency of incidentaloma findings is increasing as well. Recent studies undertaken using high-resolution computed tomography (CT) have reported an estimated prevalence of 4%.^{1,5} In autopsy studies, the prevalence ranged 0.2%-8.7%, depending on definitions used and age group, as there is an age-dependent occurrence of adrenal incidentalomas.¹⁻⁶ The estimated prevalence in patients younger than 30 years is < 1%, in contrast to a 7% frequency in patients 70 years of age or older.¹ With an aging population and advanced radiological techniques becoming more widely available, the increasing frequency of adrenal incidentalomas is of growing importance.

When an incidentaloma is found, it is of vital importance to make an early and reliable differentiation between benign and (potentially) malignant lesions, but also to assess tumor functionality. The mass can originate from either the adrenal medulla or cortex.⁴ Consequently, a spectrum of different pathological conditions may underlie an incidentaloma, all requiring a different therapeutic approach. As much as 38 different diagnoses have been reported in patients with a serendipitous discovered adrenal tumor.⁶ Most adrenal incidentalomas are clinically nonhypersecretory benign adrenal adenomas, with an estimated frequency of 70-80%, which cause no health problems. However, in 5-20% of patients who have no endocrinological signs or symptoms, analysis reveals subclinical hypercortisolism.^{1-3,5} Other frequently reported diagnoses besides a nonfunctioning adenoma include cortisolsecreting benign adrenal adenoma, adrenocortical carcinoma (ACC), pheochromocytoma, metastasis and aldosterone-producing adenoma. Although malignancy is rare, it is of great clinical concern because of the poor prognosis.^{2,3}

After recognition of an incidentaloma both patient and physician are faced with uncertainties regarding the course, likelihood of a malignancy and treatment of the adrenal mass. Unfortunately, no diagnostic or therapeutic strategy has been validated in prospective clinical trials. Thus, the diagnostic work-up as well as management of an incidentaloma is a growing public health challenge.^{1,3,5}

The goal of this chapter is to provide a diagnostic guideline, which contains information about clinical presentation, biochemical work-up and radiological imaging. In addition, this chapter offers practical recommendations for the management of adrenal incidentaloma, including follow-up, surgery, chemotherapy and radiotherapy. Furthermore, we present organisational

recommendations concerning the management of adrenal incidentaloma and emphasize the need for centralization of adrenal disease-research and patient care to improve treatment and provide patients with an opportunity to receive optimal care, as the beneficial effects of specialization have been proven multiple times in other rare diseases.

Table 1: Prevalence and clinical presentation of the most frequent types of adrenal incidentaloma.^{1,5}

Causes	Est. prevalence	Clinical presentation
Adenoma		
Subclinical Cushing's Syndrome	9 %	Weight gain with central obesity, flushes, proximal muscle weakness, polydipsia, cognitive changes
Primary aldosteronism	1.2 %	Nocturia, muscle cramps, polyuria, palpitations
Androgen overproduction	Rare	Hirsutism, acne and oligomenorrhoe
Nonfunctioning	73.9 %	-
Pheochromocytoma	4.7 %	Episodic headaches, tachycardia, generalized sweating, pallor, dyspnea, anxiety
Malignancy		
Adrenocortical carcinoma	4.8 %	Symptoms of functioning mass (see above), abdominal pain or fullness
Metastasis	2.3 %	Cancer-related symptoms (fever, unintentional weight loss)

DIAGNOSTICS OF INCIDENTALOMA

The first step in the evaluation of adrenal incidentalomas is establishing the definition of the tumor type, beginning with a thorough history taking and extensive physical examination, with attention to signs or symptoms of hormonal overproduction, a malignancy or pheochromocytoma. Furthermore, hormonal work-up and radiological imaging is required in the diagnostic evaluation of the adrenal mass.

History and physical examination

History

Signs suggestive of hormonal overproduction may include Cushing's characteristics, symptoms of hyperaldosteronism or sex hormone excess. Cushing's syndrome may be asymptomatic in the event of subclinical disease or present with weight gain and central obesity, flushes, proximal muscle weakness, and polydipsia. Furthermore, cognitive changes, such as irritability, depression or restlessness, may also be present. Hirsutism, acne, gynaecomasty and oligomenorrhoe may be symptoms of hypercortisolism or sex hormone overproduction. Features of primary hyperaldosteronism are nocturia, muscle cramps and polyuria in case of hypokalaemia and palpitations.^{1, 4, 5}

The classic triad of symptoms associated with a pheochromocytoma includes episodic headaches of variable duration, tachycardia and generalized sweating. However, this combination of symptoms is present in only in a small percentage of patients (10%).⁷ Characteristics less commonly present are pallor, dyspnea and anxiety and secondary, complaints of hyperglycemia, unintentional weight loss, arrhythmias and cardiomyopathy.^{1, 4}

An adrenocortical carcinoma may either present with signs of adrenal hypersecretion as mentioned above or symptoms related to mass effect, such as abdominal fullness or abdominal pain. Cancer-related signs (e.g. fever, unintentional weight loss) are less frequently present.^{1, 3-5}

Physical examination

Clinical features of Cushing's syndrome detected during physical examination are hypertension, central obesity, striae, facial rounding ('moon face') and supraclavicular and dorsocervical fat pads ('buffalo hump'), proximal muscle weakness, clitoris hypertrophy, acne and hirsutism. Primary aldosteronism is characterized by hypertension. In rare cases, female patients can present with signs of virilization (e.g. acne, hirsutism). In contrast, an estrogen secreting adrenal lesion can produce signs of feminization, such as gynaecomasty in the male patient. A pheochromocytoma may present with hypertension (paroxysmal or sustained), orthostatic hypotension, pallor and sweating on physical examination. Adrenocortical carcinoma may as well present the signs of hormonal overproduction mentioned above. In addition, a palpable mass may be present at abdominal examination.^{1, 3-5}

Hormonal evaluation

Additional hormonal work-up is necessary in evaluation of tumor functionality as well as the risk of malignancy or pheochromocytoma. Although an adrenal mass may appear clinically nonhypersecretory, up to 20% of patients with an incidentaloma may have hormonal dysfunction, which is associated with higher risk of morbidity, such as metabolic disorders and cardiovascular disease.^{4, 5}

Subclinical Cushing's Syndrome

The most frequently diagnosed endocrine alteration in patients with an incidentaloma is Subclinical Cushing's Syndrome (SCS), which refers to autonomous and dysregulated cortisol secretion by the tumor, which may cause mild cortisol excess without typical signs and symptoms of hypercortisolism.^{1, 4, 5} It is also known as subclinical autonomous glucocorticoid hypersecretion.² The average prevalence is 9% (range 1-29%, depending on criteria used).⁵ It is difficult to characterize, since clinical Cushing's syndrome is not present and patients may have normal 24-hour urinary free cortisol secretion.³ Therefore, late-night salivary cortisol and/or overnight dexamethasone (1 mg) suppression test is recommended to detect subclinical hypercortisolism.² ⁷ The optimal cut-off value is much discussed. A cortisol value greater than 138 nmol per liter (5 microg/dL) in response to dexamethasone is associated with glucocorticoid overproduction and has an estimated sensitivity of 98% and specificity of 80-98%.⁵ When a level between 50-70 nmol/L (1.8-2.5 microg/dL) is used as cut-off value, confirmatory testing is indicated, such as midnight plasma cortisol or serum ACTH level.^{1-3, 5} It remains controversial whether SCS is

associated with long-term morbidity as a result of continuous endogenous cortisol secretion, such as arterial hypertension, obesity, diabetes mellitus due to insulin resistance, atherosclerosis and osteoporosis that are classical signs of Cushing's syndrome. Furthermore, the natural course of SCS is still elusive, yet progression to obvious Cushing's syndrome appears to be minimal.³⁻⁵

Recent studies and own observations identify urinary steroid profiling as a very promising screening instrument for early differentiation between benign and malignant tumors. A quantitative analysis of steroid precursors by gas chromatography and mass spectrometry, reveals steroid patterns associated with particular clinical problems. A recently designed algorithm screens for nine metabolites in a 24-hour urine sample and has impressive test characteristics with high sensitivity and specificity.⁸

Primary aldosteronism

Primary aldosteronism, Conn's syndrome, is present in approximately 1.2% of patients with an adrenal incidentaloma.⁴ The textbook presentation comprises hypertension and hypokalaemia, however almost 40% of patients are normokalaemic.^{5,6} Therefore, serum potassium level is not considered a reliable screening method. Hormonal work-up includes routine measurement of ambulatory morning plasma aldosterone concentration (PAC)-to-plasma renin activity (PRA) ratio (PAC/PRA ratio) in hypertensive patients. This can be performed during treatment with antihypertensive drugs with the exception of beta blockers and aldosterone antagonists. A PAC/PRA ratio ≥ 30 and plasma aldosterone concentration greater than 0.5 nmol/L is indicative of autonomous aldosterone secretion. Since the PAC/PRA ratio is influenced by time of sampling and posture of the patient, the diagnosis needs to be confirmed by additional measurement of mineralocorticoid secretory autonomy (e.g. saline infusion test).^{1,2,5-7}

Sex hormone overproduction

Sex hormone-secreting adrenal tumors rarely present as an incidentaloma, since they are usually symptomatic (e.g. hirsutism, virilization, gynaecomasty). Androgen overproduction may be a feature of ACC, but measurement of androgens and their precursors in serum has a low diagnostic accuracy in differentiating malignant from benign adrenal masses. Routine measurement of androgen or estrogen production is not necessary in patients with an incidentaloma.^{1,6}

Nonclassic congenital adrenal hyperplasia may cause unilateral or bilateral adrenal lesions and is an uncommon cause (< 1%) of incidentalomas. Routine cosyntropin-stimulation testing with measurement of cortisol precursors is not warranted, unless the diagnosis is suspected based on clinical manifestation (hirsutism, acne, menstrual irregularities) or the presence of bilateral adrenal masses.^{1,6,7}

Silent pheochromocytoma

The estimated prevalence of a pheochromocytoma among patients with an adrenal incidentaloma is 4 - 7%. Although it is mostly a benign condition, it may cause significant mortality and morbidity. Hypertension is constantly present in only half of the patients and paroxysmal in approximately 30%. It is essential to diagnose a catecholamine-secreting pheochromocytoma, since it has

the potential to cause cardiac arrhythmias and hemodynamic instability even in asymptomatic patients. Therefore, routine measurement of fractionated metanephrines and catecholamines in 24-hour urine specimen is indicated in all patients presenting with an incidentaloma. Recent research reported the superiority of determination of fractionated plasma free metanephrines, with a diagnostic sensitivity of 99% and specificity of 89%. However, this method is not widely available.^{1-5,7}

Radiologic evaluation

Imaging studies that brought the incidentaloma to light should be reviewed with a focus on the adrenal glands, but will often be insufficient. The goal is to distinguish adenomas from malignant masses. Several imaging characteristics are used to assess the malignant potential and to provide information concerning appropriate management.

Computed Tomography

It is advised to perform an unenhanced CT-scan to help distinguish adenomas from nonadenomas, followed by a delayed contrast-enhanced sequence and computed wash-out percentage.⁹ The use of combined unenhanced CT and washout values can discriminate adenomas from other adrenal masses with 98% sensitivity and 92% specificity.⁴ Attenuation of adrenal masses is measured in Hounsfield Units. A low attenuation on CT before contrast administration indicates high lipid content and is found in adenomas. However, around 30% (range 10-40%) of adenomas do not have a large lipid content and consequently may be difficult to discriminate from nonadenomas.

Furthermore, size and appearance of the adrenal lesion may as well help to differentiate between benign and malignant tumors. The probability of an incidentaloma being an ACC is directly related to size of the lesion. A diameter greater than 4 cm is reported to have 90% sensitivity for identifying ACC, but a low specificity, since only approximately 25% of lesions greater than 4 cm are malignant. In addition, calcifications, necrosis and hemorrhage are indicative of a malignancy.^{3,6,7}

Magnetic Resonance Imaging

Magnetic Resonance Imaging (MRI) is equally effective as CT in differentiating benign from malignant adrenal masses.² A normal adrenal gland is characterized by an equal or slightly lower intensity than that of the liver on T1 and T2. In contrast, malignant lesions are hyperintense on T2-weighted images.^{4,6}

Positron Emission Tomography

Additional advanced radiological testing is generally not indicated. 18-Fluoro-2-deoxy-D-glucose positron emission tomography (PET) is highly sensitive in identifying malignant lesions. However, it is of limited use regarding the evaluation of adrenal incidentaloma (in patients without a prior history of malignancy).^{1,5,10}

Table 2: CT characteristics of the most frequent types of an incidentaloma^{1, 3, 6}

CT-characteristic	Adenoma	Pheochromocytoma	Adrenocortical carcinoma	Metastasis
Size	Usually < 4 cm	Large, usually > 3 cm	Large, usually > 3 cm	Variable, usually < 3 cm
Shape	Round, smooth margins	Round, smooth margins	Irregular, unclear margins	Oval, irregular margins
Attenuation on unenhanced CT	< 10 HU	> 10 HU	> 10 HU	> 10 HU
Washout (in 10 minutes)	Rapid, > 50%	Delayed, < 50%	Delayed, < 50%	Delayed, < 50%
Growth rate	Stable	Slow (usually)	Rapid (usually)	Variable
Other features	Rarely necrosis, hemorrhage or calcification	Hemorrhage, cystic necrotic areas	Necrosis, hemorrhage, calcification	Hemorrhage, cystic necrotic areas

HU = Hounsfield Units

Fine-Needle Aspiration

There is no evidence to support the routine use of computed tomography-guided fine-needle aspiration (FNA) in the diagnostic evaluation of an incidentaloma. It is rarely informative, since it has a high-false negative rate, and there is a risk of complications, such as hemorrhage, abdominal pain, pancreatitis and pneumothorax. Moreover, its added value over radiological imaging has not been established. In case of a suspected pheochromocytoma FNA is contraindicated, since manipulation of the tumor can potentially cause a hypertensive crisis. Furthermore, biopsy of an adrenocortical carcinoma may lead to tumorspill and consequently tumor recurrence along the needle track. The only role of FNA in the evaluation of an incidentaloma is in confirming metastatic disease in patients with a known extra-adrenal malignancy without other signs of metastases.^{1-3, 5-7, 11}

DIAGNOSTIC EVALUATION

The work-up leads to a preliminary conclusion which determines further management. The spectrum varies from benign adenoma to the presumption of malignancy or a pheochromocytoma.

Suspect adenoma

As noted before, the first step in evaluation of an adrenal incidentaloma is discrimination between a benign or malignant adrenal mass, in which radiological imaging by CT-scan has a fundamental role. Most adrenal incidentalomas exhibit characteristic features of adrenocortical adenoma (ACA). Adenomas typically present as small (< 4 cm) lesions, with clear margins and high lipid content, which is characterized by low attenuation (< 10 HU) on unenhanced CT. Furthermore, they display rapid washout of contrast medium (e.g. more than 50% after 10 minutes) (Figure 1).⁴

Figure 1: Washout sequence of an adrenocortical adenoma

In patients with non-functioning ACA, size of the adrenal mass is the major determinant in choice of management.^{4, 7} Over 60% of incidentalomas less than 4 cm in diameter are ACA, in contrast only 2% are malignant. For a small non-functioning adenoma surgical resection is not necessary, follow-up through CT-imaging and biochemical screening will suffice. In lesions larger than 6 cm the prevalence of ACC increases to approximately 25% and surgery is indicated.⁵ Management of adrenal masses with a diameter between 4 to 6 cm is less well defined. Because of a higher risk of malignancy in this subgroup of patient's surgical approach is recommended.

In about 20% of adrenal adenomas hormonal work-up reveals overproduction of aldosterone (0.5-1%) or cortisol (5-20%), which may have a negative influence on patient's health. Primary hyperaldosteronism is associated with increased risk of cardiovascular events. Additionally, patients with SCS may be at risk for potential morbidity attributable to cortisol overproduction. However, progression to clinical overt Cushing's syndrome is uncommon. Although management of ACA remains controversial, surgical resection is considered the treatment of choice when biochemical overproduction is confirmed.

Suspect pheochromocytoma

It is essential to exclude a pheochromocytoma in patients presenting with an adrenal incidentaloma, because they are potentially lethal even when clinically asymptomatic.¹

Increased metanephrines and catecholamines in 24-hour urine specimen or fractionated plasma free metanephrines in combination with features on CT, such as increased attenuation on unenhanced CT (>10 HU), prominent vascularity of the mass and delayed washout of contrast (<50% after 10 minutes), are highly suggestive of a pheochromocytoma.³ Characteristic indicative of pheochromocytoma on MRI, includes hyperintensity on T2-weighted imaging, with a approximately 92% sensitivity and 88% specificity.⁴ When a pheochromocytoma is suspected, surgical treatment is indicated. Patients should be adequately prepared pre-operatively by adrenergic blockade, to prevent a perioperative hypertensive crisis caused by manipulation of the tumor and subsequent catecholamine-release.

Suspect malignancy

Adrenocortical carcinoma

The risk of a malignancy is the main concern in patients with an incidentaloma. The prevalence of ACC in patients without a history of malignancy is estimated at 4.8%, which makes it the most commonly identified adrenal malignancy.³ Obviously, ACC significantly affects patient's health. It is an aggressive malignancy with a median survival of 19 months (range 8-29 months), as calculated from data of 191 patients diagnosed between 2000 and 2010 in The Netherlands. Prognosis of ACC is still mainly dependent on stage at diagnosis.¹² For that reason it is vital to make accurate decisions regarding the necessary diagnostic and therapeutic measurements.

A smaller tumor size corresponds with a lower tumor stage and consequently better prognosis. The risk of ACC is, as mentioned, associated with mass size. However, because the prevalence of adrenal adenoma is age-dependent, the presence of small adrenal masses in young patients should raise major concern of a potential malignancy. A malignant adrenal lesion typically presents as a larger mass (> 6 cm) and is characterized by an irregular border, high attenuation on enhanced CT (> 10 HU) and slow washout after contrast administration (see figure 2).³ Own observations from the authors show that although an ACC may appear clinically non-functioning, in about 80-95% additional hormonal work-up and urinary steroid profiling reveals presence of hormone excess.

When an adrenal malignancy is suspected, further investigation concerning cancer staging is warranted before directing the patient to surgery.

Metastasis

Tumors that frequently metastasize to adrenal glands include carcinomas of lungs, esophagus, kidney, colon, breast, liver, pancreas and stomach.¹ Metastases frequently occur bilateral and are variable in size, mostly smaller than 3 cm. Abdominal imaging may also reveal the presence of necrosis, hemorrhage or calcifications.⁶ Adrenal metastasis may cause beginning adrenal insufficiency. The suspicion of metastasis in an incidentaloma has clinical implications for prognosis and management and the search for a primary neoplasm is indicated. Resection of an isolated adrenal metastasis is associated with improved (disease-free and overall) survival. However, only in a limited number of cases an adequate treatment of adrenal metastasis is possible.³

Figure 2: Washout sequence of an adrenocortical carcinoma

SURGICAL TREATMENT OF INCIDENTALOMA

Based on results of the diagnostic evaluation of the adrenal incidentaloma, decisions are made for the required therapeutic approach. However, a prospective randomized comparison of laparoscopic versus open adrenalectomy has not been performed yet. Recommendations are made based on little known evidence and pragmatism.

Adenoma

When an adrenocortical adenoma is suspected, subsequent management is founded on size of the mass and functionality. As mentioned, in case of overproduction of cortisol, aldosterone or sex hormones, surgical resection of the mass is the treatment of choice. Mortality associated with adrenalectomy is estimated at less than 2%.² Laparoscopic approach allows for a minimal invasive procedure associated with less morbidity in the patient and shorter period of hospitalization, while surgical results are comparable if performed by an experienced surgical team.¹³ An important issue in resection of functioning adrenal masses is steroid suppletion

Table 3: Recommendations for management of adrenal incidentaloma

Suspicion	Management	Considerations
Adenoma		
Functioning	Laparoscopic resection	Post-operative steroid hormone suppletion
Nonfunctioning		
< 4 cm	Follow-up - CT-scan within 6-12 months - Hormonal work-up annually during 4 years	
4-6 cm	Risk group: - Repeat CT-scan within 6 months - Consider surgical resection - Urinary steroid profiling - Consider referral to specialized centre	Suspicious imaging phenotype: open adrenalectomy
> 6 cm	Laparoscopic resection	
Pheochromocytoma	Laparoscopic resection	Pre-operative preparation
Adrenocortical carcinoma	Referral to specialized centre Open adrenalectomy by specialized surgical team	Post-operative steroid hormone suppletion Additional treatment depending on stage

postoperatively, because of the risk of adrenal insufficiency, hemodynamic crisis and death. In most cases this can be tapered over time.

It is common practice to perform a surgical resection of incidentalomas larger then 6cm, even if they are non-functioning and there are no signs of malignancy. It is unclear whether this is a good indication for a surgical resection, as follow-up might be sufficient as well. In lesions smaller than 4 cm, surgical resection is deemed not necessary and follow-up is generally accepted as the correct management. For lesions between 4cm and 6cm in diameter, a clear recommendation is lacking. In this group, surgery might be the safest option regarding the increasing risk of malignancy, however the number needed to treat with respect to curing a carcinoma will be large. The other option is to repeat medical imaging on a shorter term, for example 3 months. We expect urinary steroid profiling to become a valuable instrument in differentiating between benign and malignant lesions in this particular subgroup.

Pheochromocytoma

In patients with an adrenal incidentaloma and suspicion of a pheochromocytoma, rapid surgical resection is the standard curative option, associated with an excellent prognosis.³ Due to potential perioperative catecholamine excess, removal of a pheochromocytoma is accompanied with unusual hemodynamic and technical conditions, which require thorough preoperative medical preparation and adrenergic blockade to minimize perioperative cardiovascular

Figure 3: Algorithm for diagnostic evaluation of adrenal incidentaloma
PAC/PRA ratio = plasma aldosterone concentration (PAC)-to-plasma renin activity (PRA) ratio. HU = Hounsfield Units. SCS = Subclinical Cushing's Syndrome.

morbidity. Furthermore, close perioperative monitoring is mandatory.^{13,14} Catecholamine release is suggested to be lower during laparoscopy than open adrenalectomy. Therefore, and because of the other benefits of laparoscopic surgery mentioned earlier, a laparoscopic approach is recommended in patients with an incidentaloma suspected for a pheochromocytoma.^{15,16}

Adrenocortical carcinoma

A radical surgical resection is the only chance of cure for patients with an adrenocortical carcinoma, so an aggressive surgical approach is warranted.^{17,18} A complete resection is possible in most cases when the diagnosis is suspected pre-operatively. Success rates drop significantly in cases where a carcinoma is not recognized before or during surgery, as follows from own observations from the authors. This emphasizes the need of a complete diagnostic work-up before the patient is directed for a surgical resection of an incidentaloma. The surgeon has to be prepared to perform an extensive resection and to keep the tumor capsule intact, as tumor spill is strongly associated with the occurrence of peritoneal carcinomatosis and a poor prognosis.¹⁷

¹⁹ Therefore, several authors recommend an open surgical approach instead of a laparoscopic

technique, which is being used increasingly in adrenal surgery.^{18, 20-22} This topic is controversial, as prospective studies are lacking and retrospective studies show contradictory results. We think that in general, a laparotomy is the safest option with respect to achieving a complete resection, although an expert surgeon in laparoscopic adrenalectomies might achieve better results than a less experienced surgeon can achieve performing a laparotomy.

We therefore recommend that these patients should be treated by a multidisciplinary team with at least an endocrinologist, a surgeon, an oncologist, a pathologist and an experienced radiologist. The team should evaluate all patients with a suspect adrenal incidentaloma and decide on which patients will be treated surgically. Peri-operative hydrocortisone suppletion is recommended in all patients. The surgical technique should be determined with respect to the preference and specific qualities of the surgeon. The pathological examination of the tumor requires special attention, as carcinomas might be difficult to recognize. Rating systems as the Weiss-score and the Van Slooten score should be applied to all adrenal tumors. Close follow-up using medical imaging is strongly recommended as the risk of recurrence is high, even after complete resection. The debate regarding adjuvant therapy with mitotane is still ongoing, but it is the opinion of the authors that this is recommended if the tumor has a ki-67 index >10%.²³

FOLLOW-UP OF NONFUNCTIONING ADENOMA

A much discussed matter in the management of patients with a non-functioning adrenal adenoma is the frequency and duration of follow-up evaluation. Recommendations regarding follow-up are aimed at identifying changes in size or functionality of the adrenal adenoma and to recognize lesions with malignant potential that have escaped detection on primary analysis. Research suggests that approximately 8% of non-functional adrenal incidentalomas increase in size by at least 1 cm during follow-up, whereas 3-4% decrease in size.^{5, 6} The majority of adrenal adenomas remain stable. In contrast, adrenocortical carcinomas usually display rapid growth. It is recommended to repeat adrenal imaging by CT-scan in patients with nonfunctioning adenomas smaller than 4 cm within 6-12 months after the initial discovery to detect size changes.²

Approximately 20% of adrenal adenomas which displayed no excess hormone secretion at time of discovery, become autonomous during subsequent period of 4 years. Lesions of at least 3 cm in diameter are more likely to develop subclinical hyperfunction in contrast to smaller tumors.⁴ It is reported that the risk seems to disappear after 3-4 years follow-up. Hyperaldosteronism or catecholamine hypersecretion occurs rarely during follow-up.² Cortisol overproduction is more likely to occur. Hence, annual repetition of hormonal work-up, including late-night salivary cortisol and/or overnight dexamethasone (1 mg) suppression test is recommended during 4 years of follow-up. Whether measurement of PAC/PRA ratio and determination of fractionated metanephrines and catecholamines in 24-hour urine specimen or fractionated plasma free metanephrines should be repeated, is left to the discretion of the clinician as the indication may vary per patient.

Further follow-up is not indicated in patients with an adrenal mass that remains stable on two imaging studies, done at least 6 months apart and do not demonstrate hormonal overproduction during 4 years of follow-up.² Recommendations regarding follow-up of patients with a functional adenoma operated on, have not been developed.

TREATMENT IN ADVANCED STAGES

Introduction

The occurrence of metastatic disease in patients with adrenocortical carcinoma is not rare, as 35% of patients present with stage 4 disease. 50% of patients who initially have had a curative resection, ultimately suffer a recurrence.^{24, 25} Even in advanced stages, a surgical debulking should be considered as our own observations indicate this might give a survival benefit. The backbone of treatment in advanced stages is formed by drug therapy with mitotane. Cytotoxic chemotherapy may be added, but response percentages vary. The role of radiation therapy remains disputed. Current experimental treatments include IGF-R blockers (OSI-906) and sunitinib.

Surgery

In their recent review article, Fassnacht and Allolio provide a flowchart for management of ACC, which advocates at least consideration of surgery in every stage of disease.¹² Surgery including metastasectomy should at least be considered in stage IV patients and should be pursued if technically feasible and if the patient is motivated and in appropriate physical condition. On the other hand, the absolute survival gain might not weigh up against morbidity after surgery in certain (older) patients. This implies that the decision to perform surgery should be tailored to individual cases and should be discussed in a multidisciplinary team including an experienced surgeon. An additional benefit in cases of hormonal overproduction is that surgery might help controlling hormonal excess.¹²

Repeat surgery should be considered individually, results indicate that this could be beneficial with regard to survival, especially if the interval between the two operations is more than 6 months.^{26, 27}

Mitotane

Mitotane is the only adrenal-specific agent available for the treatment of ACC.²⁸ The exact mechanism of action is not known, but it is proposed and generally accepted that mitotane is metabolized in adrenal mitochondria and causes cytotoxicity by oxidative damage through the production of free radicals.²⁷ Whatever the exact pathway may be, the main effect is focal degeneration of the fascicular and (particularly) the reticular zone, which clinically leads to adrenal insufficiency for which glucocorticoid substitution is needed.²⁹ When describing results of mitotane, one should differentiate between antitumor- and antihormonal effects. Regarding antitumor activity, mitotane has been assessed in several clinical studies, with variable results. Most studies were retrospective and comprised only small numbers of patients. Results show

that mitotane does have activity against ACC. Percentages vary, but most investigators report total or partial tumor responses in about 25% to 30% of cases.

Concerning hormone excess, therapy with mitotane is sufficient in the majority of patients. However, the onset of mitotane is slow due to its lipophilic properties and the resulting accumulation in adipose tissues. It can take up to three months before therapeutic levels are established, so in patients with severe hypercortisolism another agent must be used concurrently to treat this condition while mitotane levels are being built up. The recommended treatment in this situation would be ketoconazol, which is generally well tolerated. Other options, dependent of the case at hand, could be etomidate, mifepristone or metyrapone.^{27, 30}

Mitotane treatment with a plasma concentration >14mg/L is associated with prolonged survival.²⁸ Adverse effects occur in over 80% of patients and involve mainly the gastro-intestinal tract: anorexia, nausea, vomiting and diarrhea are frequently observed.²⁹ Reported symptoms caused by effects on the central nervous system are ataxia, speed disturbances, confusion and somnolence. Typically, all adverse effects are reversible after mitotane withdrawal.³¹

It is important to bear in mind that mitotane not only has adrenolytic effects, impairing adrenal steroidogenesis and thus inducing a need for replacement hydrocortisone, but also stimulates peripheral cortisol metabolism, so that hydrocortisone should be administered in higher doses. A second issue in managing patients on mitotane is monitoring thyroid hormone and thyroid stimulating hormone levels, as mitotane can decrease thyroid hormone as well. A third and possibly favorable interaction is the supposedly increased efficacy of cytotoxic chemotherapy when combined with mitotane. However, evidence on this topic is not conclusive. The proposed mechanism for this synergistic effect is the possible negative effect of mitotane on multidrug resistance proteins, as investigated in vitro, which could decrease the resistance of adrenocortical cancer cells to cytotoxic agents.^{29, 30}

Given the rarity of the indication and use of mitotane, it is recommended to leave treatment with mitotane to experienced doctors who are familiar with possible adverse events and are able to manage them.

Cytotoxic chemotherapy

Regarding cytotoxic chemotherapy, several combinations of agents have been tried so far. The highest response rates have been found in a trial with a treatment regimen combining mitotane with etoposide, doxorubicine and cisplatin (response rate 49%) and another trial with a treatment regimen combining mitotane and streptozotocine (response rate 36%).^{32, 33} Currently, these two regimens are being compared in the First International Trial in Locally Advanced and Metastatic Adrenocortical Cancer (FIRM-ACT). Results of this trial are expected in 2011.

Radiation therapy

Whether there is a place for radiation therapy in the treatment of adrenocortical carcinoma, is not clear yet according to the literature. Some authors claim to have accomplished favorable

results, like prevention of local recurrence and adequate pain relief in metastatic disease, whereas toxicity was low.³⁴⁻³⁶

Other investigators recommend a more conservative approach, seeing that re-operations in a post-radiation tumor bed would be more difficult and that the favorable results are not all too convincing, given the retrospective character of research so far.²⁷ One could argue that radiation therapy can be of use in a palliative setting, especially in alleviating pain or neurologic complaints caused by metastatic disease in bone or brain and that a prospective trial is needed to determine the efficacy in an adjuvant setting.

Future therapeutic agents

The insulin-like growth factor receptor (IGF-R) in adrenocortical carcinoma is regarded as a possible target for treatment. Both antibody and tyrosine kinase inhibitor trials targeted against IGF-R are in progress. A trial using sunitinib as therapeutic agent produced disappointing results, but a better understanding of the metabolic complexity of the disease might lead to better trials in the future. Other areas of interest are VEGFR inhibitors and FGFR inhibitors, but these have not been translated into clinical trials yet.

LIMITATIONS

Due to the limited evidence and guidelines, there are still multiple unresolved issues regarding management of incidentalomas, mainly concerning the duration of follow-up. The most important health risk in patients with an incidentaloma is related to several characteristics of the adrenal mass associated with a malignant mass or pheochromocytoma.³⁷ The rate of growth of a benign adrenal lesion remains unclear. Besides this, the percentage of patients that will develop hormonal overproduction when initial analysis was negative is uncertain as well. Furthermore, there is some concern regarding the side effects of repeated CT imaging. One report estimated the risk of fatal cancer due to exposure to ionising radiation during CT-imaging to be one in 430-2170.⁴ This is comparable to the chance of developing an adrenocortical carcinoma during 3-year follow-up of an incidentaloma. Additionally, a long follow-up period with repeated extensive hormonal work-up and radiological imaging is associated with enormous costs. Since the frequency of discovered adrenal incidentalomas is expected to increase and the use of abdominal imaging is also increasing, the cost-effectiveness of repeated hormonal work-up and imaging becomes an important issue in health care. However, in practice, choices of follow-up or treatment are based on psychological or social mechanisms, such as anxiety, doubt and risk aversion as well as cost-effectiveness. To elucidate these uncertainties prospective trials are warranted to evaluate the optimal diagnostic approach and management of an incidentaloma and provide an answer for unresolved questions.

ORGANIZATION OF CARE

The rarity of a number of adrenal disorders, such as an ACC or pheochromocytoma, and the dismal prognosis associated with an adrenal malignancy, requires a multidisciplinary approach

of each patient. In the event of an ACC, physicians often are not familiar with the disorder and its few available treatment options, resulting in inferior patient care. Given that a large part of diagnostics and management is based on pragmatism and expert opinion instead of prospective trials, additional studies concerning treatment and follow-up of adrenal tumors are necessary. In order to improve care in patients with adrenal disorders and stimulate scientific research, national and international collaboration is vital. In a number of European countries (France, Germany, Italy, The Netherlands), national networks have been set up to coordinate adrenal diseases-research and - patient care.^{38, 39}

In the southern region of The Netherlands, our hospital acts as a tertiary referral center for patients with adrenal tumors. We have provided local hospitals with a guideline for diagnostics and patient referral similar to the procedure described in this chapter. The subsequent centralization of these patients facilitates reliable pre-operative diagnostics and specialized surgery, of which the importance cannot be overemphasized. Too many patients each year see their chance of survival be ruined because an adrenal malignancy is not recognized before, during or even after surgery. Irradical resection and/or rupture of the tumor capsule in adrenocortical carcinoma is fatal without exception, but can often be prevented if treated by experienced doctors.

Therefore, we strongly support initiatives of centralization being deployed in other regions and countries, as the beneficial effects of specialization have been proven multiple times in other rare diseases.^{40, 41} Centralization and multidisciplinary approach is associated with more complete resections, improved survival and enhanced patient care. A secondary benefit is the facilitation of scientific research and participation in clinical trials in centralized populations of patients with a rare disease.

CONCLUSION

Due to the increasing discovery of adrenal incidentalomas, the diagnostic work-up as well as the management of incidentalomas is a growing public health challenge. Hormonal functionality and malignant potential of the lesion need to be evaluated. Incidentalomas are mostly benign nonhypersecretory adrenal adenomas, however important diagnoses to exclude are (subclinical) Cushing's Syndrome, primary aldosteronism, sex hormone overproduction, pheochromocytoma or malignancy (e.g. adrenocortical carcinoma, metastasis). Surgical treatment is recommended in all patients with a hormonally active tumor or a tumor larger than 6cm. Furthermore, surgery may be indicated in individual cases depending on radiological characteristics. In patients with nonfunctioning adrenal adenomas smaller than 4cm follow-up with CT-scan after 6-12 months and annual hormonal work-up for 4 years is recommended. An adrenocortical carcinoma is rare, but often lethal. Surgery is the cornerstone of initial treatment, whereas drug therapy with mitotane is inevitable in advanced stages. It is recommended that patients with adrenal disorders are treated in a multidisciplinary setting by experienced physicians. Centralization of care is strongly encouraged in order to improve patient outcome and to stimulate research and trial participation.

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CHAPTER 5

ADRENAL TUMORS WITH UNEXPECTED OUTCOME: A REVIEW OF THE LITERATURE

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ABSTRACT

The finding of an adrenal mass should induce a diagnostic work-up aimed at assessing autonomous hormone production and differentiating between benign and (potentially) malignant lesions. The common differential diagnosis in adrenal incidentaloma consists of (non-)functioning adenoma, pheochromocytoma, myelolipoma, metastasis and primary carcinoma. There remains a category of lesions that are hormonally inactive and display non-specific imaging characteristics.

We provide a succinct literature review regarding pathologies from this category. Imaging and histological characteristics are discussed, as well as clinical management.

In conclusion, an adrenal mass may present a diagnostic challenge. After exclusion of most common diagnoses, it can be difficult to differentiate between possible pathologies based on pre-operative diagnostic tests. Surgical resection of possibly harmful tumors is indicated, e.g. lesions with malignant potential or risk of spontaneous hemorrhage. Resection of an obviously benign lesion is not necessary, unless problems due to tumorsize are expected.

INTRODUCTION

Clinicians may be confronted with adrenal masses in four different scenarios. The first category comprises patients presenting with endocrinological symptoms suggesting adrenal origin, such as virilization or Cushing's syndrome as seen in selected adrenocortical adenomas and carcinomas. Hypertension, flushes and headache may be signs of pheochromocytoma or aldosterone-producing adenoma (Conn's syndrome). Secondly, patients may present with non-specific symptoms that turn out to be caused by an adrenal tumor such as pain, fatigue, weight loss or the sensation of an abdominal mass. Thirdly, adrenal metastases might be found in the work-up of another malignancy, for example lung cancer. Finally, an adrenal mass may be found incidentally during evaluation for non-related complaints: a so-called adrenal incidentaloma.

The common differential diagnosis includes six entities which account for the large majority of all adrenal masses. This will be discussed first. Secondly, we discuss a remaining category that consists of ten entities that are hormonally inactive and display non-specific imaging characteristics.

DIFFERENTIAL DIAGNOSIS

The common differential diagnosis in adrenal incidentaloma consists of non-functioning adenoma, functioning adenoma, pheochromocytoma and adrenocortical carcinoma. Myelolipomas and metastases from various malignancies are also common and should be included.^{1,2} The ranking by likelihood of these diagnoses varies depending on individual presentation. In general, most incidentalomas (70-80%) are benign adenomas which cause no symptoms. However, in 5-20% of patients who have no endocrine signs or symptoms, analysis reveals subclinical hypercortisolism.³⁻⁵ Pheochromocytoma makes up about 1.5-14% of incidentalomas, adrenocortical carcinoma (ACC) is found in 1.2-11%, aldosterone-producing adenoma in 1.6-3.3% and adrenal metastases in 1-18%.^{6,7}

DIAGNOSTIC WORK-UP

The diagnostic work-up should be aimed at assessing autonomous hormone production and differentiating between benign and (potentially) malignant lesions.

Evaluation of cortisol and (nor)metanephrine secretion should be performed in all patients presenting with an adrenal mass, even in absence of clinical signs of Cushing's syndrome or pheochromocytoma.^{3,6,8,9} Also, clinicians should be aware of the possibility of adrenal insufficiency in case of bilateral lesions. Screening for primary hyperaldosteronism by measuring plasma aldosterone concentration and plasma renin activity should be performed if hypertension and/or hypokalemia are present.^{6,8} The most accurate predictor to differentiate between benign and malignant masses is attenuation on unenhanced CT. If the lesion's attenuation value is ≤ 10 Hounsfield Units (HU), malignancy is extremely unlikely.¹⁰ In case of HU >10 , a contrast wash-

out sequence should be performed. A wash-out $>50\%$ after 15 minutes is indicative of adrenal adenoma. Combined use of attenuation measurement and washout values can be used to discriminate adenomas from other adrenal masses with 98% sensitivity and 92% specificity.¹¹

Percutaneous adrenal biopsy has high false negative rates and there is a risk of complications. Therefore, the only role of percutaneous biopsy in the evaluation of an adrenal mass is confirming metastatic disease in patients with known extra-adrenal malignancy and confirming the diagnosis of ACC when radical resection is deemed not possible.^{3,8}

REMAING PATHOLOGIES: A MIXED GROUP

There remains a category of lesions that are hormonally inactive and display non-specific imaging characteristics, which poses a diagnostic challenge. Here we discuss individual entities from this group. A summary of imaging and pathological characteristics of these lesions is provided in Table 1.

Primary adrenal lymphoma (PAL) is a rare finding with less than 200 cases described in the literature. In 70-80% of cases both adrenal glands are affected.¹²⁻¹⁶ On imaging studies, PAL typically presents as a large mass in which cystic or hemorrhagic components may be present. Homogeneous and heterogeneous lesions are reported in similar frequencies. Diffuse large-cell B cell lymphoma is the most commonly reported subtype, anaplastic large cell or T-cell lymphoma are only reported sporadically.^{12,16-18} Treatment consists of combination chemotherapy, sometimes preceded by surgery in cases of a large tumor mass.^{12,19} Prognosis depends heavily on treatment response, but a mean overall survival of 15 months has been reported.^{14,19}

Liposarcomas account for 45% of all retroperitoneal soft tissue sarcomas. Five histological subtypes are known, of which well-differentiated liposarcomas (WDLS) and dedifferentiated liposarcomas (DDLs) are most commonly found retroperitoneally.²⁰ DDLs is found as a focal lesion with low attenuation on T1-weighted MRI within a well-delineated, lipogenic and septated mass that is the WDLS in approximately 10% of all cases.²¹ Histologically, the dedifferentiated area is characterized by atypical nonlipogenic stromal cells with hyperchromatic nuclei that are scattered in fibrous septa. With increasing grade of dedifferentiation, cellularity and nuclear atypia is more prominent. Despite often severe nuclear deformities, the mitotic rate is not very high.²² Retroperitoneal liposarcomas are notorious for recurring and prognosis is poor: 5-year overall survival rates differ from 36 to 55%.²³⁻²⁵

Schwannomas originate from Schwann cells in peripheral nerve sheaths. Approximately 3% of schwannomas is located in the retroperitoneal space, where it may involve the adrenal gland and/or mimic an adrenal mass.^{26,27} All schwannomas display benign behavior, except for a poorly defined proportion of the rare subtype melanotic schwannoma.²⁸ The appearance of a schwannoma on CT-scan is a round and well-circumscribed mass, hypo or iso-intense compared to muscle that enhances after contrast administration.²⁹ On T1-weighted MRI images, signal intensity is intermediate and similar to muscle. On T2-weighted images, signal

Table 1: Summary of imaging and pathological characteristics of rare adrenal pathologies

Diagnosis	Number of cases	Imaging characteristics		Pathological characteristics		Clinical behavior
CT						
MRI						
Histology						
Immunohistochemistry +						
Primary adrenal lymphoma 12-14, 16-19, 51	< 200	Mostly hypodense tumors, aspect homo or heterogeneous, slight to moderate contrast enhancement.	Iso/hypointense in T1 and hyperintense in T2.	Atypical cells, anisokaryosis, hyperchromasia, necrosis.	Most common: CD45/CD20/CD40 (B-cell) CD3/CD30/CD43 (T-cell)	Malignant
Dedifferentiated liposarcoma 20-25, 52	>500	DDLs: Non-lipogenic, heterogeneous node within a well-delineated, lipogenic and septulated mass that is the WDLS	WDLS: >75% fat, nonlipomatous components are prominent thick septa. Nodular nonadipose areas may be present. DDLs within WDLS: low to intermediate on T1 and intermediate to high on T2.	Atypical nonlipogenic stromal cells with hyperchromatic nuclei, scattered in fibrous septa. Cellularity and nuclear atypia increase with dedifferentiation. Mitotic rate typically <8/10HPF.	MDM2, CDK4	Malignant
Schwannoma 26-30	>500	Round, well-circumscribed, hypo or iso-intense compared to muscle, enhancement post-contrast.	Intermediate on T1 (isointense to muscle), marked increase on T2.	Elongated spindle cells in areas of both high (Antoni A) and low cellularity (Antoni B).	NSE, microfilament, S100	Benign
Ganglioneuroma 31-33, 53, 54	> 60	Att. > 25 HU, homogeneous aspect, calcifications in 30%-60%.	Hypo-intense on T1, heterogen. hyperintense on T2.	Ganglion cells, spindle cells, nerve fibres.	NSE, synaptophysin, S100 and CD57.	Benign Rare: transformation to malignant nerve sheath tumor.

Table 1 continues on next page

Idiopathic adrenal haematoma 34, 55, 56	> 10	Variable: homo/heterogeneous depending on lesion's age.	High intensity on T1 in periphery of lesion suggests hemorrhage.	Hemorrhage, necrosis and hemosiderin.	-	Benign
Cavernous haemangioma 35-38	> 60	Heterogeneous, central cystic/necrotic components, calcifications, nodular peripheral enhancement post-contrast.	Homogeneous on T1, high intensity on T2.	Necrosis, cystic components, large vascular spaces, single lining of endothelium.	-	Benign Risk of spontaneous hemorrhage. Rare: transformation to angiosarcoma.
Angiomyolipoma 39-43	< 10	Heterogeneous: contains fat, possibly small enhancing foci.	-	Adipose tissue, smooth muscle fibres.	HMB45, MART1/MelanA, smooth muscle actin.	Benign Risk of spontaneous hemorrhage.
Epithelioid angiosarcoma 44, 46, 57-59	> 20	Irregular margins, nonhomogeneous density, calcifications.	High intensity on T2.	Vascular spaces lined by endothelial cells with epithelioid features, possibly pleiomorphism.	Factor VIII, also CD34 and UEA-1 (less specific).	Malignant
Leiomyosarcoma 45, 60	< 20	Heterogeneous, possibly also liquid components.	-	Spindle-shaped neoplastic cells, nuclear pleomorphism, giant cell formation.	Smooth muscle actin.	Malignant
Cyst 47, 49, 50, 61-63	> 600					

Diagnosis	Number of cases	Imaging characteristics		Pathological characteristics		Clinical behavior
		CT	MRI	Histology	Immunohistochemistry +	
Pseudocyst	39%	Fibrous wall, no endothelial/ epithelial lining, dependent on age septations, blood products, fluid—fluid level or soft tissue component.	Intermediate/high density in T1, marked bright up in T2.	Usually unilocular, no endothelium. Contains brown /reddish fluid. Connective tissue walls calcificated/ hyalinized.	-	Benign
Endothelial	45%	Thin wall (≤3.5 mm), smooth borders and pure cystic internal structure. Att. < 20 HU. No contrast enhancement.	-	Smooth endothelial lining, contains clear or milky fluid.	D2-40.	Benign
Epithelial	9%			Lined with cylindrcal epithelium.	Calretinin and WT-1.	Benign
Parasitic	7%	Floating membrane or daughter cysts, septal or mural calcifications, co-existent hydatid cysts of other organs.	-	Thick, possibly calcificated walls, parasites within.	-	Benign

DDLs: Dedifferentiated liposarcoma, WDLS: Well-differentiated liposarcoma, Att: Attenuation on unenhanced CT, HU: Hounsfield units, NSE: neuron-specific enolase, UEA-1: Ulex Europaeus Agglutinin 1, WT-1: Wilms tumor protein, Immunohistochemistry + : Positive immunohistochemical staining.

intensity is markedly increased.²⁹ Histologically, a schwannoma can be recognized by the presence of elongated spindle cells, organized in areas of both high and low cellularity, called Antoni A and B tissue.^{27, 29} Immunohistochemical staining is positive for neuron-specific enolase (NSE), microfilament proteins and S-100 protein, the neural protein in Schwann cells.^{27, 30} To our knowledge, there are no reports on recurrent retroperitoneal schwannoma after radical resection.

Ganglioneuromas typically arise from primordial neural crest cells present in the adrenal medulla.³¹⁻³³ Calcifications may be apparent on CT-scan in 30%-60% of cases. Unenhanced attenuation values are relatively high: >25HU. Biological behavior is benign in most cases, although malignant transformation is supposedly possible.³¹

Idiopathic adrenal haematomas may be discovered as incidentaloma, due to abdominal complaints or due to adrenal insufficiency. Imaging characteristics vary from well-demarcated homogeneous masses to heterogeneous lesions suspect for peri-adrenal infiltration.³⁴ Adrenalectomy is often performed in order to obtain a diagnosis.

Adrenal cavernous haemangiomas are very rare and have only been described in individual case reports.³⁵⁻³⁸ Recurrence after complete resection is not reported, however malignant transformation to angiosarcoma may be possible.

Adrenal angiomyolipomas are extremely rare with only five cases reported.³⁹⁻⁴³ These tumors are classified in the family of perivascular epithelioid tumors (so called PEComas). It may be difficult to differentiate this tumor from (ad)renal carcinomas on imaging studies and even upon histological examination. The presence of both adipose tissue and cells positively staining for muscle and melanoma markers are required for definitive diagnosis.

Adrenal leiomyosarcomas and epithelioid angiosarcomas are also exceptionally rare. Concise histomorphological examination combined with positive staining of specific immunohistochemical markers is necessary to confirm the diagnosis.^{44, 45} Invasion of peri-adrenal tissue and the occurrence of distant metastases are certainly possible, but complete resection in early stage could prevent this from happening.⁴⁶

Adrenal cysts form a subcategory which can be divided into pseudocysts, endothelial cysts, epithelial cysts, and parasitic cysts.⁴⁷ On CT imaging, differentiation from malignant cystic neoplasms or pseudocysts associated with malignant tumors is not possible.⁴⁸ Pseudocysts and endothelial cysts are both considered vascular lesions, the first originating from adrenal hemorrhage and the latter from a pre-existent vascular or lymphatic malformation.^{49, 50} Adrenal lymphangioma is a subtype of an endothelial cyst. Histologically, the diagnosis can be established by determining the endothelial origin of the cells through immunohistochemical staining (CD31, CD34, D2-40). Epithelial cysts are more difficult to characterize, as the adrenal gland lacks acini where such a cyst should originate from. An alternative explanation suggests embryonic

origin, where the cyst would develop from displaced mesothelial tissue.⁴⁹ Parasitic cysts are very rare, mostly caused by infection with echinococcus. However, the adrenal glands are involved in less than <0.5% of infected patients.⁴⁷ Of note, all adrenal pathologies may display cystic degeneration which should not be confused with these four subtypes of adrenal cysts.

CONCLUSION

An adrenal mass may present a diagnostic challenge. If a diagnosis is not established after exclusion of the most common diagnoses, a category remains that consists of rare entities. It may be difficult or even impossible to differentiate between these pathologies based on pre-operative diagnostic tests. Radical surgical resection is indicated in case of possibly harmful tumors, e.g. lesions with malignant potential, risk of spontaneous hemorrhage or increase in size over time. Clinicians should assess these issues using clinical judgment complemented with radiological evaluation of the lesion, aimed at characteristics summarized in the present study. This will result in resection of benign lesions, but this is inevitable given the uncertainty that may remain after complete diagnostic work-up. Surgical resection is not necessary if a lesion is judged to be certainly benign unless the size of the lesion causes problems, for example due to a mass effect on other abdominal organs.

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CHAPTER 6

DIAGNOSTIC VALUE OF URINARY STEROID PROFILING IN THE EVALUATION OF ADRENAL TUMORS

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ABSTRACT

Radiological examination may unexpectedly reveal an adrenal mass. Current algorithms for differentiating between benign and malignant lesions mainly rely on size and densitometry on unenhanced CT, which have limited specificity. We examined the diagnostic value of urinary steroid profiling by gas-chromatography/mass-spectrometry (GC/MS) in differentiating between benign and malignant adrenal tumors.

A retrospective study in two referral centers for patients with adrenal disease was performed. All urinary steroid profiles ordered for evaluation of an adrenal tumor between January 2000 and November 2011 were examined. Patients were diagnosed with adrenal cortical carcinoma (ACC), adrenal cortical adenoma (ACA) or other adrenal mass. Results of hormonal measurements, imaging studies, pathology reports and clinical outcome were retrieved from medical records. The diagnostic value of individual urinary steroid metabolites was determined by receiver operating characteristics analysis. Cut-off values were compared to reference values from an age and gender-standardized population of healthy controls.

Eighteen steroid metabolites were excreted in significantly higher concentrations in patients with ACC (n=27) compared to patients with ACA (n=107) or other adrenal conditions (n=18). Tetrahydro-11-deoxycortisol (THS) at a cut-off value of 2.35 $\mu\text{mol}/24\text{h}$ differentiated ACC from other adrenal disorders with 100% sensitivity and 99% specificity.

Elevated urinary excretion of tetrahydro-11-deoxycortisol (THS) was associated with a very high sensitivity and specificity to differentiate between an ACC and a benign adrenal mass. Urinary steroid profiling might be a useful diagnostic test for the evaluation of patients with an adrenal incidentaloma.

INTRODUCTION

An adrenal incidentaloma is an adrenal mass found coincidentally during a radiologic examination performed for reasons other than evaluation for adrenal disease.¹ The estimated prevalence of adrenal incidentalomas ranges from about 0.1% for general health screening with ultrasonography to 4.4% in older subjects examined with high-resolution CT scanning.² Optimal clinical management of adrenal incidentalomas has not been established as none of the proposed diagnostic algorithms has been validated prospectively.^{1,3-5} Key objectives of these algorithms are to determine whether hormonal overproduction and/or malignant disease is present. The reported frequency of adrenocortical carcinoma (ACC) among patients with adrenal incidentaloma varies from 1.2–12%.⁵ Assessment of malignancy risk is predominantly based on radiological characteristics such as size, shape and attenuation value of the adrenal mass. In addition, follow-up by monitoring growth rate with repeat CT or MRI-scans at various intervals during 1 to 2 years after discovery is generally advised for those patients not undergoing surgery after the initial evaluation.^{1,4-7} However, a strategy of repeat imaging is associated with certain risks for the patient and is unlikely to be cost-effective.⁸ Urinary steroid profiling (USP) might offer an alternative diagnostic tool for discriminating between ACC and non-ACC in individuals presenting with an adrenal incidentaloma, as it has been shown that ACC is often accompanied by alterations in the urinary steroid metabolome.⁹⁻¹¹ Currently, only few studies have examined the potential diagnostic value of USP in individuals harboring an adrenal tumor.¹²⁻¹⁵ Except for the study by Arlt et al¹³, these studies described a small number of subjects. Therefore, the primary objective of our study was to determine the diagnostic performance of USP in differentiating ACC from non-ACC in a large cohort of patients with adrenal tumors.

PATIENTS AND METHODS

Both Máxima Medical Center (MMC) and University Medical Center Groningen (UMCG) are referral centers for patients with adrenal disease. We retrospectively analyzed all urinary steroid profiles ordered for evaluation of an adrenal tumor at UMCG and MMC from January 1st, 2000 until November 1st, 2011. In addition, we included all adult patients with ACC diagnosed from January 1st, 2011 until January 1st, 2014 in whom a baseline urinary steroid profile was performed. The corresponding medical records were examined and the following data were retrieved: age, gender, symptoms and signs, laboratory measurements (i.e. hormonal tests), imaging studies, treatment, pathology reports, clinical outcome and follow-up.

Endocrine activity of the tumor was determined based on the results of locally applied hormonal assays. Glucocorticoid excess was defined as an abnormal result of the 1 mg overnight dexamethasone suppression test (cut-off value serum cortisol at 50 nmol/L) and/or as an elevated 24h urinary free cortisol excretion. Serum aldosterone to plasma renin ratio was determined to screen for primary aldosteronism, serum dehydro-epiandrosteronesulfate (DHEAS) and testosterone were measured to demonstrate hyperandrogenism (cut-off values based on individual age and gender-related upper reference limits). Plasma free metanephrines or urinary fractionated metanephrines were measured to detect a pheochromocytoma. Tumor

size and unenhanced CT-attenuation values were collected from the original radiological examination report. In case these were not reported, the original CT studies were re-examined by an experienced radiologist.

The final diagnosis was based on either the pathological examination of the resected adrenal gland or on the clinical course including the results of follow-up imaging studies in patients who did not undergo surgery. In patients with ACC, the Weiss-score (a set of nine histopathological criteria with prognostic value in adrenocortical tumors) was extracted from the original pathology reports and disease progression was staged according to the European Network for Study of Adrenal Tumors (ENSAT) staging system.^{16, 17} Patients in whom a final diagnosis of pheochromocytoma was made, were excluded from the present study.

Gaschromatography/mass-spectrometry (GC/MS) for determination of the USP was performed at the department of laboratory medicine of the UMCG. In summary, free and conjugated steroids were extracted from 1 mL urine by liquid-liquid extraction. Enzymatic hydrolyzation, re-extraction, and chemical derivatization formed methyloxime-trimethyl-silyl ethers from steroid conjugates. An Agilent 5973 instrument operating in selected-ion-monitoring (SIM) mode was used to achieve sensitive and specific detection and quantification of 22 selected steroid metabolites.¹⁸

Reference values for USP were established in a group of healthy volunteers (n=240) recruited from the LifeLines cohort, a three-generation population-based study.¹⁹ These subjects were stratified according to gender and age, with age ranging from 20 to 79 years and each decade comprising 40 subjects (male-to-female ratio 1:1).

Statistical analysis

Demographical characteristics were assessed using ANOVA for continuous variables and Pearson's chi-squared test for categorical variables. Between-group differences in steroid excretion were evaluated using the Kruskal-Wallis test followed by post-hoc analysis using the Mann-Whitney test with Bonferroni adjustment ($\alpha=0.008$). Receiver operating characteristics (ROC) curves were generated for those individual urinary metabolites which displayed a significant between-group difference. Sensitivity and specificity were calculated at cut-off values providing highest sensitivity. Cut-off values were compared with age- and gender-dependent reference values. Correlation between histological characteristics and steroid excretion in patients with ACC was calculated using Pearson's correlation analysis and expressed as Pearson's *r*. A two-sided P-value <0.05 was considered to be significant. Data management and statistical analyses were performed using Prism 6.0 (Graphpad Software, La Jolla, USA) and SPSS 19.0 (IBM, Armonk, USA).

RESULTS

Patients

We evaluated 152 patients with an adrenal tumor (52 males, 100 females), demographical characteristics are summarized in Table 1. The following diagnoses were established:

Table 1: Clinical and radiological characteristics of 152 patients who were evaluated for an adrenal tumor.

	Total (n=152)	ACC (n=27)	ACA _{functioning} (n=22)	ACA _{non-functioning} (n=85)	Other (n=18)	P-value
Age (years) [mean ± SD]	56 ± 13	57 ± 14	50 ± 12	58 ± 12	54 ± 17	0.043*
Sex						
[n (%)]						
Male	52 (34)	8 (30)	6 (27)	28 (33)	10 (56)	0.219
Female	100 (66)	19 (70)	16 (73)	57 (67)	8 (44)	
BMI (kg/m2) [mean ± SD]	28.0 ± 5.6	26.7 ± 4.9	29.1 ± 4.4	28.0 ± 5.7	28.8 ± 7.1	0.552
Hormonal overproduction						
[n (%)]						
Glucocorticoids	38 (25)	18 (67)	19 (86)	0	1 (6)	-
Androgens	14 (9)	14 (52)	0	0	0	
Estrogens	1 (1)	1 (4)	0	0	0	
Mineralocorticoids	4 (3)	0	3 (13)	0	1 (6)	
Tumor size (cm) [median (range)]	3.5 (0.8-17.0)	10.0 (5.3-17.0)	3.0 (0.9 - 5.0)	2.8 (0.8-10.0)	7.4 (2.3-14.0)	<0.001**
CT Densitometry (HU)						
[n]						
≤10	37	0	6	28	3	-
>10	41	13	2	22	4	
n/a	74	14	14	35	11	

ACC: adrenocortical carcinoma; ACA: adrenocortical adenoma; BMI: body mass index; P-value for comparison between groups; n/a: unenhanced CT-scan was not performed.
*Mann-Whitney U test with Bonferroni correction for comparison between individual groups (α=0.008) between ACA_{functioning} and ACA_{non-functioning}; P=0.005; other comparisons did not show significant results. ** Mann-Whitney U test with Bonferroni correction for comparison between individual groups (α=0.008) between ACC and ACA_{functioning}; P<0.001; between ACC and ACA_{nonfunctioning}; P<0.003; between ACA_{functioning} and Other: P<0.001; between ACA_{nonfunctioning} and Other: P=0.001.

adrenocortical carcinoma (ACC; n=27) and adrenocortical adenoma (ACA; n=107). In addition, there was a mixed group of 18 patients with a variety of diagnoses: benign myelolipoma (n=4), metastasis from an extra-adrenal primary tumor (n=4), adrenal hyperplasia (n=2), multinodular hyperplasia (n=2), cavernous lymphangioma (n=1), ganglioneuroma (n=1), hemangioma (n=1), cyst (n=1), non-Hodgkin lymphoma (n=1) and leiomyosarcoma (n=1). The majority of these patients were analyzed because of an adrenal incidentaloma (n=109, 72%).

A diagnosis of ACC was histologically confirmed in 25 subjects (93%). Median Weiss-score was 6 (range 4-8). In the remaining 2 patients, a clinical diagnosis of ACC was made based on imaging studies showing an adrenal tumor with metastases in combination with biochemical evidence of steroid hypersecretion. At presentation, the tumor stages according to the ENSAT classification were II (n=10), III (n=6) and IV (n=11).¹⁶ Hormonal overproduction was present in 20 patients (74%); hypercortisolism (n=18), hyperandrogenism (n=14) and hyperestrogenism (n=1). Histological confirmation was obtained in 28 subjects with ACA (26%) and in 13 patients from the mixed group (68%). In the remainder of subjects, a diagnosis of ACA was established by biochemical and imaging studies as well as by clinical follow-up (median 52 months; range 3-167 months). Patients with functioning ACA demonstrated hypercortisolism (n=19) or primary aldosteronism (n=3).

Radiological characteristics

Patients underwent radiological imaging with CT (n=126) and/or MRI (n=29). Median tumor size in patients with ACC was 10.0 cm (range 5.3-17.0 cm), significantly larger than the lesion size in patients with ACA (2.9 cm; range 0.8-10.0 cm) or in patients from the mixed group (7.4 cm; range 2.3-14.0 cm, P<0.001 for both; Table 1).

An unenhanced abdominal CT-scan was performed in 78 patients (51%). All adrenal lesions with attenuation value ≤ 10 HU (n=37) were found to be benign. The 41 adrenal tumors with an unenhanced attenuation value >10 HU included ACC (n=13), ACA (n=24), metastatic lesions (n=2), benign myelolipoma (n=1) and a leiomyosarcoma (n=1). At a cut-off value of 10 HU, unenhanced CT-densitometry was 100% sensitive and 57% specific for the presence of ACC.

Steroid metabolite excretion

Eighteen steroid metabolites were excreted in significantly larger quantities by patients with ACC compared to patients with non-ACC related adrenal masses (Figure 1). In contrast, patients with ACC excreted less of the metabolite allo-tetrahydrocorticosterone (allo-THB) compared to subjects without ACC (P=0.019). There were no significant differences in individual metabolite excretion between the groups with functioning ACA, non-functioning ACA and the mixed group.

ROC-analysis demonstrated that fifteen steroid metabolites had a sensitivity >90% for detecting ACC, of which 7 had a sensitivity of 100% (i.e. tetrahydro-11-deoxycortisol (THS), pregnanediol (P2), pregnanetriol (P3), etiocholanolone (E), androsterone, tetrahydrocortisol and tetrahydrocortisone, Table 2). The ROC curve for THS was found to have the largest AUC (1.0), with a sensitivity of 100% and a specificity of 99% at a cut-off value of 2.35 μmol/24h

(Figure 2). This cut-off value significantly exceeded each of the age and gender specific upper reference limits. Median THS excretion in patients with ACC, ACA or those belonging to the mixed group was 13.10 $\mu\text{mol}/24\text{h}$ (interquartile range 6.20-35.90 $\mu\text{mol}/24\text{h}$), 0.30 $\mu\text{mol}/24\text{h}$ (IQR 0.20-0.65 $\mu\text{mol}/24\text{h}$) and 0.40 $\mu\text{mol}/24\text{h}$ (IQR 0.20-0.63 $\mu\text{mol}/24\text{h}$), respectively. Excluding patients in whom the adrenal tumor had a CT attenuation value <10 HU ($n=37$) did not affect the diagnostic performance of THS excretion (AUC 1.0). Figure 3 displays the relationship between cut-off values, reference intervals and median excretion of four steroid metabolites showing the highest sensitivity and specificity (THS, P2, P3, E).

Median THS excretion in patients with ACC and ENSAT stage II, III or IV was 8.70 $\mu\text{mol}/24\text{h}$ (range: 2.40-45.20 $\mu\text{mol}/24\text{h}$), 10.00 $\mu\text{mol}/24\text{h}$ (range: 3.30-39.20 $\mu\text{mol}/24\text{h}$) and 30.40 $\mu\text{mol}/24\text{h}$ (range: 6.20-250.00 $\mu\text{mol}/24\text{h}$), respectively ($P=0.025$). Histopathological description of diameter and weight of the ACC was available for 23 and 13 patients, respectively. We found a significant correlation between THS excretion and ACC diameter ($r=0.477$, $P=0.021$), and a near-significant correlation between THS excretion and ACC weight ($r=0.553$, $P=0.050$).

Table 2: Receiver operating characteristics for individual steroid metabolites with sensitivity for detecting ACC $>90\%$.

Metabolite	AUC	Cut-off value ($\mu\text{mol}/24\text{h}$)	Sensitivity	Specificity
THS	1.000	2.35	100%	99%
P2	0.975	0.66	100%	60%
P3	0.960	1.45	100%	61%
E	0.960	2.47	100%	53%
A	0.839	0.35	100%	14%
THF	0.806	2.10	100%	5%
THE	0.700	3.20	100%	4%
PDL	0.884	0.15	96%	26%
11-KE	0.733	0.49	96%	18%
β -cortolone	0.793	0.45	96%	7%
α -cortolone	0.760	0.75	96%	5%
α -cortol	0.789	0.15	96%	4%
11-HA	0.699	0.15	96%	2%
11-HE	0.761	0.49	93%	25%
Polone	0.922	0.15	91%	83%

AUC: Area under curve. THS: tetrahydro-11-deoxycortisol; P2: pregnanediol; P3: pregnanetriol; E: etiocholanolone; A: androsterone; THF: tetrahydrocortisol; THE: tetrahydrocortisone; PDL: pregnanediolone; 11-KE: 11-keto-etiocholanolone; 11-HA: 11-hydroxy-androsterone; 11-HE: 11-hydroxy-etiocholanolone; Polone: epi-pregnanolone.

DISCUSSION

In a relatively large cohort of patients with primary adrenal tumors, we found that urinary steroid profiling (USP) by gaschromatography-mass spectrometry discriminates ACC from non-ACC with high diagnostic accuracy. In particular, we demonstrated that measurement of tetrahydro-11-deoxycortisol (THS) had the highest diagnostic test performance with a sensitivity and specificity of 100% and 99%, respectively. In addition, THS excretion was significantly correlated with ACC tumor size and stage.

It has been demonstrated in several studies that USP may often reveal an increased excretion of steroid metabolites in patients with ACC, even in those without clinical signs of hormonal overproduction.^{10, 12, 14, 15, 20} These observations suggest that USP might be a useful diagnostic tool in order to determine whether an adrenal tumor is either malignant or benign. Nearly all studies included a small number of patients with an adrenal tumor, limiting the external validity of these data. So far, only the study by Arlt et al. had a size comparable to the current series.¹³ These investigators established a specificity and sensitivity of both 88% for the nine most differentiating steroid metabolites after conducting a more complex analysis, i.e. generalized matrix learning vector quantization. In addition, they also identified THS as the most discriminative steroid in differentiating benign from malignant adrenal tumors. There are some differences between

Figure 1A-D: Median urinary excretion of 22 metabolites in patients with ACC, functioning adenoma, non-functioning adenoma or other adrenal tumors, organized per steroid class.

A: androsterone; E: etiocholanolone; DHEA: dehydroepiandrosterone; 11-KE: 11-keto-etiocholanolone; 11-HA: 11-hydroxy-androsterone; 11-HE: 11-hydroxy-etiocholanolone; THE: tetrahydrocortisone; THF: tetrahydrocortisol; allo-THF: allo-tetrahydrocortisol; THA: tetrahydro-11-dehydrocorticosterone; THB: tetrahydrocorticosterone; allo-THB: allo-tetrahydrocorticosterone; allo-P2: allo-pregnanediol; P2: pregnanediol; P3: pregnanetriol; Polone: epi-pregnanolone; THS: tetrahydro-11-deoxycortisol; PDL: pregnanediolone; PTL: pregnanetriolone. Whiskers indicate range, * indicate significant difference between ACC and other groups.

Figure 2A-D: Receiver operating characteristic (ROC) curve for tetrahydro-11-deoxycortisol, pregnanediol, pregnanetriol and etiocholanolone for the diagnosis of adrenocortical carcinoma.

the present study and the one by Arlt et al. which might explain why we established a higher diagnostic performance for USP. In all our subjects, USP was performed before removal of the primary ACC. In contrast, 22% of patients with ACC in the study by Arlt et al. were examined after removal of the primary tumor because of the presence of metastases. In view of the here described correlation between tumor size and THS excretion, it seems likely that the difference in diagnostic performance of USP can be partially explained by variation in tumor burden between the two populations at study. Presumably, a gradual increase in urinary THS occurs during development of metastatic disease. During follow-up after primary resection, THS levels below this cut-off value may not exclude the presence of residual or recurrent disease.

Figure 3A-D: Relationship between cut-off values, reference values and measured urinary excretion of tetrahydro-11-deoxycortisol, pregnanediol, pregnanetriol and etiocholanolone in 27 ACC patients.

The etiology of THS overproduction in ACC remains speculative. Under physiological conditions, ACTH stimulates the conversion of free cholesterol to pregnenolone in mitochondria. Further steroid hormone biosynthesis takes place on the endoplasmic reticulum, except for the final steps in glucocorticoid and mineralocorticoid synthesis.²¹ These reactions, catalyzed by CYP11B1 (11-deoxycortisol → cortisol) and CYP11B2 (11-deoxycorticosterone → aldosterone) take place on the inner mitochondrial membrane (IMM) and are stimulated by ACTH.²² The abundant presence of THS, a metabolite of 11-deoxycortisol, suggests a relative deficiency of otherwise normally functioning CYP11B1 and/or dysfunction of the enzyme itself.^{13, 23} Possible explanations for a relative deficiency include increased production of steroid precursors upstream due to malignant proliferation or impaired access of substrate to the IMM. Dysfunction of CYP11B1 may be caused by mutational changes inherent to ACC or diminished ACTH secretion due to negative feedback by increased levels of steroid precursors.

The results of our study suggest that USP could be a valuable diagnostic tool in analyzing an adrenal incidentaloma. Current diagnostic algorithms mainly depend on radiological characteristics for differentiation between benign and malignant adrenal tumors.^{1, 4-7} CT-scanning, however, has several disadvantages such as exposure to ionizing radiation, adverse effects of radiocontrast (nephropathy, allergic reactions) and costs.⁸ Thus, USP might offer a patient friendly, entirely safe and less expensive alternative diagnostic test for the evaluation of an adrenal incidentaloma. Further validation of this test is warranted and is the main objective of a recently started multicenter prospective study in the Netherlands (NCT02324647).

Like previous studies on this subject, the value of our study is limited by its retrospective design. In addition, selection bias could have influenced the results since it cannot be guaranteed that all patients with an adrenal tumor were evaluated with USP. Moreover, not all diagnoses were confirmed by histopathological examination. This is, however, in agreement with the clinical practice to exclude the diagnosis of ACC in case symptoms or signs suggestive for adrenal malignancy have not occurred during long term follow-up of the patient.

In conclusion, USP might be a useful diagnostic tool for discriminating between benign and malignant adrenocortical tumors. In particular, increased urinary excretion of tetrahydro-11-deoxycortisol (THS) was associated with an almost perfect diagnostic power. Prospective studies are required to establish the true diagnostic value of USP in patients with an adrenal incidentaloma.

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PART III: TREATMENT OF ADRENOCORTICAL CARCINOMA

CHAPTER 7

SURGERY FOR ADRENOCORTICAL CARCINOMA IN THE NETHERLANDS: ANALYSIS OF NATIONAL CANCER REGISTRY DATA

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ABSTRACT

Objective

Adrenocortical carcinoma (ACC) is a rare disease with an estimated incidence of 1-2 cases per 1 million inhabitants. The Dutch Adrenal Network (DAN) was initiated with the aim to improve patient care and to stimulate scientific research on ACC. Currently, not all patients with ACC are treated in specialized DAN-hospitals. The objective of our current investigation was to determine whether there are differences in survival between patients operated on in DAN and non-DAN hospitals.

Design

The study is set up as a retrospective, population-based survival analysis.

Methods

Data on all adult ACC patients diagnosed between 1999 and 2009 were obtained from The Netherlands Cancer Registry (NCR). Overall survival was calculated and a comparison was made between DAN- and non-DAN hospitals.

Results

The NCR contained data of 189 patients. Median survival of patients with ENS@T disease stage I-III was significantly longer for patients operated on in a DAN hospital (n=46) compared to patients operated on in a non-DAN hospital (n=37, 5-year survival 63% versus 42%). Survival remained significantly different after correction for sex, age, year of diagnosis and stage of disease in multivariate analysis (hazard ratio 1.96 (95% CI 1.01-3.81), $P=0.047$).

Conclusion

The results associate surgery in a DAN-center with a survival benefit for patients with local or locally advanced ACC. We hypothesize that a multidisciplinary approach for these patients explains the observed survival benefit. These findings should be carefully considered in view of the aim for further centralization of ACC treatment.

INTRODUCTION

Carcinoma of the adrenal cortex (ACC) is a rare disease with an estimated incidence of 1-2 cases per 1 million inhabitants.¹⁻³ The prognosis is stage-dependent with overall 5-year survival ranging from 84% for stage I disease to 15% for stage IV disease.⁴⁻⁷ Aggressive surgery is the treatment of choice, since radical resection is the only chance of cure.^{7,8} Specialization of surgical treatment in rare conditions has been a controversial issue for many years, but increasing evidence suggests that treatment outcome is improved by centralization of care in specialized, high-volume centers.⁹⁻¹² There is supporting evidence that this is also the case for adrenal surgery.^{13, 14}

In the Netherlands, ACC patient care has been concentrated in the (eight) University Medical Centers for many years, due to regional patterns in the assignment of patient care and the historic role of the university hospitals as a referral center for complex pathologies. The Dutch Adrenal Network (DAN) was founded in 2004 by local specialists in the Máxima Medical Center (MMC) out of special interest and expertise in ACC. The DAN consists of the eight university hospitals and MMC. The aim of the DAN was to formalize the existing practice of collaboration among these centers and to improve patient care, primarily for patients with adrenocortical carcinoma. The DAN stimulates (international) trial participation, facilitates research in adrenal diseases and organizes meetings of experts, where research progress and clinical cases are discussed. Currently, treatment of ACC-patients in the Netherlands is not performed exclusively in DAN-hospitals. Whether a patient is directed to a DAN-hospital is at the discretion of the treating physician.

Optimal treatment of ACC should be approached in a multidisciplinary way and possible combinations of surgery, adjuvant therapy with mitotane and cytotoxic chemotherapy for advanced stages must all be considered.^{7,15-18} Lack of adequate expertise and/or facilities could be an argument for centralization of treatment.^{4,19} A previous study from our group demonstrated that treatment in specialized centers was beneficial to ACC patients, because improved survival was observed in patients initially operated on in DAN-hospitals compared to patients initially treated in non-DAN hospitals.²⁰ That study included patients treated between 1965 en 2008 (n=175) and recruited patients only from DAN-centers, resulting in a selected population. The absence of data on patients that were treated in general hospitals and were never directed to a DAN-center was regarded as a serious limitation of that study. The objective of our current investigation was to confirm and strengthen the previous results using a population-based study design that excludes selection bias in the best possible way. The aim was to determine whether overall survival differs between ACC patients who underwent surgical treatment in a DAN-hospital and patients who were operated on in non-DAN hospitals.

SUBJECTS AND METHODS

The Netherlands Cancer Registry (NCR) is a nation-wide, population-based registry, which has been collecting clinical data on cancer patients since 1989. It contains data on all patients

who have histopathologically proven disease as well as most patients with cancer diagnosed otherwise. The registry also records the hospital where the patients were treated.

In the Netherlands, hospital pathology departments all participate in a nationwide network (PALGA), which supplies the NCR with lists of patients and their corresponding diagnoses. In addition, the offices of hospital medical records supply the NCR with lists of the diagnoses of outpatients and hospitalized cancer patients. Trained registrars from the NCR extract patient and tumor characteristics from the medical records. Topography and histology are coded according to the International Classification of Diseases for Oncology (ICD-O).²¹ For this study we selected adult patients who were diagnosed with ACC between 1st January 1999 and 31st December 2008. Pediatric patients were excluded because ACC in young children has been proposed to be a different disease entity than ACC in adolescents and adults. This is based on differences in presentation and survival and the strong association with p53 mutations in young children but not in adults.^{22, 23} The following items were used: sex, age at time of diagnosis, tumor laterality, disease stage, type of hospital in which surgery of the primary tumor was performed (DAN or non-DAN hospital), method of establishing diagnosis and overall survival. Notably, the use of mitotane is not registered in the NCR. Vital statistics in the NCR are updated on a yearly basis through a link with the Municipal Personal Records Database, which contains personal files for everyone who lives or has lived in the Netherlands. In order to have at least one year of follow-up, the end of the observation period was 31st December 2009. Disease stage of ACC is registered in the NCR according to the ‘Extent of Disease’ (EoD) staging, based on clinical information and histopathological examination of the tumor.²⁴ In order to facilitate comparison of our data with other studies, the EoD staging was converted to the system proposed by the European Network for the Study of Adrenal Tumors (ENS@T-staging), which is currently accepted as the ‘gold-standard’ in ACC research (Table 1).^{5, 25} Because the NCR does not register tumor size for ACC, it was not possible to differentiate between ENS@T-stage I and II.

Statistical analysis

Between-group differences for the continuous variable ‘age’ were evaluated using the Kruskal-Wallis test followed by post-hoc analysis using the Mann-Whitney test with Bonferroni adjustment ($\alpha=0.02$). The chi-square test was used for comparison of the remaining categorical variables.

Table 1: Staging systems in adrenocortical carcinoma: conversion from Extent of Disease-code to ENS@T-stage of disease

EoD-code	Explanation	ENS@T-stage (n)
2	Localized in tissue of origin	I-II (n=67)
3	Tumor infiltration into surrounding tissue	III (n=19)
4	At least one positive lymph node	III (n=7)
5	Tumor infiltration into surrounding tissue and at least one positive lymph node	III (n=1)
6	Presence of distant metastasis	IV (n=72)

EoD: Extent of disease. ENS@T: European Network for the Study of Adrenal Tumors. Sample size is indicated bij *n*.

The Kaplan-Meier estimator was used for univariate analysis of survival data. The log-rank test was used for unadjusted comparison of survival data between DAN and non-DAN hospitals. Cox-proportional hazards regression analysis was used for uni- and multivariate analyses for patients with ENS@T stage I-III disease. The proportional hazards assumptions were verified graphically. Stage IV patients were left out of the Cox-model because clinically this is a different category. Only palliative treatment is possible for these patients, which means that the influence of the covariable 'surgery' is not expected to be constant in time nor comparable to the other subgroups. Patients for whom stage of disease was unknown were also left out of the Cox model. The results of the Cox model are reported as a hazard ratio (HR) with 95% confidence interval (CI). A two-sided *P*-value < 0.05 was considered significant. All statistical analyses were performed using SPSS 19.0.

RESULTS

Patients

Included were 189 patients who were diagnosed with ACC between 1st January 1999 and 31st December 2008. The diagnosis was confirmed by histological examination in 183 patients (97%) and by cytological examination in five (3%). The diagnosis was based on clinical grounds in one patient.

Median age at the time of diagnosis was 55 years (range 22-84, Table 2). Median age was significantly higher in the non-operated group compared to the DAN-group (*P*<0.001) and in the non-DAN group compared to the DAN-group (*P*=0.009).

There were 111 female patients (59%). Left-sided tumors were seen in 103 patients (55%), there was one patient with bilateral tumors. Sixty-seven patients (35%) had disease stage I-II at time of diagnosis. Stage III was confirmed in 27 patients (14%) and stage IV in 72 patients (38%). There was no information on tumor stage for 23 patients (12%). Among all patients who underwent surgery (*n*=139), 70 had primary surgery in a DAN hospital (50%), 54 patients were operated on in a non-DAN hospital (39%) and 15 patients underwent surgery, but there were no data on the hospital in which the patients were operated on (11%). Fifty patients did not undergo surgery.

Survival

Overall median survival was 19 months (95% CI 8-29 months). Among patients with ENS@T stage I-II disease, median survival was not reached (Figure 1). In the ENS@T-stage III group, median survival was 22 months (95% CI 5-39 months) and in the stage IV group, this was 6 months (95% CI 2-10 months). Median survival was 19 months (95% CI 0-55 months) in the group with unknown stage of disease (not shown in figure).

Stage I-III

Among patients with ENS@T-stage I-III who were operated on in a DAN-center (*n*=46), median survival was not reached. After one year, 93% of patients were alive and five-year survival was 63% (Table 3).

Table 2: Patients' characteristics

	Total (n=189)	DAN surgery (n=70)	non-DAN surgery (n=54)	Surgery, unspec. (n=15)	No surgery (n=50)	P-value
Age (yr) [median (range)]	55 (22-84)	52 (22-74)	57 (28-80)	55 (29-79)	63 (30-84)	0.001*
Sex [n (%)]						
Male	78 (41)	26 (37)	25 (46)	6 (40)	21 (42)	0.783
Female	111 (59)	44 (63)	29 (54)	9 (60)	29 (58)	
Side [n (%)]						
Left	103 (55)	33 (47)	34 (63)	10 (67)	26 (52)	0.381
Right	84 (44)	37 (53)	20 (37)	5 (33)	22 (44)	
Bilateral	1 (1)	-	-	-	1 (2)	
Unknown	1 (1)	-	-	-	1 (2)	
ENS@T-stage						
I-II	67 (35)	35 (50)	26 (48)	6 (40)	-	<0.001
III	27 (14)	11 (16)	11 (20)	3 (20)	2 (4)	
IV	72 (38)	23 (33)	9 (17)	2 (13)	38 (76)	
Unknown	23 (12)	1 (1)	8 (15)	4 (27)	10 (20)	

Sample size is indicated bij N. DAN: Dutch Adrenal Network. Surgery, unspec.: Patients underwent surgery, but data on the hospital involved were not available. No surgery: Patients did not undergo surgery. ENS@T: European Network for the Study of Adrenal Tumors. *P*-value for comparison between all groups (chi-square test or Kruskal-Wallis test used where appropriate). *Mann-Whitney U test with Bonferroni correction: between DAN-center and non-DAN center, *P*=0.009; between DAN-center and no surgery, *P*<0.001; other comparisons did not show significant results.

Table 3: Survival according location of surgery in patients with ACC stage I-III

Stage I-III	Total (n=94)	DAN surgery (n=46)	non-DAN surgery (n=37)	Surgery, unspec. (n=9)	No surgery (n=2)	P-value
Median survival [months (95% CI)]	58 (10-106)	N.R.	49 (24-75)	26 (3-49)	-	0.044
1-year survival	84%	93%	78%	78%	-	
5-year survival	49%	63%	42%	22%	-	

DAN, Dutch Adrenal Network; Surgery unspec., patients underwent surgery, but data on the hospital involved were not available; NR, median survival not reached. *P* value for comparison between DAN surgery and non-DAN surgery (log-rank test).

Figure 1: Overall survival according to stage of disease

In the group operated on in non-DAN hospitals, median survival was 49 months (95% CI 24-75 months) ($n=37$, $P=0.044$, Figure 2). One- and five-year survival were 78% and 42%, respectively. The patients who underwent surgery, but for whom the hospital of surgery was unknown, had a median survival of 26 months (95% CI 3-49 months), with 78% and 22% 1- and 5-year survival, respectively. All patients for whom the hospital of surgery was unknown were operated on before 2004.

In univariate Cox proportional hazards analyses, age at diagnosis, stage of disease, and surgery in a non-DAN-hospital were significantly associated with the risk of death. In multivariate analysis, these effects remained significant: surgery in a non-DAN hospital, HR 1.96 (1.01-3.81), $P=0.047$; age at diagnosis, HR 1.05 (1.01-1.08), $P=0.005$; stage of disease, HR 3.08 (1.56-6.10), $P=0.001$ (Table 4).

Stage IV

Patients with stage IV disease who did not undergo surgery ($n=38$) had a median survival of 2 months (95% CI 1-3 months, Table 5). In comparison, patients who did undergo surgery regardless

of the type of hospital ($n=34$) had a median survival of 10 months (95% CI 4-16 months, $P<0.001$). There was no significant difference in survival between patients with ENS@T stage IV disease operated on in a DAN-hospital compared to patients operated on in a non-DAN hospital (median survival 9 months versus 20 months, $P=0.955$, not shown in table).

DISCUSSION

The results of our population-based study confirm that surgical removal of the primary tumor in a DAN-hospital is associated with a survival benefit compared to primary surgery in a non-DAN hospital for patients with local or locally advanced ACC.

Our previous study showed a survival benefit for patients who were operated on in DAN-centers compared to patients operated on in non-DAN centers, similar to the one observed in the present study in stage I-III patients.²⁰ The prior study contained only data of patients who had been treated in a DAN-center at any time during the course of their disease. This design resulted in a selected population because patients who are treated and followed up in non-DAN hospitals are missing. The present study is based on the independent Netherlands Cancer Registry and

Figure 2: Overall survival of patients with stage I-III ACC; surgery in DAN hospital compared to surgery in non-DAN hospitals

Table 4: Univariate and multivariate analyses of overall survival of 92 surgically treated patients with stage I-III ACC.

Variable			Univariate analyses		Multivariate analysis	
			HR	P-value	HR	P-value
Sex	female	(n=56)	1		1	
	male	(n=36)	0.93 (0.50-1.74)	0.815	0.80 (0.42-1.51)	0.489
Age			1.04 (1.02-1.07)	0.002	1.05 (1.01-1.08)	0.005
Year of diagnosis			1.08 (0.96-1.21)	0.180	0.97 (0.84-1.12)	0.656
ENS@T-stage	stage I-II	(n=67)	1		1	
	stage III	(n=25)	2.68 (1.43-5.03)	0.002	3.08 (1.56-6.10)	0.001
Surgery in DAN-hospital	Yes	(n=46)	1		1	
	Unknown	(n=9)	2.79 (1.16-6.75)	0.023	1.80 (0.69-4.65)	0.227
	No	(n=37)	1.94 (1.01-3.75)	0.047	1.96 (1.01-3.81)	0.047

ENS@T: European Network for the Study of Adrenal Tumors. DAN: Dutch Adrenal Network. HR: Hazard ratio for mortality. P-value for Cox-regression analysis (univariate and multivariate as indicated).

Table 5: Survival in patients with ACC stage IV with and without surgical treatment

Stage IV	Total	Surgery	No surgery	P-value
	(n=72)	(n=34)	(n=38)	
Median survival [months (95% CI)]	6 (2-10)	10 (4-16)	2 (1-3)	<0.001
1-year survival	25%	47%	5%	
5-year survival	4%	9%	0%	

Sample size is indicated by n. P-value for comparison between Surgery and No surgery (Log-rank test).

includes patients diagnosed between 1999 en 2009. This results in a population that approaches as close as possible the true number of all ACC patients in The Netherlands. Compared to the prior study, an additional 98 patients from 1999 to 2008 are included, from which 30 were operated on in a non-DAN center, 16 in a DAN-center and 15 in an unspecified hospital. Thirty-seven patients did not undergo surgery.

A German study suggested that patients who were initially treated in a specialized center had a better outcome than patients initially treated in a general hospital.⁴ However, statistical power was limited and a survival benefit was only demonstrated for stage II patients. Arguments in favor of centralization were that patients would benefit from the more frequent use of adjuvant therapy, expertise of experienced physicians and a more individually tailored follow-up. Another recent series reports superior disease-free survival for a group of 28 patients who underwent primary surgery in a specialized center compared to 190 patients who were transferred to that institution after treatment in other hospitals.²⁶ Similar to the previous Dutch study, these studies are compromised by selection and referral bias, because all patients were included solely from specialized centers. In such a design, the number of patients curatively treated in other hospitals without recurrent or metastatic disease remains unknown. The strength of the current study design is that selection bias is virtually ruled out, as all confirmed cases of ACC are included.

Notably, the total number of 189 patients matches with an expected incidence of 1-2 per million for a population of about 16 million inhabitants during a ten-year period.

The population-based setup also has a drawback: it does not facilitate determination of the underlying cause for the survival benefit because the cancer registry does not hold many details about individual patients. We speculate that the explanation lies beyond a mere increase in surgical volume. In a DAN-hospital, ACC patients are discussed pre-operatively in a multidisciplinary team including an endocrinologist, surgeon, oncologist, radiotherapist and radiologist. Adjuvant treatment is carried out according to up-to-date guidelines and most DAN-centers offer the possibility of participation in international trials regarding ACC.²⁷ All aspects of treatment, from disease staging to follow-up visits, benefit from a multidisciplinary approach by physicians who are experienced in treating patients with ACC. If it is assumed that patients who are operated on in a DAN-hospital have received additional treatment and follow-up there as well, the observed survival benefit for primary surgery in a DAN-center can be extrapolated to ‘treatment in DAN’. Whether or not patients who are operated on in non-DAN hospitals received additional treatment later in the course of their disease would not change the interpretation of the present results.

The concept that rare diseases should be treated in a limited number of specialized hospitals is intuitively logical and is increasingly instituted in health care systems. In the Netherlands, a centralized approach has been introduced for other rare diseases like esophageal/gastric cardia cancer and pancreatic cancer and has proven to be effective.²⁸⁻³⁰ In high-volume oncological diseases such as breast or colon cancer centralization of care is a big issue as well. The effects of introducing minimum volume requirements for oncological surgery have recently been investigated in the Netherlands. Interestingly, this report shows that low-volume centers do not necessarily perform worse than high-volume centers. This suggests that besides surgical volume other factors are responsible, which is in agreement with our hypothesis regarding treatment of ACC in DAN-centers.³¹

The survival benefit in stage IV patients who underwent surgery versus those who did not has been observed before.²⁰ Because this observation is probably in part attributable to selection bias, these data have to be interpreted with caution. Based on theoretical reasoning, a radical resection might be beneficial in stage IV patients. Debulking surgery might alleviate symptoms and yield a survival benefit in patients with slowly progressive disease. Surgery of stage IV patients is mainly performed in specialized centers. In contrast to the stage I-III group, no significant difference in survival was observed between DAN- and non-DAN hospitals for stage IV patients.

The present study has limitations. As mentioned, detailed information on individual treatments or course of disease were not available, which means that it was not possible to confirm the cause of the observed survival benefit nor to compare disease-free survival between the two groups. Also, the lack of a central pathology review makes the NCR dependent on accurate

registration of the true diagnosis by the local pathologist and physician. Finally, referral bias cannot be excluded.

The present population-based study associates surgical removal of a primary local or locally advanced ACC in a DAN-center with a survival benefit compared to patients treated in a non-DAN hospital. The study design does not permit determination of the cause of the difference. However, the authors believe that these findings underscore the need for further centralization of ACC-treatment. Initiatives have been undertaken to raise awareness of the disease and the DAN and to intensify collaboration between the DAN and the ENS@T.

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CHAPTER 8

COMPARISON OF TWO MITOTANE STARTING DOSE REGIMENS IN PATIENTS WITH ADVANCED ADRENOCORTICAL CARCINOMA

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ABSTRACT

Context

Mitotane is the only approved drug for treatment of adrenocortical carcinoma (ACC). Its pharmacokinetic properties are not fully elucidated and different dosing regimens have never been compared head-to-head.

Objective

To investigate the relationship between mitotane dose and plasma concentration comparing two dosing regimens.

Design/Setting

Prospective open-label multicenter trial of a predefined duration of twelve weeks.

Patients/Interventions

Forty mitotane-naïve patients with metastatic ACC were assigned to a predefined low- or high-dose regimen by the local investigator. Thirty-two could be evaluated in detail.

Main Outcome Measure

Difference in median mitotane plasma levels between both treatment groups.

Results

Despite a difference in mean cumulative dose (440 ± 142 g versus 272 ± 121 g), median maximum plasma levels were not significantly different between the two groups (high-dose 14.3mg/L (6.3-29.7, n=20) versus 11.3mg/L (5.5-20.0, n=12), $p=0.235$). Ten out of twenty patients on the high-dose regimen reached plasma concentrations ≥ 14 mg/L after 46 days (18-81 days) compared to four of twelve patients on the low-dose regimen after 55 days (46-74 days, $p=0.286$). All patients who reached 14mg/L at 12 weeks displayed a level ≥ 4.1 mg/L on day 33 (100% sensitivity). There were no significant differences in frequency and severity of adverse events. Among patients not receiving concomitant chemotherapy mitotane exposure was higher in the high-dose group: 1013 ± 494 mg.d/L versus 555 ± 168 mg.d/L, $p=0.080$.

Conclusions

The high-dose starting regimen did neither result in significantly different mitotane levels nor in a different rate of adverse events, but concomitant chemotherapy influenced these results. Thus, for mitotane monotherapy the high-dose approach is favorable, whereas for combination therapy a lower dose seems reasonable.

INTRODUCTION

Current medical treatment of advanced adrenocortical carcinoma (ACC) is based on mitotane given either as monotherapy or combined with cytotoxic chemotherapy.^{1,2} Mitotane is registered in the United States since 1970 for the treatment of advanced ACC and was in 2004 also authorized by the European Medicines Agency. Despite this long history of use, its mechanism of action and pharmacokinetic properties are not fully elucidated.³

There is evidence of a correlation between mitotane plasma levels and anti-neoplastic efficacy. Studies have demonstrated an objective response rate of 55-66% in patients whose plasma levels were above 14mg/L, while lower levels were associated with lack of efficacy.⁴⁻⁶ The concept that mitotane levels predict treatment response has been confirmed in a large multicenter study.⁷ Accordingly, monitoring plasma levels is considered to be as standard of care.

Information on mitotane pharmacokinetics primarily originates from a study performed in 1960.⁸ It appears that 35 to 40% of the drug is absorbed from the gastro-intestinal tract and is stored primarily in adipose tissues. This may explain the time-lag of four weeks to several months necessary for reaching target plasma levels.⁹⁻¹² Thus, higher starting doses have been proposed to earlier reach the therapeutic window.¹³ In addition, some groups suggested that mitotane plasma level after 2-4 weeks might predict whether the patient reaches the target level within a short time justifying monotherapy in these patients.^{2, 13}

Metabolism of mitotane results in the formation of two metabolites, 2,4-dichlorodiphenyl acetic acid (DDA) and 1,1-(o,p'-dichlorodiphenyl)-2,2-dichloroethene (DDE), of which DDA has been identified as the major circulating and excreted metabolite.¹⁴ A recent retrospective study in a group of 91 patients with ACC demonstrated that DDA plasma levels >92 mg/L may be associated with tumor response.⁷

Tolerability is a matter of concern, as adverse effects occur frequently and are also related in part to drug levels. However, the number of patients who discontinue therapy can be minimized by regular counseling about management of adverse effects, careful adjustment of hormone replacement therapies and tailoring of mitotane dosage based on plasma levels and side-effects.¹⁵

The recently completed first randomized trial in advanced ACC (FIRM-ACT) compared two chemotherapy regimens and established etoposide, doxorubicin, cisplatin plus mitotane (EDP-M) as first-line cytotoxic chemotherapy for patients with ACC.¹⁶ The present study was nested within FIRM-ACT with the aim to better understand the relationship between mitotane dose (daily and cumulative) and plasma concentrations by comparing two pre-defined starting regimens (highdose and lowdose). Secondary objectives were to evaluate the safety of mitotane and its impact on various hormonal parameters. Time to reach a mitotane plasma level of 14mg/L was determined as post-hoc endpoint. In addition we examined whether mitotane levels assessed

early in therapy were predictive for reaching 14mg/L within 12 weeks. This is the first prospective multicenter study aimed at improving knowledge on the pharmacokinetic properties of mitotane.

METHODS

Patients

The main eligibility criteria were histologically confirmed locally advanced or metastatic adrenocortical carcinoma not amenable to radical surgical resection, radiologically monitorable disease, ECOG performance status 0-2, life expectancy >3 months, age ≥18 years, adequate hematologic and biochemical function, effective contraception, written informed consent and ability to comply with study procedures. Exclusion criteria were previous treatment with mitotane, cytotoxic chemotherapy or experimental drugs for ACC, history of prior malignancy, renal or hepatic insufficiency, pregnancy, breast feeding, presence of active infection, decompensated heart failure, myocardial infarction or revascularization procedure in the last six months. The protocol was approved by the institutional ethics committees and all patients gave informed consent.

Study design and treatment

The study was an international prospective open-label multicenter study. Treatment with mitotane was initiated at least two weeks prior to the start of cytotoxic chemotherapy. Assignment to one of two dosing regimens was at the discretion of the local investigator without detailed inclusion criteria. A detailed summary of both regimens is provided in Table 1. Mitotane was taken orally in three daily doses with food. The predefined study duration for each patient was twelve weeks.

Measurement of mitotane and metabolites

Mitotane plasma level was assessed weekly during the first eight weeks and then at weeks 10 and 12. Sampling was performed in the morning at least twelve hours after the last dose. Quantitative analyses of mitotane, DDA and DDE were performed by Parexel (Bloemfontein, South Africa) using high performance liquid chromatography.¹⁷

Safety assessments

Adverse events (AE) were monitored throughout the study and assessed with the use of the National Cancer Institute Common Terminology Criteria (NCI-CTC) for Adverse Events version 2.0. Dosing of intravenous chemotherapy in case of hematologic, renal and neurologic toxicity was done according to the study protocol. Mitotane dose was adjusted in case of grade 3/4 gastro-intestinal AE and/or grade 2-4 central nervous system AE (Table 1). In case of >grade 3 non-hematological toxicity (except alopecia) both mitotane and chemotherapy were temporarily discontinued. Liver enzymes (serum glutamic pyruvic transaminase, (SGPT), serum glutamic oxaloacetic transaminase (SGOT), gamma-glutamyltransferase (GGT)) were analyzed at baseline, week 4 and week 12.

Table 1: Starting dose and dose adjustments according to low-dose and high-dose regimen.

Low-dose regimen		High-dose regimen
Time (days)	Dose (gram)	Dose (gram)
1	1.0	1.5
2	1.0	3
3	1.5	4.5
4	1.5	6
5	1.5	6
6	2.0	6
7	2.0	6
8	2.0	6
9	2.5	6
10	2.5	6
11	2.5	6
12	3.0	6
13	3.0	6
14	3.0	6
Weeks 3-12	Dose adjustment at least every two weeks according to adverse effects and plasma level, see below:	
Mitotane plasma level	CNS (grade 2) and/or GI (grade 3/4)	CNS (≥ grade 3)
	Absent	Present
< 14 mg/L	Increase by 1g*	Reduce by 1g
14 – 20 mg/L	Maintain dose	Reduce by 1.5 g
> 20 mg/L	Reduce by 50-75%	Interrupt mitotane#

CNS: Central nervous system. GI: Gastro-intestinal. * Maximum daily dose permitted was 12 gram.
interrupt until recovery of adverse effects and restart with reduced dose (50-75% of most recent dose).
Final decision about dose adjustments are at the discretion of the local investigator.

Endocrine work-up

All hormone analyses were performed using validated assay methods by Bicêtre Hospital (Endocrinology Department, Paris, France). Thyroid-stimulating hormone (TSH), free triiodothyronine (FT3), free thyroxine (FT4), thyroxine-binding globulin (TBG) and sex hormone binding globulin (SHBG or SBP) were measured at baseline and at week 4 and 12. Total and free testosterone, follicle-stimulating hormone (FSH) and luteinizing hormone (LH) were measured before treatment and at week 12.

Statistical analysis

Only limited data were available on inter and intra-subject variability of mitotane pharmacokinetics, precluding a classical sample size calculation. It was estimated that it would be necessary to recruit eight patients per subgroup (high/low dose, EDP/Sz chemotherapy) in order to investigate differences between both dosing regimens and to evaluate safety. Assuming some drop-outs, the study aimed at inclusion of 40 patients. The following pharmacokinetic parameters were calculated per protocol: plasma concentration (C) before morning dose; highest C observed

(C_{Max}); time to reach C_{Max} (t_{max}) and area under the concentration-time curve (AUC_{0-t}). The post-hoc endpoint median time to reach mitotane plasma level of 14mg/L was measured in days among patients in both regimens. The predictive value of early mitotane levels was examined using receiver operating characteristic (ROC) analyses. Data on thyroid and sex hormones are displayed according to mitotane plasma level. Adverse events are displayed according to severity and dosing regimen. Results are expressed as mean ± standard deviation (SD) or median and range as indicated. Data were analyzed by the Biostatistics Unit of BIOTRIAL.

RESULTS

Patient characteristics

Forty patients were enrolled in the study between 2004 and 2009. There were 19 men (48%) and 21 women (52%), mean age was 51 years, mean BMI was 25.3kg/m2. Table 2 displays demographic data according to the subgroups. As all participants had to sign informed consent for the FIRM-ACT study at inclusion, the intention was to administer chemotherapy to all participants. However, seventeen patients were not randomized in FIRM-ACT and did not receive chemotherapy during this substudy due to indolent disease (n=8), rapid progression of disease (n=3), patient’s refusal (n=2), early death (n=2), revision of tumor staging (n=1) or violation of inclusion criteria (n=1).

As stated in the study protocol, all 40 patients were included in safety analyses. Eight patients were excluded from the per protocol analysis of pharmacokinetics: three patients died after 19, 21 and 24 days due to disease progression (n=2) and pulmonary embolism (n=1), respectively; in two patients protocol deviations resulted in missing at least three consecutive PK-results, two patients did not respect the inclusion criteria and were rapidly excluded (after 4 and 6 days of treatment, respectively) and one patient refused to continue mitotane after 14 days (Figure 1).

From the remaining 32 patients, four patients were excluded from analyses concerning cumulative doses as they discontinued the study before the projected end-point at week 12: one patient died after 39 days, one patient had severe central nervous system disorders (day 68) and two patients failed to comply with the protocol (day 65 and 71).

Mitotane cumulative dosage and plasma concentrations

Mean cumulative doses over the treatment period were substantially higher in the high-dose group (440g±142g) compared to the low-dose group (272g±121g). Ten out of 20 patients (50%) on the high-dose regimen reached plasma concentrations above or equal to 14mg/L compared to 4 of 12 patients (33%) on the low-dose regimen. In the high-dose group, this level was reached after a median of 46 days (18-81 days) compared to 55 days (46-74, p=0.286) in the low-dose group. ROC curves identified a plasma level of 6.0mg/L on day 33 as the best compromise between sensitivity (86%) and specificity (61%) to predict attainment of the therapeutic level within 12 weeks. All patients who reached 14mg/L displayed a level ≥ 4.1 mg/L on day 33 (100% sensitivity).

Table 2: Patient characteristics of the study population according to dosing regimen

Characteristic		Overall (n=40)	Low-dose (n=13)	High-dose (n=27)
Age (years) [mean ± SD]		51.3 ± 12.1	51.9 ± 13.0	51.4 ± 11.9
Sex [n (%)]	male	19 (48%)	5 (39%)	14 (52%)
	female	21 (53%)	8 (62%)	13 (48%)
BMI (kg/m2) [mean ± SD]		25.3 ± 4.0	24.6 ± 3.3	25.7 ± 4.3
Endocrine symptoms [n (%)]	Cushing's syndrome	16 (40%)	5 (38%)	11 (41%)
	Virilization only	2 (5%)	0	2 (7%)
	No symptoms	21 (53%)	8 (62%)	13 (48%)
	Unknown	1 (3%)	0	1 (4%)
ECOG performance status [n (%)]	0	16 (40%)	6 (46%)	10 (37%)
	1	14 (35%)	3 (23%)	11 (41%)
	2	8 (20%)	3 (23%)	5 (19%)
	3	2* (5%)	1 (8%)	1 (4%)

BMI: Body Mass Index. ECOG: Eastern Cooperative Oncology Group.
*The two patients suffered from severe Cushing's syndrome and were judged by the local investigators to be able to participate in the present study despite their ECOG status >2

Figure 1: Overview of patient assignment to dosing regimens, patient's exclusion and the administration of cytotoxic chemotherapy

Mean cumulative dose needed to reach 14mg/L was 291±126g (high-dose) versus 137±39g (low-dose, p=0.020). Median C_{max} was 14.3mg/L (6.3-29.7) in the high-dose group (n=20) and 11.3mg/L (5.5-20.0) in the low-dose group (n=12, p=0.235). It should be noted that mitotane plasma levels in most patients were still rising and no plateau was reached within twelve weeks (Figure 2A). At the last visit in week 12, median plasma level was 14.2mg/L (2.1-29.7) in the high-dose group and 10.6mg/L (2.3-18.1) in the low-dose group (p=0.120). Mitotane exposure was higher in the high-dose group, but did not reach statistical significance (AUC₀₋₁₂ weeks 790±444mg.d/L vs. 543±215mg.d/L, p=0.171).

Metabolites of mitotane

Median DDA C_{max} was 99.7µg/mL (range 23.2-185.0 µg/mL) in the high-dose group (n=20) and 88.0µg/mL (35.4-102.0µg/mL) in the low-dose group (n=12, Figure 2B).

Twelve out of twenty high-dose patients (60%) reached DDA levels above 92mg/L on at least one occasion compared to 3/12 (25%) in the low-dose regimen (p=0.076). Median time to reach a DDA level of 92mg/L was 11 days after start of treatment (high-dose, n=12) versus 46 days (low-dose, n=3). Of fifteen patients reaching DDA plasma levels >92mg/L, nine (60%) also had mitotane plasma levels above 14mg/L, eight of whom were treated according to the high-dose schedule.

Plasma concentrations of DDE were below the limit of quantification on most occasions. Nine patients on the high-dose regimen and two on the low-dose regimen presented measurable DDE values. In week 12, mean DDE plasma concentration among the high-dose patients was 0.67±0.17mg/L and 0.68±0.15mg/L in the low-dose group.

Effect of concomitant cytotoxic chemotherapy on mitotane plasma level

Mean cumulative mitotane dose in patients receiving chemotherapy in addition to mitotane (n=19) was comparable to the dose administered to thirteen patients who did not receive cytotoxic chemotherapy (358g±143g vs. 389g±132g; p=0.574). Within the chemotherapy-group, patients on the high-dose regimen had a median plasma level in week 12 of 9.2 mg/L (2.1-27.1, n=12), four patients reached plasma levels of 14mg/L or higher. Median plasma level was not significantly different from patients on the low-dose regimen (10.8mg/L (2.3-18.1), n=7), of whom two patients reached the threshold of 14mg/L.

Among patients not receiving chemotherapy, higher median plasma levels were observed in the high-dose group: 18.3mg/L (4.3-29.7, n=8) compared to 10.3mg/L (7.4-15.7, n=5) in the low-dose group, but results were not statistically significant. The observation was reflected by a larger AUC₀₋₁₂ in this subgroup: 1013±494mg.d/L versus 555±168mg.d/L (p=0.081).

Six out of eight patients from the high-dose group reached a plasma level of 14mg/L, in the low-dose group this was reached by two out of five patients.

Whiskers indicate interquartile range.

Figure 2A and 2B: Median mitotane (A) and DDA (B) plasma concentration throughout the study in both dosing regimens

Safety and Toxicity

All forty patients were evaluated for safety. A total of 200 adverse events (AE) was reported, most of them were NCI-CTC grade 1 or 2 (n=166, Table 3). The remaining AEs were grade 3 (n=28) or grade 4 (n=6). The rate of AE per patient was similar in both groups (5.0 vs. 5.2 AE/patient).

Almost half of all AEs (n=96, 48%) were related to the gastro-intestinal system, mostly nausea (n=30), diarrhea (n=23) and vomiting (n=15). One grade 4 event was reported: gastro-intestinal bleeding. The rate of gastro-intestinal AEs per patient was slightly higher in the low-dose group (2.8 AE/patient versus 2.1 AE/patient).

There were eighteen AEs (9%) related to general disorders. General discomfort NCI-CTC grade 1 (n=6), asthenia (n=4) and fatigue (n=4) were most common events in this category. Most events were reported among patients on the high-dose regimen (n=16).

Fourteen AEs were related to metabolism and nutrition (7%), ten occurred in the high-dose group. There were two grade 4 AEs: these were episodes of hypomagnesaemia and hypokalaemia, both probably related to concomitant cytotoxic treatment with EDP. There were four events in the low-dose group, all hypercholesterolemia $\leq$ grade 2.

Eighteen events were related to disorders of the nervous system (9%). Vertigo (n=6) and dizziness (n=5) were most common. Regarding psychiatric AEs, there were four reports of anxiety attacks and two reports of insomnia. Depression and hallucination were both reported once.

Changes in hepatic enzymes were seen frequently. After twelve weeks, the mean level of GGT was 361 ± 248 IU/L compared to 123 ± 191 IU/L at baseline. All patients had elevated GGT levels with 89 IU/L being the lowest level. The mean level of SGOT in week 12 was 46 ± 24 IU/L, mean SGPT was 52 ± 36 IU/L.

Four patients died during the study. Two patients died due to disease progression, one patient died following pulmonary embolism and one patient died following severe cardiac failure, these events were not considered drug-related.

Table 3: Summary of adverse events reported in the study population according to dosing regimen

NCI-CTC Category		Overall (n=40)	High-dose regimen (n=27)	Low-dose regimen (n=13)
Gastro-intestinal				
[n (%)]	Grade ≤2	84 (42%)	50 (38%)	34 (50%)
	Grade 3	11 (6%)	9 (7%)	2 (3%)
	Grade 4	1 (1%)	1 (1%)	-
	Total	96 (48%)	58 (45%)	36 (53%)
General				
[n (%)]	Grade ≤2	17 (9%)	15 (12%)	2 (3%)
	Grade 3	1 (1%)	1 (1%)	-
	Total	18 (9%)	16 (12%)	2 (3%)
Metabolism and nutrition				
[n (%)]	Grade ≤2	11 (6%)	7 (5%)	4 (6%)
	Grade 3	1 (1%)	1 (1%)	-
	Grade 4	2 (1%)	2 (2%)	-
	Total	14 (7%)	10 (8%)	4 (6%)
Nervous system				
[n (%)]	Grade ≤2	15 (8%)	11 (9%)	4 (6%)
	Grade 3	3 (2%)	3 (2%)	-
	Total	18 (9%)	14 (11%)	4 (6%)
Psychiatric				
[n (%)]	Grade ≤2	7 (4%)	3 (2%)	4 (6%)
	Grade 3	1 (1%)	1 (1%)	-
	Total	8 (4%)	4 (3%)	4 (6%)
Other				
[n (%)]	Grade ≤2	32 (16%)	22 (17%)	10 (15%)
	Grade 3	11 (6%)	5 (4%)	6 (9%)
	Grade 4	3 (2%)	1 (1%)	2 (3%)
	Total	46 (23%)	28 (22%)	18 (27%)
Total [n (%)]	Grade ≤2	166 (83%)	106 (82%)	58 (85%)
	Grade 3	28 (14%)	20 (15%)	8 (12%)
	Grade 4	6 (3%)	4 (3%)	2 (3%)
	Total	200	130	68

NCI-CTC National Cancer Institute Common Terminology Criteria. n: number of patients. Adverse events from the ‘other’ category were laboratory abnormalities (n=7), endocrine disorders (n=6), vascular disorders (n=6), skin/subcutaneous tissue disorders (n=5), infections (n=5), musculoskeletal disorders (n=4), respiratory disorder (n=4), cardiac disorders (n=4), eye disorders (n=2), reproductive system disorders (n=2), and blood and lymphatic system disorder (n=1).

Hormonal parameters

Mean TSH did not change significantly over the course of this 12-week study, both in patients with high and low mitotane plasma levels (Table 4). In patients who did not reach a mitotane level of 14mg/L (n=14), mean FT4 level in week 12 was 5.7±4.5pmol/L lower compared to baseline. The decrease was greater in patients with mitotane plasma levels >14 mg/L: 7.7±3.2pmol/L (n=11). Three patients already were on thyroid replacement therapy prior to the study. In three other patients, this was started during the study; two in the high-dose regimen and one in the low-dose regimen.

Mean TBG levels increased during the trial. Among patients who were evaluated in week 12, a mean increase of 5.1±8.8 IU/mL was observed in those with mitotane levels below 14mg/L (n=14). A mean increase of 7.2±7.5 IU/mL was seen in patients with mitotane levels >14mg/L (n=11).

Analysis of sex hormones in males evaluated at week 12 showed a mean increase in LH level in week 12 of 4.5±3.8 IU/L for those with mitotane levels below 14mg/L (n=5). In patients with mitotane levels >14mg/L, the mean increase was 5.7±3.8 IU/L (n=6).

Mean level of free testosterone in males tended to decrease during the study, whereas the mean level of total testosterone in week 12 was not different from baseline. Mean SHBG levels were significantly higher in week 12 compared to baseline.

In females, mean concentrations of FSH and LH in week 12 compared to baseline show variable results (not shown). Total and free testosterone remained stable in female patients, whereas the mean concentration of SHBG was significantly increased.

DISCUSSION

This is the largest prospective study on the pharmacokinetic properties of mitotane published so far. Despite the expected difference in cumulative dose at 12 weeks neither the plasma concentrations of mitotane nor the time to reach a therapeutic level differed significantly between two starting dose regimens. Area under the mitotane plasma concentration/time curve was larger in the high-dose starting group, but this was not statistically significant. Also, the proportion of patients achieving the target plasma concentration within 12 weeks was higher in the high-dose group (10/20 versus 4/12, respectively). The beneficial effect of the high-dose schedule on mitotane exposure was stronger in a subgroup of patients not receiving chemotherapy.

Reaching the target concentration of ≥14mg/L is a major goal during mitotane treatment and has been associated with better response both in advanced disease and in adjuvant setting.^{4-6, 18} Consequently, it seems important to reach such concentrations as early as possible to rapidly establish antiproliferative efficacy, although this reasoning is not underpinned by clinical data.

Table 4: Summary of thyroid and sex hormone levels according to mitotane plasma concentration at baseline and in week 12

Thyroid hormone levels		Baseline	Week 12		Normal range
			Mitotane <14mg/L	Mitotane >14mg/L	
TSH	[mean in mIU/L ± SD (n)]	1.08 ± 0.98 (34)	0.97 ± 0.70 (13)	0.30 – 4.50	1.60 ± 1.78 (11)
FT3	[mean in pmol/L ± SD (n)]	3.61 ± 1.04 (35)	4.11 ± 0.90 (14)	2.0 – 5.5	4.40 ± 0.49 (11)
FT4	[mean in pmol/L ± SD (n)]	15.58 ± 5.05 (35)	10.73 ± 4.84 (14)	11.0 – 25.0	8.09 ± 2.52 (11)
TBG	[mean in IU/mL ± SD (n)]	14.40 ± 5.80 (35)	21.3 ± 11.4 (14)	9 – 24	19.85 ± 7.52 (11)
Gonadotropin and sex hormone levels (males only)					
FSH	[mean in IU/L ± SD (n)]	6.40 ± 4.95 (12)	4.98 ± 3.80 (5)	3.00 – 7.00	8.55 ± 6.86 (6)
LH	[mean in IU/L ± SD (n)]	3.92 ± 2.44 (13)	7.72 ± 4.37 (5)	3.00 – 8.00	13.03 ± 7.41 (6)
Testosterone	[mean in ng/mL ± SD (n)]	4.14 ± 2.04 (15)	6.53 ± 4.48 (6)	3.50 – 8.50	6.63 ± 1.47 (6)
Free testosterone	[mean in pg/mL ± SD (n)]	5.67 ± 3.06 (15)	3.67 ± 1.21 (6)	20.0 – 100.0	2.07 ± 0.89 (6)
SHBG	[mean in nmol/L ± SD (n)]	32.10 ± 17.00 (15)	191.7 ± 214.0 (6)	15 – 40	170.8 ± 80.10 (6)

n: number of patients. SD: standard deviation.
Gonadotropin and sex hormones in female patients are not listed, because information on the patients' menstrual cycle or menopausal status was not available.

In a previous study with thirteen patients taking mitotane as first-line therapy, a correlation was observed between plasma levels and cumulative dose of mitotane.⁴ This suggests dose-proportionality, which means that drug elimination is independent of the concentration present in the body (zero-order reaction) rather than dependent (first-order reaction). Therefore, we speculate that in clinical practice elimination of mitotane proceeds at a constant rate independent of the plasma concentration. This is supported by the stable levels of DDA that were observed after 4 weeks of treatment in both groups. Consequently, administration of higher doses should result in higher plasma levels in a shorter period of time. As steady state levels of mitotane had not been reached at week 12 it is tempting to speculate that a longer study duration would have demonstrated that high-dose mitotane reaches steady state conditions earlier.

Our data confirms previous observations that the increase in plasma concentrations is in general slow and highly variable. Obviously this behavior cannot be sufficiently explained by differences in dosing only, indicating that other factors contribute to this observation. Potential explanations are related to effects on enzyme induction by concomitant drugs and differences in intestinal absorption which may also be affected by ingested food. Also, recent data shows that individual genetic differences are significantly associated with mitotane levels¹⁹ as this is known for other drugs.^{20, 21} In the absence of baseline predictors, early measurements constitute the only signal whether a given patient has a high probability of reaching the therapeutic level in time.¹³

The observation that the high-dose schedule results in higher exposure in patients not receiving concomitant chemotherapy may be explained by multiple factors. It is possible that patients do not tolerate high mitotane doses in addition to chemotherapy or that physicians anticipate on the addition of chemotherapy by lowering mitotane dosage. However, cumulative doses were not different among these subgroups. Of note, treatment compliance could be a limitation in this respect. Also, administration of cytotoxic chemotherapy could result in lower plasma levels due to drug interactions or impaired intestinal uptake.²²

No significant difference was detected between the two groups regarding the frequency and severity of adverse events. This suggests that actual dosage is not the key determinant of adverse effects. Of note, we did not observe any (significant) correlation between adverse events and mitotane plasma level. However, it has to be acknowledged that 18 patients (45%) never reached a plasma level above 14mg/L and only 6 patients had measurements above 20mg/L. Nevertheless, in our personal experience adverse events can usually be managed by adequate support, even when given in parallel to cytotoxic chemotherapy. An increase of gamma-GT is well known and appeared in 100% of our patients. Observations in patients treated adjuvantly demonstrated that this is reversible.^{15, 23}

Mitotane can induce hormonal effects soon after the onset of treatment. After 12 weeks a significant decrease in free T4 was detectable with a trend for a negative correlation between mitotane and free T4 levels. TSH and free T3 remained unchanged or were increased. It is known from previous research that mitotane use can lead to biochemical findings mimicking central hypothyroidism, i.e. low FT4 with roughly normal FT3 and TSH levels.¹⁵ Previously proposed

explanations are a direct effect on the pituitary level, induction of thyroid hormone metabolism, an increase in TBG levels or changes in the thyroid hormone receptor's affinity for FT₄.^{22, 24, 25} Also, interference with FT₃, FT₄ and/or TSH assays cannot be excluded.

Data on the influence of mitotane on sex hormones are scarce. In vitro studies showed that mitotane increases the synthesis of steroid binding protein.^{26, 27} In our study, SHBG was markedly increased while in males free testosterone was decreased and LH was increased. Mitotane has been shown to bind the human estrogen receptor- α with weak affinity, approximately 1000-fold weaker than that of estradiol itself.²⁸ Although the concentration of mitotane in plasma is about 5.0x10⁵ times higher than estradiol levels in men, the presence of increased levels of LH makes a strong estrogenic effect of mitotane at the pituitary level not likely. A recent study demonstrated significantly decreased levels of dihydrotestosterone in mitotane treated patients, which might be the reason for the increase of gonadotrophins.²⁹ Another possibility is mitotane-induced inhibition of testosterone secretion, set off at least partially by an increase in SHBG and by decreased production.³⁰

We acknowledge the limitations of our study. First, patients were not randomized to a dosing regimen, which could have introduced bias. However, due to the embedment of the study within the FIRM-ACT protocol, a second randomization was judged to be not feasible. Second, protocol deviations produced missing data which made the study underpowered in several analyses. Furthermore, we acknowledge that more patients in the high-dose group had to be excluded from the pharmacokinetic analyses. However, as mentioned under Results, the reasons for exclusion are unlikely treatment-related and therefore, we do not expected that this fact influenced the conclusions. A forth limitation is that steady state levels of mitotane were not reached in all patients. However, due to the aggressiveness of the disease a longer, comprehensive pharmacokinetic study would be very challenging for patients and investigators. Finally, the study was underpowered to assess differences in subgroups.

In conclusion, the high-dose starting regimen led to higher mitotane plasma levels within twelve weeks of treatment and more patients reached the target level of 14 mg/L. Observed differences were greater in the subgroup of patients who did not receive concomitant cytotoxic chemotherapy, but results were not statistically significant due to lack of power. The rate of adverse events was similar between both groups, leading us to conclude that the high-dose approach is the preferred strategy in patients with mitotane monotherapy. However, in patients with reduced tolerability (e.g. due to concomitant cytotoxic chemotherapy), a less aggressive regimen might be reasonable.

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CHAPTER 9

DEVELOPMENT OF A PHARMACOKINETIC MODEL OF MITOTANE: TOWARDS PERSONALIZED DOSING IN ADRENOCORTICAL CARCINOMA

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ABSTRACT

Background

Mitotane is the drug of choice in medical treatment of adrenocortical carcinoma. The anti-neoplastic effect appears to be correlated with a minimum plasma level of 14mg/L, but plasma concentration build-up is in general slow due to the long elimination half-life. Consequently, the therapeutic effect sets in after weeks or even months. The objective of the present study was to develop a pharmacokinetic model that enables clinicians to adjust dosing based upon a target drug exposure, which facilitates personalized therapy.

Methods

Data on dosing and plasma level measurements performed throughout mitotane therapy were retrospectively collected in a population of 29 patients from two hospitals. A population pharmacokinetic model was constructed based on data from 20 patients using iterative two-stage Bayesian fitting (ITSB, MWPharm). The model was validated in an independent sample of 9 patients.

Results

The concentration-time data were best described by a three-compartment model. The model estimated mitotane clearance at 0.94 ± 0.37 L/h and volume of distribution in steady state at 161 ± 68 L/kg·LBM. Mean prediction error was $14\% \pm 13\%$.

Conclusions

A pharmacokinetic model was developed which characterized mitotane by slow clearance and large volume of distribution. The model appears to be able to predict mitotane levels in individual patients with an error margin of 14%. The model enables to adapt dosing based on individual plasma level measurements in prospective setting, which improves the prediction's accuracy. We expect that individualization of mitotane dosing leads to anticipated and more rapid attainment of the therapeutic levels and potentially to improved clinical management of mitotane treatment.

INTRODUCTION

Mitotane is the drug of choice in patients with adrenocortical carcinoma (ACC). It can be combined with cytotoxic chemotherapy in patients with extensive and/or rapidly progressive disease and is recommended as adjuvant therapy in patients at high risk of recurrence.¹⁻⁴ In fact, withholding mitotane should only be considered after radical resection of the primary tumor and ki-67 index <10%.^{4,5}

Little is known about pharmacodynamic and pharmacokinetic properties of mitotane.⁶ The anti-neoplastic effect appears to be correlated with mitotane plasma levels: several studies demonstrated an objective response in patients whose plasma levels were above 14mg/L, while lower levels were associated with lower efficacy.⁷⁻⁹ The time to reach steady state plasma concentrations takes months due to its long elimination half-life. In most patients steady state is reached after three months of treatment with a daily dose of about 6.0 gram.^{10,11} Due to the slow build-up of mitotane plasma levels, the therapeutic effect sets in after several weeks or months of treatment. This complicates timing of therapy evaluation and the decision to add cytotoxic chemotherapy to the treatment.

Dosing regimens are based on clinical experience and adjusted according to plasma concentration and tolerability. The inability to predict mitotane levels may lead to relative underdosing and a prolonged build-up phase in some patients, while others unexpectedly demonstrate high plasma levels early in therapy, causing increased toxicity. A recent study on mitotane initiation in a population of patients with advanced ACC describes the relationship between plasma levels and two different dosing regimens during the first twelve weeks of treatment.¹² Despite a confounding effect of concomitant chemotherapy and the fact that steady state was not reached during the study, a trend towards higher plasma levels using a higher initial dose was described.

Research has indicated that individual genetic differences are significantly associated with mitotane levels achieved after three and six months of treatment.¹³ Also, a previous study demonstrated a semi-logarithmic relationship between mitotane concentration in plasma and adipose tissue.¹⁴ This suggests that individual body fat percentages influence the time to reach therapeutic mitotane plasma levels.

To our knowledge, there are no studies describing mitotane plasma levels and maintenance doses in long-term users. Also, there are no studies describing the influence of basic patient characteristics such as gender, age and body mass index on mitotane pharmacokinetics.

The aim of the present study was to develop a pharmacokinetic model of mitotane taking into account clinical patient characteristics. This model should enable clinicians to adjust dosing based upon a target drug exposure, which facilitates personalized therapy.

METHODS

Patients

This retrospective study was carried out among patients from Máxima Medical Center (MMC) and Leiden University Medical Center (LUMC) who were treated with mitotane between 2002 and 2012. In order to be eligible for this study, dosing information from the start of therapy had to be available. All dose modifications and all plasma level measurements were extracted from the medical records. All measurements were routinely performed throughout the course of mitotane therapy. Furthermore, the following patient characteristics were extracted: age, gender, weight (baseline), height and the setting in which mitotane was administered (adjuvant therapy, monotherapy or in combination with cytotoxic chemotherapy). All mitotane plasma concentrations were determined by gas liquid chromatography (GLC) at the Department of Clinical Pharmacy and Toxicology in LUMC.¹⁵ In the Netherlands, anonymous use of retrospective clinical data is permitted without explicit informed consent from the patient.

Modeling

The model was created based on the medication history of 20 patients from MMC. Data were analyzed using MW-Pharm Computer Aided Therapeutic Drug Monitoring version 3.81 (Mediware, Zuidhorn, The Netherlands).¹⁶ This program is able to calculate 1-, 2- and 3-compartmental models using iterative two-stage Bayesian fitting (ITSB).^{17,18} Before the actual modeling starts, rough estimates of the model parameters and their standard deviation (SD) are entered. In the first stage of modeling, the software calculates individual pharmacokinetic parameters $\pm$ SD that best fit the actual plasma measurements in every given patient. The previously entered estimates are used as starting point. In the second stage, all individual values are pooled resulting in (1) a population mean for every parameter, (2) a covariance matrix with values for inter-parameter associations and (3) an estimate of the residual standard deviation. Then, the cycle is repeated using the newly found population values as starting point. The calculation is finished when the new population values and the residual standard deviation are similar (0.001% difference) to the values from the previous cycle.

Drug clearance was normalized according to the equation $k_{el} = k_{elm} + k_{elr} \cdot Cl_{cr}$, where k_{el} is the total elimination rate constant, k_{elm} is the metabolic elimination rate constant, k_{elr} is the renal elimination rate constant and Cl_{cr} is the creatinine clearance. The model includes the covariates age, weight, height, gender, body surface area (BSA) and lean body mass (LBM). Influence of the covariates on the pharmacokinetic parameters was calculated by performing regression analysis of each individual parameter against each patient covariate and was displayed using the correlation coefficient (r). The modeling approach based on rate constants combines both size and function-related parameters which potentially complicates the evaluation of correlations. However, this approach resulted in a better fit to the data than a model in which clearance was individualized according to body weight, while there was no difference in strength of correlations between both models.

For matters of simplification, twice and thrice daily doses were cumulated and registered as a single daily dose at a fixed timepoint (08:00h AM). Based on the extremely long half-life of mitotane, we do not expect this to be of significant influence on the outcome. Also, all plasma measurements were presumed to have been performed at fixed timepoints (08:00h AM). It is standard procedure in both hospitals to perform mitotane sampling in the morning at least twelve hours after the last dose. The estimate for bio-availability was fixed in model calculations. Based on existing literature, this value was set at 30%.¹⁹

Validation was performed by comparing predicted mitotane plasma levels with observed levels using three different methods: (1) by fitting the model to individual patients (n=9) from an independent group who were treated in another hospital (LUMC), (2) by fitting the model to individual patients (n=20) from the same development group, and (3) by fitting the model to individual patients (n=100) from a simulated population that was generated using the Monte Carlo method. This latter technique randomly assigned individual pharmacokinetic parameters $\pm$ SD to virtual patients based on a 'standard' patient (male, age 55, height 1.75m, weight 75kg) with a medication history of two years, monthly plasma measurements during the first year and bi-monthly measurements during the second year.

Definitions

The prediction error was quantified by calculating the proportional difference of the predicted value relative to the observed value as follows:

$$\text{prediction error} = \sqrt{\left(\frac{\text{predicted} - \text{observed}}{\text{observed}} \cdot 100\right)^2}$$

Body mass index (BMI) was calculated as (weight/height²). Lean body mass was calculated as 50.0 + 0.9 · (height - 152) in males and 45.5 + 0.9 · (height - 152) in females.²⁰ Body surface area was calculated as (weight^{0.425} · height^{0.725} · 0.007184).²¹ The volume of distribution in steady state (V_{ss}) follows from the equation $V_{ss} = V_1 \cdot \left(1 + \frac{k_{12}}{k_{21}} + \frac{k_{13}}{k_{31}}\right)$.

RESULTS

Patient characteristics

Data on mitotane treatment of patients from the development group and the LUMC validation group are specified in Table 1. There were no significant differences in age, gender, BMI and the setting in which mitotane was administered. Figure 1A-C displays plasma concentration-time curves from both real populations and the virtual Monte Carlo population. There were 302 plasma level measurements in the development group and 112 measurements in the validation group. All patients except two (one from every group) reached the therapeutic level during mitotane treatment. Cumulative dose was 2640 gram for simulated patients in the Monte Carlo group.

Model characteristics

The concentration-time data were best described by a three compartment model. Parameters of the model are summarized in Table 2. Mean volume of distribution in the central compartment

(V_1) was 1.216 ± 0.511 L/kg·LBM and volume of distribution in steady state (V_{ss}) was 161 ± 68 L/kg·LBM. Analysis of covariates showed that weight is significantly correlated with V_1 ($r = -0.505$), k_{21} ($r = 0.478$) and $k_{a(po)}$ ($r = -0.528$); height with k_{12} ($r = -0.487$) and k_{21} ($r = 0.607$); gender with k_{12} ($r = 0.555$); BSA with k_{elm} ($r = 0.446$), V_1 ($r = -0.576$), k_{21} ($r = 0.626$) and $k_{a(po)}$ ($r = -0.509$); LBM with V_1 ($r = -0.469$), k_{12} ($r = -0.600$) and k_{21} ($r = 0.688$).

Prediction and treatment simulation

Analysis of observed and predicted values in the validation group resulted in a mean prediction error of $14\% \pm 13\%$. Figure 2A displays a goodness-of-fit plot and a plot of weighted residuals versus time of the validation group. Analysis of the development group resulted in a mean prediction error of $25\% \pm 39\%$, goodness-of-fit and weighted residuals are displayed in Figure 2B. In the Monte Carlo population, mean prediction error was $6\% \pm 6\%$.

Three individual examples from the validation group of good, average and poor fits are displayed in Figure 3A-C. Mean prediction error in a patient with a good fit was $11\% \pm 11\%$, in a patient with an average fit $14\% \pm 14\%$ and in a patient with a poor fit $19\% \pm 16\%$.

Bayesian fitting of the model to individual patients in the LUMC validation group resulted in a mean clearance of 0.94 ± 0.37 L/h. Mean clearance was 1.12 ± 0.41 L/h among patients in the MMC development group.

Table 1: Comparison of both development and validation populations regarding demographic characteristics and mitotane treatment

	Total (n=29)	Development group (MMC patients (n=20)	Validation group (LUMC patients) (n=9)	P-value
Age (years) [median (range)]	53 (20-76)	54 (20-76)	47 (33-72)	0.741
Gender [n (%)]	13 16	8 (40%) 12 (60%)	5 (56%) 4 (44%)	0.688
BMI (kg/m ²) [median (range)]	23.8 (18.1-40.8)	25.1 (18.1-40.8)	23.0 (19.4-31.2)	0.322
Treatment Setting [n (%)]	10 8 11	7 (35%) 5 (25%) 8 (40%)	3 (33%) 3 (33%) 3 (33%)	0.890
Time on mitotane (months) [median (range)]	11 (1-54)	11 (2-54)	11 (1-24)	0.587
Total dose administered (gram) [median (range)]	1491 (121-3685)	1704 (210-3685)	1140 (121-2928)	0.157
Number of measurements [median (range)]	14 (4-28)	15 (5-28)	11 (4-23)	0.202
Time to reach 14 mg/L (days) [median (range)]	116 (21-321)	116 (50-212)	137 (21-321)	0.923
Dose needed to reach 14mg/L (gram) [median (range)]	626 (168-1621)	628 (214-1621)	691 (168-1097)	0.815

MMC: Máxima Medical Center, LUMC: Leiden University Medical Center, BMI: Body Mass Index, P-value for comparison between groups (Mann-Whitney U test).

Figure 1A-C: Plasma concentration-time curves from (A) the validation group; (B) the development group; and (C) the Monte Carlo group. C, Forty from 100 curves are shown for the sake of legibility.

Figure 1A-C continues on the next page

Continuation of Figure 1A-C

Figure 1A-C: Plasma concentration-time curves from (A) the validation group; (B) the development group; and (C) the Monte Carlo group. C, Forty from 100 curves are shown for the sake of legibility.

Table 2: Pharmacokinetic parameters calculated by iterative two-stage Bayesian population procedure

Model parameter	Estimate
V_1 (L/kg·LBM) [mean ± SD]	1.216 ± 0.511
k_{elim} (hr ⁻¹) [mean ± SD]	0.014 ± 0.001
k_{12} (hr ⁻¹) [mean ± SD]	0.150 ± 0.010
k_{21} (hr ⁻¹) [mean ± SD]	0.002 ± 0.0001
k_{13} (hr ⁻¹) [mean ± SD]	0.023 ± 0.001
k_{31} (hr ⁻¹) [mean ± SD]	0.0004 ± 0.0001
$k_{a(po)}$ (hr ⁻¹) [mean ± SD]	0.005 ± 0.005
$F_{(po)}$ [mean ± SD]	0.30

V_1 : Volume of distribution in central compartment, k_{elim} : Elimination rate constant, k_{12} : Rate constant from compartment 1 to compartment 2, k_{21} : Rate constant from compartment 2 to compartment 1, k_{13} : Rate constant from compartment 1 to compartment 3, k_{31} : Rate constant from compartment 3 to compartment 1, $k_{a(po)}$: Absorption rate constant (oral administration), F_{po} : Bio-availability (oral administration). LBM: Lean body mass, SD: standard deviation, hr: hour. Estimates are accurate to four decimals and truncated to three decimals. The fourth decimal is displayed if the value would otherwise have been zero.

Figure 2A-B: Goodness-of-fit plots and weighted residuals versus time for a 3-compartment model of mitotane: (A) in the validation group and (B) in the development group.

DISCUSSION

This study was aimed at developing a pharmacokinetic model of mitotane using data from patients on long-term treatment. The data were best described by a three-compartment model. The model enables individualization of mitotane dosing in prospective setting based on plasma level measurements.

Two previous studies prospectively investigated mitotane pharmacokinetics during the first three months of treatment.^{12, 22} In the first study, a high dose strategy was deployed in 22 patients, median cumulative dose administered within 3 months was 405 gram (range 157-546g).²² Ten out of 22 patients (45%) reached the therapeutic level of 14mg/L within 3 months. In the second

Figure 3A-C: Individual plots of three patients from the validation group: (A) good fit; (B) average fit; (C) poor fit.

Figure 3A-C continues on the next page

Figure 3A-C: Individual plots of three patients from the validation group: (A) good fit; (B) average fit; (C) poor fit.

study, a low-dose strategy was prospectively compared to a high-dose strategy in 40 patients.¹² Median cumulative doses in both regimens were 495 gram (range 163-593g, high dose) and 242 gram (range 97-541g, low dose), respectively. Fourteen out of 32 patients who completed the study (44%) reached the therapeutic level within 3 months: 10/20 from the high dose regimen and 4/12 from the low-dose regimen. These results illustrate that plasma concentration build-up is highly variable among patients. The strength of the proposed pharmacokinetic model is adaptability to individual patients in daily clinical practice by taking into account individual plasma level measurements. This learning aspect improves the accuracy of individual predictions and facilitates tailored dose adjustments in order to reach and maintain the therapeutic level within a certain period of time. We expect that this leads to early dose increments in patients who require high mitotane doses, which speeds up attainment of the therapeutic plasma level. Also, patients who display a rapid increase in mitotane plasma level can be identified. Timely dose reductions in these patients are expected to prevent an overshoot to potentially toxic levels. In patients with advanced disease cytotoxic chemotherapy is usually added in case of disease progression and/or failure of mitotane therapy. Timing of chemotherapy initiation can be complicated if therapeutic levels of mitotane have not been reached yet, because a therapeutic effect could still be expected and chemotherapy might not be necessary at that time. We expect that improved prediction of mitotane levels leads to rapid and anticipated attainment of 14mg/L and therefore to adequately timed and more accurate therapy evaluation.

The model performed well predicting mitotane levels in a validation group from another hospital. Distribution of observed versus predicted values indicates a slight tendency towards

underestimation of plasma levels in or above the therapeutic range, predominantly in the development group. In a prospective setting, this could lead to dose increments where continuation would be appropriate or dose continuation where reduction would be appropriate. However, this is assumed to be less harmful to patients than the opposite, which could result in subtherapeutic levels. The plasma concentration-time data contained several outliers, i.e. surprisingly high mitotane levels during the maintenance phase of therapy. The model could not adequately account for these values because mitotane plasma levels usually increase gradually over the course of weeks. In most cases a satisfying clinical explanation could not be found. We hypothesize that the outliers were caused by medication interactions or undocumented weight loss triggering redistribution of mitotane to the central compartment.

Previous research indicates that mitotane is a highly lipophilic drug.¹⁹ The estimated volume of distribution is relatively high and the estimated clearance is relatively low. These findings are compatible with the drug's lipophilicity and suggest that the plasma concentration is determined by distribution processes rather than elimination processes. Previous observations of fatty tissue concentrations which were approximately 200-fold higher than plasma concentrations are in agreement with this hypothesis.¹⁴ Analysis of covariates showed considerably weak correlations ($r \approx -0.50$) between weight (both total weight and LBM) and V_1 . Other covariates (age, gender, height, BSA) displayed correlations in the same order of magnitude. We hypothesize that factors not accounted for in the model, particularly differences in genetic constitution, are of importance to explain the residual variation of mitotane pharmacokinetics in the population. This assumption is supported by recent research suggesting that a polymorphism in the gene coding for the CYP2B6 enzyme is associated with higher plasma concentrations after three months of treatment.¹³

We acknowledge the limitations of our study. We propose a pharmacokinetic model based on retrospective data. The predictive value will have to be confirmed in a prospective setting. In both hospitals, the standard operating procedure for mitotane level measurement is to assess trough levels. Due to the retrospective nature of the data we cannot guarantee that all levels were trough levels. Selection bias might be of influence, as all patients (except two) had reached the therapeutic level during treatment. Because there were no patients with liver or kidney failure, we could not assess the influence of these factors on mitotane pharmacokinetics. Finally, interindividual dietary differences could have influenced the model through drug absorption.

CONCLUSION

A three-compartment pharmacokinetic model of mitotane was developed, which estimated mean volume of distribution in steady state (V_{ss}) at 161 ± 68 L/kg-LBM and mean mitotane clearance at 0.94 ± 0.37 L/h. The model appears to be able to predict mitotane levels in individual patients with reasonable accuracy considering an error margin of $14\% \pm 13\%$ in an independent validation group. Residual variance in population pharmacokinetic parameters remains to be elucidated. Future research should be aimed at exploring genetic factors involved in mitotane pharmacokinetics.

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CHAPTER 10

SHORT-TERM VARIATION IN PLASMA MITOTANE LEVELS CONFIRMS THE IMPORTANCE OF TROUGH LEVEL MONITORING

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ABSTRACT**Objective**

Mitotane is the drug of choice in patients with adrenocortical carcinoma. The anti-neoplastic effect is correlated with mitotane plasma levels, which renders it crucial to reach and maintain the concentration above 14mg/L. However, mitotane pharmacokinetics are poorly understood. Aim of the present study was to investigate the variation of plasma mitotane during the day and the influence of a single morning dose.

Design

A prospective case–control study was conducted to investigate the variation in plasma mitotane levels.

Methods

Patients who had been treated for at least 24 weeks and had reached the therapeutic plasma level (14mg/L) at least once were eligible. In the first group mitotane levels were determined hourly for the duration of eight hours after administration of a single morning dose. In the second group mitotane levels were assessed similarly without administration of a morning dose.

Results

Ten patients were included, three patients participated in both groups. Median plasma level at baseline was 16.2mg/L (range 11.3–23.3mg/L) in the first group (n=7) and 17.0mg/L (13.7–23.8) in the second group (n=6). Plasma levels displayed a median increase compared to baseline of 24% (range 6–42%) at t=4 after morning dose and a change of 13% (range -14–33%) at t=4 without morning dose ($P=0.02$).

Conclusion

A substantial increase in mitotane plasma levels was observed in steady-state patients within a period of eight hours after morning dosing. Without morning dose, mitotane curves showed a variable profile throughout the day. This implies random sampling could yield incidentally high levels. For this reason, we recommend early-morning trough sampling as standard management in monitoring mitotane treatment.

INTRODUCTION

The adrenolytic drug mitotane (2,2(1-chlorophenyl, 2-chlorophenyl)1,1-dichloroethane [o,p'DDD]) is registered for treatment of adrenocortical carcinoma (ACC).^{1,2} Mitotane affects the adrenal steroid synthesis which results in diminished cortisol secretion, but the exact mechanism of action is unknown.³ It also exerts an anti-neoplastic effect on ACC's, which is the main indication for treatment in ACC patients. The anti-steroidogenic effect is a fortuitous side-effect in patients suffering from hypercortisolism due to a functional ACC.

In addition to the well established use of mitotane in irresectable and metastasized ACC, there is increasing evidence that adjuvant mitotane after radical resection of the primary tumor increases recurrence free survival.⁴⁻⁶ In anticipation of prospective confirmation, the international consensus is to recommend mitotane in patients with high risk of recurrence, i.e. after irradical resection and/or ki-67 index >10%.⁷

The anti-neoplastic effect appears to be correlated with plasma levels: several studies reported an objective response in patients whose plasma trough levels were above 14mg/L.⁸⁻¹⁰ Although this does not rule out the possibility of response at lower plasma levels, a major goal during mitotane therapy is to reach and maintain plasma levels above 14mg/L. In order to limit drug toxicity, 20mg/L is considered the upper limit of the therapeutic window.⁹ The build-up of plasma levels is in general slow, taking on average 3 to 4 months but interindividual differences are large.¹¹⁻¹³ Because knowledge on mitotane pharmacokinetics is sparse, current dosing regimens are based on clinical experience and adjusted according to plasma levels and tolerability. In some hospitals including our own, it is common practice to monitor mitotane plasma levels by performing trough measurements, i.e. at least twelve hours after the last dose. In other centers, random measurements throughout the day are accepted. Due to mitotane's extremely long half life of 18-159 days, it is hypothesized that plasma levels show little variation between doses and trough sampling is not necessary. However, this has never been studied in patients who reached steady state.¹⁴ The aim of the present study was to investigate the variation of plasma mitotane during the day and the influence of a single morning dose.

SUBJECTS AND METHODS

The variation in mitotane plasma levels was prospectively investigated in ACC patients who had been treated for at least 24 weeks and who had reached the therapeutic plasma level (>14 mg/L) at least once. The study protocol was approved by the medical research ethics committee in Máxima Medical Center and all patients signed informed consent.

In the first group patients took their regular morning dose (RMD) of mitotane with a minimum of 1000mg (range 1000mg-1500mg) orally at baseline (08:00h AM). Two patients (ID 4 and 6) were on a dosing regimen with no or 500mg mitotane at breakfast. In these patients, the total daily dose was administered at baseline during the study (1000 and 1500mg, respectively).

Time since prior dosing was at least twelve hours. Mitotane plasma levels were determined at baseline and hourly for the duration of eight hours. Patients did not take other mitotane doses during this time. Concomitant medication was allowed and registered. All patients were served the same breakfast (at 08:00h, after blood withdrawal for baseline measurement) and lunch (at 12:00h, after t=4 measurement) to rule out the influence of food on drug absorption as a confounding factor.

In the second group mitotane was not administered on the day of study (nRMD), but patients had taken their regular doses the day before. Determination of mitotane plasma levels and food intake was identical to the first group. Patients did not engage in strenuous physical activities and spent the day mostly reading or watching television.

Liver enzymes (gamma-glutamyltransferase [γ -GT], aspartate aminotransferase [ASAT], alanine aminotransferase [ALAT]), lactate dehydrogenase (LDH), alkaline phosphatase and serum creatinine were analyzed at baseline.

Measurement of plasma levels

Mitotane plasma concentrations were determined by high performance liquid chromatography (HPLC) with UV detection at Atlanbio laboratory (Saint-Nazaire, France) using the Lysosafe service offered by HRA Pharma (Paris, France). The HPLC assay is calibrated and verified every two months. The method is validated with an accepted variability of $\pm 15\%$ on concentration values. Mean intra-assay coefficient of variation (CV) was 3.7% and mean inter-assay CV was 2.7% in test runs performed during this study with a validation sample concentration of 20mg/L. Since July 2013, a gas chromatography/mass-spectrometry (GC/MS) assay is used for mitotane plasma concentration measurement. Mean intra-assay CV was 2.7% and mean inter-assay CV was 2.0% in test runs performed with a quality control sample concentration of 25mg/L. Previous research determined good agreement between both assays.¹⁵ Because medium to high mitotane levels were anticipated, a change in plasma levels ≥ 1.0 mg/L was considered clinically significant in the present study. This translates to a CV of 5% given a mean concentration of 20mg/L (data provided by HRA Pharma).

Statistical analysis

Main parameter in this study was the variation of mitotane plasma levels during the day. All individual measurements were displayed. In addition, median change from baseline was calculated per group and displayed. The non-parametric Mann-Whitney U test was used to analyze median change in plasma levels between both groups.

Data management and statistical analyses were performed using Prism 6.0 (Graphpad Software, La Jolla, USA) and SPSS 19.0 (IBM, Armonk, USA).

RESULTS

Ten patients were analyzed, there were eight women and two men (Table 1). Three patients (ID 11, 12 and 13) participated in both the RMD and the nRMD group, resulting in 13 individual mitotane curves. Time between both study days for patients who were analyzed twice was 81, 65 and 62 weeks, respectively.

Median time since start of mitotane therapy was 54 weeks (range 29-114 weeks) in the RMD group and 80 weeks (46-121 weeks) among patients in the nRMD group. Median BMI was 28.4 kg/m² (21.1-36.8 kg/m²) in the first group and 27.6 (20.8-36.4 kg/m²) in the second. Four patients used a proton-pump inhibitor during the study day (ID 1, 2, 4, 13), one patient used an H₂-receptor antagonist (ID 3). Three patients had mitotane concentrations <14mg/L at baseline, all patients reached this threshold at some point during the day they were studied (Figure 1A and 2A). Median plasma level at baseline was 16.2 mg/L (range 11.3-23.3 mg/L) in the RMD group and 17.0 mg/L (range 13.7-23.8) in the nRMD group. Among patients who did take a morning dose, individual maximum plasma concentrations were reached after 2 (n=1), 3 (n=1), 4 (n=2), 5 (n=2) or 7 hours (n=1). In five out of six patients without morning dose, mitotane plasma levels increased during the day as well: maximum levels were reached after 4 (n=2), 6 (n=2), or 8 hours (n=1). One patient demonstrated the maximum level at baseline (Patient 11).

In the RMD group, median change in plasma level compared to baseline was +3% at t=1 (range -8-15%) to +24% at t=4 (range 6-42%) and +3% at t=8 (range -10-19%, Figure 1B). The greatest individual increment observed was 54% between t=0 and t=5 (Patient 7).

In the nRMD group, median change in plasma level compared to baseline was -2% at t=1 (range -5;4%), +13% at t=4 (range -14-33%) and -2% at t=8 (range -8-21%, Figure 2B). The greatest individual increment observed was 26% between t=0 and t=5 (Patient 8). Median change in mitotane plasma level at t=4 was significantly different between both groups ($P=0.02$).

In all patients elevated γ -GT levels were observed, median level was 234 U/L (120-430) in the first group and 80 U/L (63-572) in the second group (Table 2). Other laboratory investigations did not indicate impaired liver or kidney function in any of the patients.

Table 1: Demographic and mitotane dosing characteristics of patients in the maintenance phase of mitotane treatment.

Patient ID	Sex	BMI (kg/m ²)	Time on mitotane (weeks)	Normal dosing regimen (mg)	Dose at baseline (mg)	Last dose prior to study day (mg)	Time since last dose (h)
With morning dose							
1	F	23,9	114	1500-500-1000	1500	1000	13
2	F	30,9	81	1000-0-500	1000	500	14
3	M	25	54	1000-1000-1000	1000	1000	12
4	F	32,4	40	500-500-500*	1500	500	15
5	M	21,1	29	1500-1000-1500	1500	1500	12
6	F	28,4	86	0-500-500*	1000	500	13
7	F	36,8	35	1500-1500-2000	1500	2000	15
Median		28,4	54		1500	1000	13
Range		21,1-36,8	29-114		1000-1500	500-2000	12-15
Without morning dose							
8	F	26,1	57	1000-500-500	n/a	500	13
9	F	25,6	46	500-0-500	n/a	500	14
10	F	29,1	66	500-500-500	n/a	500	13
11 (5)	M	20,8	94	1000-1000-1000	n/a	1000	10
12 (7)	F	36,4	97	500-500-500	n/a	500	15
13 (4)	F	30,8	121	500-0-500	n/a	500	13
Median		27,6	80		n/a	500	13
Range		20,8-36,4	46-121		n/a	500-1000	10-15

* Two patients were on a dosing regimen with no or a single tablet of mitotane at breakfast. In these patients, the total dose was administered at baseline during the study.

Figure 1A: Individual variations in mitotane plasma levels after morning dose in seven patients.

Figure 1B: Median change from baseline mitotane plasma level after morning dose in seven patients.

Figure 2A: Individual variations in mitotane plasma levels without morning dose in six patients.

Figure 2B: Median change from baseline mitotane plasma level without morning dose in six patients.

Table 2: Results from baseline laboratory investigations in patients in steady state of mitotane treatment.

Pt. ID	γ-GT (U/L)	ASAT (U/L)	ALAT (U/L)	LDH (U/L)	Alk. Phos. (U/L)	Kreat. (μmol/L)
With morning dose						
1	430	21	17	220	77	82
2	120	16	16	170	100	47
3	192	28	26	200	87	108
4	234	40	60	230	208	65
5	122	25	15	200	50	60
6	295	12	9	130	51	64
7	375	116	75	280	102	51
Median	234	25	17	200	87	64
Range	120-430	12-116	9-75	130-280	50-208	47-108
Without morning dose						
8	63	19	11	220	112	78
9	572	34	85	170	114	53
10	65	28	13	210	54	53
11 (5)	91	23	15	170	66	61
12 (7)	68	38	22	210	66	41
13 (4)	156	19	20	170	196	63
Median	80	26	18	190	89	57
Range	63-572	19-38	11-85	170-220	54-196	41-78

γ-GT: gamma-glutamyltransferase, ASAT: Aspartate aminotransferase, ALAT: Alanine aminotransferase, LDH: Lactate dehydrogenase, Alk. Phos.: Alkaline phosphatase, Creat: Creatinine.

DISCUSSION

Our results show a median mitotane plasma level increment of 24% four hours after a single morning dose. This implies that random sampling could yield significantly higher levels compared to trough sampling. When a morning dose is skipped the mitotane curve displays a variable profile with significant increments during the day in half of the patients studied. The variability is greater than expected given the extremely long half-life of the drug. Physicians should be aware of the variability, especially since the lower limit of the therapeutic window (14 mg/L) was established based on research with trough levels.^{8, 9} For these reasons, we recommend trough sampling as standard management in monitoring mitotane treatment. The early morning appears to be the most suitable moment to assess trough levels.

Our results illustrate that random plasma level sampling could cause inappropriate dosing adjustments. For example, if patient 5 would have had a routine mitotane assessment in outpatient setting at 12:00h, it is likely that the dose would have been reduced based on the result of 22mg/L. The trough level of 15mg/L is only just within the therapeutic window, which would justify dose continuation.

To our knowledge, the variation of mitotane levels after a single dose has never been studied in patients who are in steady state. In a study from 1981, plasma levels in mitotane-naïve patients were evaluated after a standard dose given in various vehicles (tablets, granules, emulsion, chocolate and milk)¹⁴ Mean plasma levels measured 5 and 10 hours after ingestion of 2000mg (as commercially available tablets) were 1.8 ± 1.1 mg/L and 1.5 ± 1.0 mg/L, respectively. The concentration-time curves showed more than one maximum, similar to our observations in 5 out of 7 patients. In the present study, the observed difference between both groups in plasma level increment at t=4 must be caused by administration of the morning dose. However, five out of six patients from the nRMD group displayed an increase in mitotane plasma level during the day despite absence of a morning dose. In three of these patients the increase constituted >20% of the baseline level. This finding cannot be explained by delayed gastro-intestinal absorption given the time of approximately seventeen hours since the last dose. Also, enterohepatic reabsorption seems unlikely since previous research could not detect unmetabolized mitotane in bile.¹⁶ Therefore, we have to speculate on another explanation. Redistribution of mitotane between fatty tissues and plasma might cause fluctuation of mitotane levels. This mechanism is mainly influenced by transport in both directions, binding of mitotane to plasma lipoproteins and metabolism into 2,4-dichlorodiphenyl acetic acid (DDA)^{17, 18} Mitotane clearance is probably of minor impact on the plasma level. This is in agreement with the drug’s known lipophilicity and with earlier findings regarding the development of a pharmacokinetic model of mitotane.¹³ The model estimated volume of distribution relatively high (161 ± 68 L/kg-LBM) and clearance relatively low (1.12 ± 0.41 L/h). Also, previous observations of fatty tissue concentrations which were approximately 200-fold higher than plasma concentrations are in agreement with this hypothesis.¹⁹ Since the time from baseline to peak variability in both groups seems comparable (3-5 hours), the influence of food intake should be considered. As this was standardized in both groups, the present study does not permit conclusions on this topic.

There was a broad distribution of plasma level increments between patients in both groups despite the standardization of food intake and daytime activities. Also, we could not identify a correlation between plasma level increment and proton-pump inhibitor use, hydrocortisone dose nor patient characteristics such as BMI, duration of treatment or baseline plasma level. Genetic differences in absorption, distribution, metabolism and excretion (ADME) genes may be crucial for explaining differences in mitotane pharmacokinetics between patients. Research has indicated that a single nucleotide polymorphism in the gene coding for the CYP2B6 enzyme was significantly associated with the mitotane level achieved after three and six months of treatment.²⁰ Observations from that study suggest CYP2B6 is influential on mitotane metabolism, possibly through first-pass metabolism in the intestinal mucosa. Increased levels of γ-GT are often reported in patients on mitotane, but the cause is unknown. Studies on patients treated adjuvantly have shown the increase is reversible.²¹ We could not find a satisfactory explanation for the apparent difference in γ-GT levels between both groups in the present study, but we deemed it unlikely this influenced our results. The present study was not designed to examine the frequency or severity of side effects in patients with plasma levels >20mg/L. Patients were questioned orally during the day and none reported adverse events grade 3 or higher according to the Common Terminology Criteria for Adverse Events (CTCAE). We speculate that incidental

peaks above 20mg/L during the day are acceptable in terms of toxicity, but that baseline levels >20mg/L should be avoided. During steady state, the primary aim should be to keep the baseline level above 14mg/L with the lowest possible maintenance dose. Given the observation that a rise in mitotane plasma levels after a single dose lasts about eight hours, we advise an administration schedule with three daily doses.

We acknowledge the limitations of our study. Inpatient variability could influence our results since measurements were performed on one day per patient only. Early morning measurements might be subject to variation as well. In clinical practice, it could be considered to perform multiple early measurements shortly after one another, for example on three days during one week. This could yield a reliable impression of the true minimum plasma level. Finally, assay variation should be considered as a factor of influence on the observed variability.

In conclusion, our study shows that mitotane plasma levels display short-term variability that is greater than expected and of clinical importance because random sampling could yield incidentally high levels. Also, mitotane levels may increase during the day even when a morning dose is skipped. Previous research showed that mitotane plasma trough levels $\geq 14\text{mg/L}$ are associated with maximum therapeutic efficacy. For this reason, we recommend trough sampling as standard management in monitoring mitotane treatment. In order to prevent measurement of inappropriately high levels, sampling in the early morning appears to be the most suitable moment to assess trough levels.

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CHAPTER 11

GENERAL DISCUSSION AND FUTURE PERSPECTIVES

GENERAL DISCUSSION

This thesis focuses on several clinical aspects of ACC with special attention to epidemiology, diagnostics and treatment. In this final chapter the main findings are discussed. In addition, topics and directions for future research are discussed/provided.

There appears to be a decreasing incidence rate of adrenocortical carcinoma.

Our population-based study on the incidence of ACC in the Netherlands (**chapter 2**) detected a trend towards a decreasing incidence rate. This was quite a surprising finding, given the increasing detection rate of adrenal tumors in general. What could be the explanation behind this finding?

First, it should be acknowledged that these figures should be interpreted with caution since absolute numbers are low. The absolute difference between an incidence of 1.3/million in 1993 and 1.0/million in 2010 in the Netherlands is about 5 patients, depending on the method of standardization.

Secondly, the method of inclusion of this retrospective database-study should be critically reviewed. The diagnosis of ACC can be difficult to establish even on histological examination of the tumor itself.^{1, 2} The National Cancer Registry relies on the diagnosis made by the local pathologist in the hospital where the patient was treated and central pathology review by an expert pathologist was not performed. For this reason, it could be argued that some tumors in our study have been wrongfully classified as ACC or that some true ACCs have not been recognized and were thus missing in our study. However, we have no reason to assume that the diagnosis of ACC by the pathologist has changed significantly through the years covered by our study (1993-2010), since the Weiss and Van Slooten scoring systems were established long before our study period.^{2, 3}

Thirdly, it could be speculated that patients with suspect and/or 'pre-malignant' adrenal adenomas increasingly undergo surgery, resulting in a lower number of true ACCs. This hypothesis assumes the concept of the adenoma-carcinoma sequence as has been proposed for several other carcinomas such as colorectal carcinoma.⁴ The theory of dedifferentiation from adrenal adenoma to carcinoma has not yet been proven, although anecdotal evidence can be found.^{5, 6} It is estimated to occur very rarely; in 1/1000 ACA.⁵ For the Dutch situation, it seems unlikely that increased adenoma surgery is a major factor contributing to the decreasing incidence rate. The influence of environmental factors on the etiology of adrenocortical cancer has never been investigated. This should not be ruled out as potential explanation for a decrease in incidence, although a strong correlation with environmental or dietary carcinogens seems unlikely since very few people develop ACC. Also, geographical patterns that could be related to exposure to certain carcinogens or a particular genetic susceptibility do not appear to be present. Moreover, there are no significant differences in ACC incidence between countries on different continents.

There is one exception: in southern Brazil there is a region where the incidence of pediatric ACC is up to 15 times greater due to a prevalent germline mutation of the TP53 gene (R337H TP53).^{7,8}

In conclusion, explaining the observed decreasing incidence rate of ACC remains difficult. It is the intention of the Dutch Adrenal Network to follow-up on this trend and re-assess the incidence rate at 5-year intervals. The NCR is a dynamic database, which means that patients may be added or removed based on new insights or revisions. A central pathology review by expert pathologists could improve the methodological quality of future studies.

There are two different entities in pediatric adrenocortical carcinoma.

In a population-based study on pediatric ACC, a significant correlation between clinical outcome and age at presentation was observed (**chapter 3**). All patients younger than 5 years at diagnosis were alive without evidence of disease after at least 5 years follow-up, whereas all patients older than 10 years died of their disease. Traditional histological criteria included in the Weiss and Van Slooten score are defined by the presence of necrosis, nuclear atypia, mitoses, changes in architecture and invasion of normal tissue. The difference in survival between both age groups could not be explained by the presence or absence of these criteria. Although the study's small sample size precludes a definite conclusion, the strong correlation suggests that there might be a fundamental difference in ACC behavior between these two pediatric age groups. Interestingly, a structural change in the human adrenal cortex takes place at around 4 years of age, i.e. development of the zona reticularis (ZR). It has been shown with morphological, immunohistochemical and enzymatic studies (examining reactivity to the ZR-specific enzyme 17 β -hydroxysteroid-dehydrogenase, HSD17B5) that the zona reticularis is absent in adrenal glands of children younger than 3 years.⁹ In summary, there is a theoretical rationale for a structural difference in adrenal cortex histology between both age groups. However, in studies on pediatric adrenal tumors, a histological difference has not yet been identified. The absence of a zona reticularis might be related to a more favorable outcome of ACC in younger children. Gene expression profiling studies aimed at adrenal tumors in infants could elucidate differences in tumorigenesis between both age groups.

In selected cases, it may be impossible to differentiate between adrenal pathologies based on pre-operative diagnostic tests.

Existing diagnostic algorithms used to determine type, hormonal activity and malignant potential of adrenal tumors were discussed in **chapter 4 and 5**. Although useful in the majority of patients, no algorithm, laboratory test or imaging study is able to correctly diagnose all patients or exclude malignancy with 100% confidence.¹⁰⁻¹³ As discussed in **chapter 6**, urinary steroid profiling might represent a useful addition to the diagnostic armamentarium. However, there remains a subgroup of adrenal lesions that are hormonally inactive and display non-specific imaging characteristics. The main concern among these patients is whether their tumor is potentially harmful, which translates to whether there is a risk of malignancy or a risk of rupture and subsequent bleeding (in case of cystic lesions). The risk of malignancy other than ACC or malignant pheochromocytoma is very low. Possible causes are adrenal lymphoma and several sarcoma subtypes (angiosarcoma, liposarcoma, leiomyosarcoma). Nonetheless, the patient's concern is understandable. In addition,

the physician may be equally worried and inclined towards definite confirmation of the diagnosis. As a consequence, a certain number of these patients will be directed for adrenalectomy in order to obtain a diagnosis. This will include patients with lesions that turn out to be benign. The question whether the surgical approach should be laparoscopy or laparotomy is an interesting one. According to the literature, there is consensus that in ACC laparotomy should be regarded as standard treatment due to the supposedly increased risk of tumor spill and subsequent peritonitis carcinomatosa after laparoscopy.^{14, 15} However, the morbidity associated with laparotomy might be disproportionate to the low a priori risk of malignancy in patients with undetermined lesions. Therefore, in selected cases a laparoscopic approach seems reasonable despite the fact that the indication for surgery is risk of malignancy. It is acknowledged that this is an inconsistency based on clinical judgment without strong evidence-based arguments. In order to assess whether laparoscopic resection is safely feasible, tumor size and radiological characteristics such as heterogeneity of the mass, presence of calcifications and suspicion of adherence to surrounding tissue should be taken into consideration. All patients should be discussed in a multidisciplinary team of specialists involved in the care for patients with adrenal tumors. For the future, there is an obvious need to improve the pre-operative determination of adrenal lesions. In this respect, urinary steroid profiling might be a promising technique.

Urinary steroid profiling might become a valuable addition to the diagnostic work-up of adrenal tumors.

Urinary steroid profiling (USP) discriminates ACC from non-ACC related tumors with high diagnostic accuracy (**chapter 6**). In particular, we demonstrated that measurement of tetrahydro-11-deoxycortisol (THS, a metabolite of 11-desoxycortisol) had the highest diagnostic test performance with a sensitivity and specificity of 100% and 99%, respectively.

The strong correlation between high urinary levels of THS and a final diagnosis of ACC is an interesting observation. High THS levels were found across all patients with ACC. High THS both in patients with localized and metastasized disease suggests that disturbances in steroidogenesis occur early in ACC pathogenesis. Also, high THS levels were observed regardless of clinical signs of hormonal overproduction. This suggests that excess THS might be a result of malignant proliferation of adrenocortical cells rather than specific disturbances in the cascade of steroidogenesis. It should be noted that downstream from the formation of pregnenolone, products are shuttled between mitochondria and the smooth endoplasmatic reticulum (SER).¹⁶ Hydroxylation of 17 α -hydroxyprogesterone to 11-deoxycortisol occurs in the SER and afterwards 11-deoxycortisol moves back to the mitochondria. In the zona fasciculata the enzyme 11 β -hydroxylase (also known as P450c11 or CYP11B1) catalyzes the formation of cortisol.¹⁷ We hypothesize that due to malignant changes in ACC cells re-entry of 11-deoxycortisol in the mitochondria is impaired and/or the mechanism becomes saturated, resulting in accumulation of the metabolite THS.

Our study was limited by its retrospective nature, although we aimed to minimize bias by including all urinary steroid profiles performed within the given period. This design could not prevent bias introduced by the physician's decision to order a

steroid profile. However, it is common practice in both centers that participated in the study to perform steroid profiling in all patients presenting with an adrenal tumor. The results justify a prospective trial. A large-scale prospective Dutch multicenter study examining the cost-effectiveness of USP compared to medical imaging has recently started (NCT02324647). We expect that if trials like these confirm the diagnostic value of USP, the number of CT-scans in the follow-up of adrenal tumors can be reduced considerably.

Surgery in specialized centers yields a survival benefit in patients with adrenocortical carcinoma.

The concept that rare diseases should be treated in a limited number of specialized hospitals seems intuitively logical. Our results presented in **chapter 7** associate primary surgery in a specialized Dutch Adrenal Network (DAN) center with a survival benefit for patients with local or locally advanced ACC.

Interestingly, a better outcome of oncological treatment in specialized centers is not necessarily correlated with volume requirements.¹⁸ Moreover, common minimum volume requirements as instituted for other cancers are not feasible for ACC in the Netherlands even if all patients with an ACC were to be treated in a single center. For example, according to current guidelines for breast cancer surgery, every center should perform at least 50 operations per year (in order to remain accredited). The number of new patients with ACC per year in the Netherlands is about 20 (median 21, range 13-26 between 1993 and 2010). Although the number of surgical procedures for suspect adrenal malignancy will be higher, the grand total is expected to be too low to institute meaningful minimum volume requirements.

Quality criteria other than volume are of greater importance in rare diseases. A possible approach could be the development of a set of criteria regarding pre-operative management, surgical resection and post-operative treatment for ACC. An international panel of experts should formalize that a given center is deemed ‘expert center’ if it meets these criteria. In addition, participation in international networks and clinical trials could be encouraged. A so-called *care pathway* describes the entire pathway a patient with a specific disorder (i.e. adrenocortical carcinoma) follows in the hospital. It describes which diagnostic tests a patient should undergo, determines discussion of the patient in a multidisciplinary meeting and it sets limits for the maximum time each additional step should take. All specialists involved (oncologist, surgeon, radiologist, radiotherapist, etc) should agree to commit to this care path. Table 1 contains a brief summary of key points from the care pathway that should be present in an ACC-specialized center.

In The Netherlands, the eight university hospitals have always been (at least since decades) a tertiary referral center for complex and rare pathologies. Traditionally, these centers harbor expertise and facilitate a multidisciplinary approach towards patients with complex and rare diseases such as ACC. There are four university hospitals situated in the densely populated mid-western part of the country. There are four additional university hospitals in the less densely populated north, central, eastern and southern parts, respectively. Regarding ACC, patients can

be treated in all university hospitals or in MMC, which is situated in the central/southern part of the country.

Without instituting minimum volume requirements, it still seems logical to reduce the number of centers for ACC treatment. Given the rarity of the disease and the short traveling times in a small country such as the Netherlands, a total number of four expert centres seems reasonable. This way, high-quality care can be combined with short-distance traveling and frequent contact between patient and physician.

Table 1: Summary of key points that characterize an expert center regarding adrenocortical carcinoma.

Phase	Subject
Diagnostic	Unenhanced abdominal CT-scan and urinary steroid profile are performed. Multidisciplinary meeting attended by all specialists involved, <2 weeks after completion of diagnostic tests.
Surgical treatment	Adrenalectomy <3 weeks of multidisciplinary meeting.
Post-operative	Pathology report contains itemized Weiss-score and ki-67 index.
Adjuvant treatment	Adjuvant mitotane treatment instituted and maintained for at least 2 years. Adjuvant treatment with radiotherapy available. Possibility to enroll patients in (international) trials.
Metastatic disease	Mitotane treatment instituted according to FIRM-ACT protocol. Treatment with EDP chemotherapy available. Treatment with second-line chemotherapy available. Palliative treatment with radiotherapy available.

Predicting mitotane plasma levels is helpful in clinically managing mitotane treatment.

Obtaining a plasma mitotane concentration above 14mg/L is a major goal during treatment as it has been associated with a better therapeutic response.^{19, 20} Consequently, it might be important to achieve such concentrations as early as possible in order to rapidly establish antiproliferative efficacy, although this reasoning is not underpinned by clinical data. A problematic aspect is that the time to reach an effective plasma concentration of 14mg/L usually takes several months.^{21, 22} Especially in patients presenting with stage IV disease (+/- 50% of patients), this long build-up phase delays effective treatment.

As displayed in **chapter 8**, we observed higher plasma levels and more patients attaining the target level after administration of higher doses of mitotane. However, the results were not statistically significant and interindividual variability was high. It was suspected that concomitant administration of chemotherapy influenced the results, because differences were greater in the subgroup of patients who did not receive chemotherapy. The co-administration of chemotherapy might affect mitotane pharmacokinetics by inducing or inhibiting enzymes involved in drug metabolism. In addition, it is possible that patients on chemotherapy were more vulnerable to drug

toxicity resulting in more mitotane-related side effects, thereby preventing optimal treatment. In order to investigate mitotane pharmacokinetics further, we developed a population pharmacokinetic model (**chapter 9**). A three-compartment model best described the data. The estimated volume of distribution was relatively high and clearance was relatively low. This is compatible with the profile of mitotane, i.e. a highly lipophilic drug with an extremely long half-life and concentrations in fatty tissue that are approximately 200-fold higher than in plasma. Translated to clinical practice, these findings suggest dose proportionality, i.e. drug elimination proceeds at a constant rate independent of the plasma concentration (zero-order kinetics). In fact, drug distribution appears to be far more important than drug elimination. Consequently, administration of higher doses should result in higher plasma levels. Using the proposed pharmacokinetic model, clinicians can predict the mitotane plasma level in an individual patient by taking into account previous plasma level measurements. This learning aspect improves the accuracy of individual predictions and facilitates tailored dose adjustments in order to reach and maintain the therapeutic level within a certain period of time. We suggest that might result in earlier dose increments in patients who require high mitotane doses, which is expected to speed up attainment of the therapeutic plasma level. Also, patients who display a rapid increase in mitotane plasma level can be identified. Timely dose reductions in these patients could be helpful to prevent an overshoot to potentially toxic levels.

Since co-administration of chemotherapy during mitotane build-up may be problematic, this situation may be avoided by postponing initiation of chemotherapy until the therapeutic mitotane level is reached. This facilitates the evaluation of mitotane monotherapy, which might be effective in approximately 60% of patients.^{19, 23, 24} An additional argument is that chemotherapy (EDP) may be more effective in combination with adequate mitotane levels due to an interaction at the drug resistance protein.^{25, 26} Some patients present with a very aggressive course of disease not amenable to surgical treatment, in which case short-term reduction of tumor volume is warranted to save vital organ functions. In these patients, administration of EDP chemotherapy might stabilize disease, which yields time to optimize mitotane treatment.

It is important to realize that despite optimization of dosing schedules, the key factor influencing build-up of mitotane plasma levels is patient tolerability. From this perspective, the importance of adequate supportive treatment can not be stressed enough, i.e. administration of hydrocortisone, anti-emetic and anti-diarrheal drugs if necessary. A possible strategy is to start concomitant treatment on day 1 including hydrocortisone 20mg, metoclopramide 10mg 3 times daily (as needed) and loperamide 2mg 4 times daily (as needed). Hydrocortisone should be increased as needed along with the increasing mitotane plasma level. Psychological and social aspects of treatment should not be neglected, i.e. professional counselling may be warranted. Follow-up on patient's well-being may be performed by using the EORTC QLQ-C30 questionnaire upon start of treatment and by repeating this assessment every 3 months. In our experience educating patients about side-effects, taking time to register side-effects and providing instruments to alleviate them improves compliance and patient motivation to stay on treatment.

Mitotane plasma levels should be assessed as trough level in the early morning.

As discussed above, monitoring plasma levels is an important aspect of mitotane treatment. Due to mitotane's extremely long half-life, it was hypothesized that plasma levels show little variation between doses. Surprisingly, our results in **chapter 10** show significantly increased plasma levels after a single morning dose. In addition, variable profiles with significant increments were observed when a morning dose was skipped.

Our study was designed to investigate the influence of a single dose on the plasma level throughout the day. Therefore, patients were only administered a single dose in the morning. This was the dose they would normally have taken in the morning, which is not the total dose on a normal day. Usually, an administration regimen of 3 times daily (with meals) is advised. We had chosen to administer only one dose because we suspected that repeat dosing would blur the view on the pharmacokinetic behavior of a single dose. Furthermore, we acknowledge that our study was not designed to demonstrate that measuring the trough level is preferable nor that sampling should be done in the early morning. However, we do know that the threshold of 14mg/L was established in studies with trough levels (i.e. at least twelve hours after last dose).^{19, 23} Therefore, the question is when the 'true' trough level should be measured. Our results demonstrate that plasma levels may increase during the day even without dosing, hence we suppose that plasma levels are at their lowest in early-morning. Consequently, we assume that if the early morning trough level is >14mg/L, the plasma level will be in adequate range during the rest of the day, especially when three doses are administered normally. In clinical practice, it could be considered to perform several early trough measurements shortly after one another, for example on three days during one week (Monday, Wednesday, and Friday). This could yield a reliable impression of the true minimum plasma level. This might be somewhat cumbersome for patients, but could be acceptable if performed once every two or three months.

FUTURE PERSPECTIVES

As always, completed studies generate new questions, hypotheses and openings for further research. Regarding the specific topics covered in this thesis, there are several items in need of follow-up investigation.

The predictive value of the pharmacokinetic model should be prospectively investigated. By comparing model-based dose adaptations with the existing practice of the FIRM-ACT dosing schedule, it can be evaluated whether patients reach the therapeutic threshold earlier with acceptable toxicity. It is expected that selected patients can tolerate higher mitotane doses than commonly applied in current clinical practice. Higher dosing in combination with adequate supportive therapy and therapeutic drug monitoring could result in earlier achievement of the therapeutic plasma concentration. Setting realistic short-term treatment goals (i.e. plasma levels) using the predictive model could help patients to remain motivated which is presumed to have a positive effect on tolerating drug toxicity. In a later stage, the model can be rewarding to patients as it is expected to be helpful in maintaining steady state on the lowest possible mitotane dose.

The model could be ameliorated by incorporating pharmacogenetic variables. It is expected that this will further improve the ability to perform individual dosing in mitotane treatment. Also, this might elucidate which ADME-genes (genes involved in drug absorption, distribution, metabolism and excretion) are important in mitotane pharmacokinetics. According to our pharmacokinetic model, drug excretion is only a minor factor in mitotane kinetics. It is expected that interindividual differences in drug absorption and distribution account for most of the variance observed. Future research on mitotane kinetics could benefit greatly from measuring mitotane tissue concentrations and standardizing mitotane intake with food, for example using lipid-rich carriers. Unfortunately, determination of mitotane concentration in tissue is currently considered 'a lost art', which should be re-invented by laboratories involved.

Prognostication of ACC has improved with the introduction of the ENSAT-staging system in 2009. However, patients with stage IV disease still form a heterogeneous group with respect to their prognosis. It will be investigated whether differentiation based on synchronous metastatic disease (i.e. at presentation or within the first six months thereafter) versus metachronous metastatic disease (i.e. at least six months after presentation) yields improved prognostication, which could potentially influence therapeutic management. Presumably, the first group performs worse in terms of overall survival. In a follow-up study, it should be investigated whether patients in the first group may benefit from combination therapy (EDP+mitotane) started immediately upon establishing stage IV disease, whereas patients with metachronous metastasis may be started on mitotane monotherapy combined with local control using surgery or radiotherapy if possible.

Difference in overall survival between patients with stage I and stage II disease was not significant in validation studies. Histological and clinical data from patients in the Dutch cohort might contribute to an improved staging system. In this group, the main question is which patients should receive adjuvant treatment. One of the hypotheses is that in addition to a high ki-67 index, vascular invasion of tumor cells puts patients at increased risk for recurrent or metastasized disease. This would warrant aggressive adjuvant treatment. Robust retrospective data could form the rationale for a prospective study on adjuvant treatment in high-risk patients, which could randomize between treatment with mitotane and EDP.

Recently, studies started focusing on epigenetic changes associated with adrenocortical tumors. Epigenetics refers to the non-sequence-based modifications of DNA that are maintained during cell division without altering the DNA sequence. Epigenetic alterations occur in cancer cells as often as genetic mutations and even have the ability to mimic the effects of the latter. Since epigenetic research in ACC is in a very early stage, it is difficult to predict possible developments. Of course, finding potential targets for treatment would be the ultimate goal, but it seems unrealistic to expect this in the near future. In the following years, research should be focused at detecting and understanding epigenetic changes in ACC. Studying the Dutch cohort might contribute to general knowledge in this promising field of research.

In a broad sense, significant improvement of ACC patient care can only be achieved through improved understanding of adrenocortical tumorigenesis. This has certainly improved in recent years, but has not yet resulted in improved clinical care. Unraveling the disease pathogenesis harbors the opportunity to discover new treatment options and is also expected to improve individual prediction of survival. International collaboration is crucial to support large-scale studies with state-of-the-art techniques (whole genome sequencing). The European Network for the Study of Adrenal Tumors (ENSAT) is an expanding collaboration formed by enthusiastic researchers and clinicians. It holds a database with clinical data of >2100 ACC patients (as of February 2015), which is of great potential for future studies. International collaboration is the key to further progress in understanding the disease, improving clinical care and keeping hope for the future.

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SUMMARY

SUMMARY

The present thesis was designed to investigate adrenocortical carcinoma (ACC) from a clinical perspective with a focus on epidemiology, diagnostics and treatment.

ACC is a rare disease. **Chapter 2** contains a population-based study on incidence and survival rates in the Netherlands. All ACC patients registered in the Netherlands Cancer Registry (NCR) between 1993 and 2010 were included. The total number of ACC patients was 359. The 5-year age-standardized incidence rate decreased from 1.3 to 1.0 per one million person-years. Median survival for patients with stage I-II, stage III and stage IV disease was 159 months (95% CI 93-225 months), 26 months (95% CI 4-48 months) and 5 months (95% CI 2-7 months), respectively ($P < 0.001$). Improvement in survival during the observed period was not observed.

In **chapter 3** incidence, histological characteristics, treatment and survival of pediatric patients with ACC in the Netherlands is described. All patients aged <20 years at diagnosis and registered in the NCR between 1993 and 2010 were included. Clinical data were extracted from the medical records and archival histology slides were reviewed. Twelve patients were identified. The population-based age-standardized incidence rate for patients <20 years was 0.18 per million person-years. For all patients histological examination displayed malignant characteristics. All patients aged ≤4 years at diagnosis survived, median follow-up was 97 months (57-179 months). All patients aged ≥5 years died, median survival was 6 months (0-38 months). Clinical outcome was remarkably better in patients aged ≤4 years. This is in accordance with less advanced stage of disease at presentation, but contrasts with the presence of adverse histological characteristics. In absence of adequate evidence regarding pediatric ACC, clinical management in advanced disease is adapted from the adult practice.

Chapter 4 contains practical recommendations for clinical management of adrenal tumors based on current guidelines. The common differential diagnosis in adrenal incidentaloma consists of (non)functioning adenoma, pheochromocytoma, myelolipoma, metastasis and primary carcinoma. By performing hormonal work-up, (over)production of glucocorticoids, mineralocorticoids and androgens can be assessed. Important radiological criteria in assessing malignant potential are tumor size, shape, unenhanced attenuation value and growth rate. There remains a category of lesions that are hormonally inactive and display non-specific imaging characteristics. In **chapter 5**, a succinct literature review regarding pathologies from this category is presented. It may be difficult or even impossible to differentiate between these pathologies based on pre-operative diagnostic tests. Radical surgical resection is indicated in case of possibly harmful tumors, e.g. lesions with malignant potential, risk of spontaneous hemorrhage or increase in size over time. Clinicians should assess these issues using clinical judgment complemented with radiological evaluation of the lesion.

Urinary steroid profiling is a promising tool for discriminating between benign and malignant adrenocortical tumors. **Chapter 6** contains a retrospective study including urinary steroid profiles

performed in 152 patients with various adrenal tumors. The results show that urinary excretion of tetrahydro-11-deoxycortisol (THS), a metabolite of 11-deoxycortisol, is associated with very high sensitivity (100%) and specificity (99%) to differentiate between ACC and a benign adrenal mass. Since the technique is patient friendly, entirely safe and less expensive compared to the current practice of repeat imaging studies, it is expected that urinary steroid profiling will become standard procedure in the evaluation of adrenal tumors in the near future.

Surgery is the primary treatment of choice in ACC, since radical resection is the only chance of cure. Specialization of surgical treatment in rare conditions has been a controversial issue for many years, but increasing evidence suggests that treatment outcome is improved by centralization of care in specialized, high-volume centers. **Chapter 7** describes a population-based study aimed at determining whether there are differences in survival between patient operated on in specialized Dutch Adrenal Network (DAN) hospitals and non-DAN hospitals. Data on all adult patients diagnosed between 1999 and 2009 were obtained from the NCR (n=189). Median survival of patients with ENS@T disease stage I-III was significantly longer for patients operated on in a DAN hospital (n=46) compared to patients operated on in a non-DAN hospital (n=37, 5-year survival 63% versus 42%). Survival remained significantly different after correction for sex, age, year of diagnosis and stage of disease in multivariate analysis (hazard ratio 1.96 (95% CI 1.01-3.81), $P=0.047$). These results show that surgery in a DAN-hospital is associated with a survival benefit for patients with local or locally advanced ACC. The number of ACC patients in the Netherlands is too small to estimate minimum volume requirements for individual centers. It is hypothesized that criteria other than surgical volume are associated with the observed survival benefit. Examples are a multidisciplinary approach in several stages of treatment and adjuvant treatment according to up-to-date guidelines.

The adrenolytic drug mitotane is the cornerstone of ACC treatment in advanced stages and is increasingly used as adjuvant treatment in patients at high risk for recurrence. The drug's anti-neoplastic effect is correlated with plasma levels. A plasma level of 14mg/L is considered the lower limit of the therapeutic window. In **chapter 8**, the relationship between mitotane dose and plasma concentration is prospectively investigated by comparing two dosing regimens. Forty mitotane-naïve patients with metastatic ACC were assigned to a predefined low- or high-dose regimen. Median maximum plasma levels were not significantly different between the two groups (high-dose 14.3mg/L (6.3-29.7) versus 11.3mg/L (5.5-20.0), $p=0.235$). However, the administration of chemotherapy might have influenced these results. The observed differences were greater in a subgroup of patients who did not receive chemotherapy. Because the rate of adverse events was similar between both groups, the high-dose approach is the preferred strategy in patients with mitotane monotherapy. In patients with reduced tolerability (e.g. due to concomitant cytotoxic chemotherapy), a less aggressive regimen might be reasonable.

The study described in **chapter 9** was aimed at developing a pharmacokinetic model that enables clinicians to predict mitotane levels and adjust dosing based on a target drug exposure. A population pharmacokinetic model was constructed based on detailed data on mitotane dosing and plasma level measurements from 20 patients using iterative two-stage Bayesian fitting

(MWPharm). The concentration-time data were best described by a three-compartment model. The model estimated mitotane clearance at 0.94 ± 0.37 L/h and volume of distribution in steady state at 161 ± 68 L/kg·LBM. The model appears to be able to predict mitotane levels in individual patients with an error margin of 14%. The model enables clinicians to adapt dosing based on individual plasma level measurements in prospective setting, which improves the prediction's accuracy. It is expected that individualization of mitotane dosing leads to anticipated and more rapid attainment of the therapeutic levels and potentially to improved clinical management of mitotane treatment.

In **chapter 10**, the variation of plasma mitotane during the day and the influence of a single morning dose are investigated. Patients who had been treated for at least 24 weeks and had reached the therapeutic plasma level (14mg/L) at least once were included. Mitotane plasma levels displayed a median increase compared to baseline of 24% (range 6-42%) 4 hours after morning dose. Surprisingly, a median increase of 13% (range -14 to 33%) after 4 hours of observation without administration of a morning dose was seen. This implies random sampling could yield incidentally high levels. For this reason, it is recommended to perform early-morning trough sampling as standard in monitoring mitotane treatment.

SAMENVATTING

NEDERLANDSE SAMENVATTING VOOR NIET-INGEWIJDEN.

Het doel van dit proefschrift is het onderzoeken van kanker van de bijnierschors. Het vóórkomen van de ziekte in de bevolking wordt onderzocht, evenals het herkennen en behandelen ervan.

Kanker van de bijnierschors, ook wel *bijnierschorscarcinoom*, is zeldzaam. In **hoofdstuk 2** wordt een studie beschreven naar het vóórkomen en naar de overlevingspercentages van deze ziekte in Nederland tussen 1993 en 2010. Deze studie bevat gegevens uit de gehele Nederlandse bevolking, omdat ze is gebaseerd op de Nederlandse kankerregistratie. Het totale aantal geïnccludeerde patiënten is 359. De *incidentie* (=het aantal nieuwe patiënten per jaar), gestandaardiseerd naar leeftijd, daalde in de onderzochte periode van 1.3 per miljoen inwoners naar 1.0 per miljoen inwoners. Van alle patiënten die zich presenteerden met een niet-uitgezaaide tumor (stadium I-II) was 50% nog in leven na 159 maanden, bij patiënten met een lokaal doorgroeiende tumor (stadium III) was deze termijn (ook wel de *mediane overleving* genoemd) 26 maanden. Van alle patiënten met uitzaaiingen bij presentatie (stadium IV) was 50% nog in leven na 5 maanden (95% BI 2-7 maanden). Er werd geen verbetering in overleving waargenomen in de onderzochte periode.

Hoofdstuk 3 bevat een studie naar het vóórkomen, de microscopische kenmerken, de behandeling en de overleving van kinderen met bijnierschorscarcinoom in Nederland. Alle patiënten met een leeftijd onder de 20 op het moment van diagnose werden geïnccludeerd. Ook deze studie is gebaseerd op de Nederlandse kankerregistratie en omvat de jaren 1993 t/m 2010. Gegevens werden ontleend aan de patiëntendossiers en archiefmateriaal van de tumoren werd opnieuw bekeken. Er werden twaalf patiënten geïdentificeerd. De incidentie werd berekend op 0.18 per miljoen inwoners onder de 20 jaar oud. Microscopisch onderzoek van de tumoren toonde diverse kwaadaardige kenmerken bij alle patiënten. Alle patiënten die vier jaar of jonger waren bij diagnose waren in leven aan het einde van de observatieperiode, de mediane observatieduur was 97 maanden. Alle patiënten van vijf jaar of ouder bij diagnose zijn overleden, de helft van de patiënten overleed binnen 6 maanden (spreiding 0-38 maanden). De patiënten van vier jaar of jonger hadden weliswaar een minder ver gevorderd stadium van ziekte bij presentatie, maar bij microscopisch onderzoek van het tumorweefsel werd geen verklarend patroon gezien in de aan- of afwezigheid van kwaadaardige kenmerken. Bij gebrek aan gegevens over de behandeling van bijnierschorscarcinoom in gevorderde stadia bij kinderen, wordt de behandeling overeenkomstig die bij volwassenen toegepast.

Hoofdstuk 4 bevat aanbevelingen voor de klinische praktijk wat diagnostiek en behandeling betreft bij patiënten die zich presenteren met een per toeval gevonden bijniergezwel van onduidelijke origine. De lijst van meest waarschijnlijke uiteindelijke diagnoses (=differentiaaldiagnose) behorend bij dit zogenaamde *bijnierincidentaloom* bestaat uit *adenoom* (hormoonproducerend of niet-producerend), *feochromocytoom* (=gezwel uitgaande van het *bijniermerg*), *myelolipoom* (=goedaardig vethoudend gezwel), *uitzaaiing* van een primaire tumor elders, of een *carcinoom* uitgaande van de bijnier zelf. Door middel van laboratoriumonderzoek kan overproductie van bijnierhormonen worden vastgesteld. Belangrijke radiologische criteria om (potentieel)

kwaadaardig gedrag aan te tonen zijn afmeting, vorm, dichtheid op CT-scan en groeisnelheid. Er resteert een categorie met tumoren zonder hormonale overproductie en zonder specifieke radiologische kenmerken. **Hoofdstuk 5** bevat een literatuurstudie naar tumorsoorten die in deze restcategorie vallen. Het kan moeilijk of zelfs onmogelijk zijn om door middel van pre-operatief onderzoek te differentiëren tussen deze mogelijke diagnoses. Complete chirurgische verwijdering is aangewezen bij potentieel gevaarlijke tumoren, zoals tumoren met risico op kwaadaardige ontaarding, risico op spontane bloedingen of een toename in afmeting. Klinische beoordeling gecombineerd met radiologische criteria zouden moeten leiden tot een afgewogen oordeel hierover.

Een steroïdprofiel is een laboratoriumonderzoek waarbij in de urine van een patiënt de concentratie wordt bepaald van 22 verschillende soorten pro-hormonen en afbraakproducten hiervan. In **hoofdstuk 6** wordt een retrospectieve (=terugkijkende) studie naar de diagnostische waarde van steroïdprofilering onder 152 patiënten beschreven. Uit de resultaten blijkt dat uitscheiding in de urine van het hormoon-afbraakproduct *tetrahydro-11-deoxycortisol (THS)* een zeer hoge *sensitiviteit* (=de kans op een positieve test gegeven aanwezigheid van de ziekte, in dit geval 100%) en *specificiteit* (=de kans op een negatieve test gegeven afwezigheid van de ziekte, in dit geval 99%) heeft voor het onderscheiden van een bijnierschorscarcinoom en een goedaardige bijniertumor. Dit urine-onderzoek is patiëntvriendelijk, volledig ongevaarlijk en goedkoper dan het maken van meerdere CT-scans, wat in de huidige praktijk gebruikelijk is. Al met al lijkt dit dan ook een veelbelovend diagnostisch instrument voor het differentiëren tussen goed- en kwaadaardige tumoren. Het is te verwachten dat het urine-steroïdprofiel een plek zal verwerven in het diagnostisch proces bij bijniertumoren na bevestiging van deze resultaten in een *prospectieve* (=vooruitkijkende) en *gerandomiseerde* studie (=indeling binnen de studie bepaald door loting).

Complete chirurgische verwijdering is de primaire behandeling van het bijnierschorscarcinoom, tevens de enige mogelijkheid tot genezing. Specialisatie van de chirurgische behandeling van zeldzame ziekten is al enige tijd een controversieel onderwerp. Gaandeweg komt er steeds meer bewijs vóór de stelling dat de uitkomst van de behandeling beter is indien deze uitgevoerd wordt in specialistische centra, die over het algemeen een grotere patiëntenpopulatie behandelen. **Hoofdstuk 7** beschrijft een onderzoek naar verschil in overleving tussen patiënten die geopereerd zijn in een gespecialiseerd ziekenhuis aangesloten bij het Bijnier Netwerk Nederland (BNN) en patiënten die in niet-BNN ziekenhuizen zijn geopereerd. De Nederlandse kankerregistratie is geraadpleegd en alle volwassen patiënten gediagnosticeerd tussen 1999 en 2009 zijn geïnccludeerd (n=189). De overleving van patiënten met niet-uitgezaaid bijnierschorscarcinoom (stadium I-III) bleek significant langer voor patiënten die waren geopereerd in een BNN-ziekenhuis vergeleken met patiënten die waren geopereerd in een niet-BNN centrum (5-jaars overleving 63% versus 42%). Dit verschil bleef significant, ook na correctie voor geslacht, leeftijd, jaar van diagnose en stadium van ziekte. Concluderend lijkt er een overlevingsvoordeel te zijn voor patiënten met niet-uitgezaaid bijnierschorscarcinoom die worden geopereerd in een BNN-ziekenhuis. Het totale aantal patiënten met bijnierschorscarcinoom in Nederland is echter te klein om zinvolle minimale patiëntenaantallen af te spreken voor individuele ziekenhuizen.

Bovendien wordt het geobserveerde verschil in overleving mogelijk niet verklaard door verschil in patiëntenaantal alleen. Een multidisciplinaire aanpak in alle stadia van de ziekte en *adjuvante behandeling* (=nabehandeling na een succesvolle operatie om de kans op terugkeer van ziekte te verkleinen, bijvoorbeeld met chemotherapie of bestraling) conform de huidige richtlijnen zouden hier zeer wel aan bij kunnen dragen.

Het medicament mitotaan is de hoeksteen van de behandeling bij *uitgezaaid* bijnierschorscarcinoom. Het wordt ook steeds vaker ingezet als adjuvante behandeling na een succesvolle operatie bij patiënten zonder uitzaaiingen, maar met een hoog risico op recidief. Het therapeutisch effect van mitotaan hangt samen met de concentratie van dit middel die in het bloed wordt opgebouwd: een concentratie van 14mg/L wordt tijdens de behandeling nagestreefd als ondergrens. **Hoofdstuk 8** bevat een onderzoek naar de relatie tussen de opbouw van deze concentratie en twee verschillende doseringsregimes. Een hoge-dosis opbouwschema werd vergeleken met een lage-dosis opbouwschema bij veertig patiënten. De mediane maximale plasmaspiegels waren niet significant verschillend tussen de twee groepen. De toediening van chemotherapie tijdens de studie kan de bevindingen hebben beïnvloed, omdat de verschillen in plasmaspiegels groter waren in de groep zonder chemotherapie. Ten aanzien van bijwerkingen waren de profielen van beide groepen vergelijkbaar, derhalve wordt toch geadviseerd te kiezen voor het hoge-dosis opbouwschema. Bij patiënten met een verminderde tolerantie voor mitotaan, bijvoorbeeld tijdens chemotherapie, kan het lage-dosis schema een goede keuze zijn.

De studie in **hoofdstuk 9** beschrijft het ontwikkelen van een *farmacokinetisch model* (=farmacokinetiek, de wetenschap die onderzoekt wat het lichaam doet met een geneesmiddel), dat de behandelend arts in staat moet stellen mitotaan bloedspiegels te voorspellen en de dosis van mitotaan aan te passen aan een doelconcentratie. Het model is berekend op basis van gedetailleerde gegevens betreffende mitotaan dosering en concentratie-metingen in twintig patiënten. Het model kan worden aangepast aan individuele patiënten op basis van lengte, gewicht en nierfunctie. Ook kunnen tijdens de behandeling dosisaanpassingen worden ingevoerd, evenals tussentijds gemeten bloedspiegels. Zodoende voorspelt het model mitotaan concentraties in individuele patiënten met een gemiddelde foutmarge van 14%. Gedurende de behandeling neemt de foutmarge af, omdat het model 'leert' van iedere ingevoerde meting. Met behulp van dit model kunnen artsen de mitotaandosis optimaliseren op basis van de gemeten en de gewenste concentraties. Het is te verwachten dat individualisering van mitotaandosis leidt tot het eerder en gepland bereiken van de therapeutische mitotaanspiegel en dat minder patiënten 'doorschieten' naar te hoge concentraties met de daarbij behorende verergering van de bijwerkingen.

Hoofdstuk 10 beschrijft de variatie van mitotaanspiegels gedurende de dag, met daarbij de invloed van een enkele dosis in de ochtend. Patiënten die waren behandeld gedurende minimaal 24 weken en daarin minimaal één keer boven 14mg/L hebben gezeten, werden geïnccludeerd. Mitotaanspiegels toonden na vier uur een mediane toename van 24% boven de uitgangswaarde. Verrassend genoeg werd ook in patiënten zonder ochtenddosering een mediane toename na 4 uur gezien van 13%. Deze resultaten tonen dat willekeurige bloedafnames kunnen leiden tot meting

van hogere spiegels, met het risico dat die niet juist geïnterpreteerd worden. Het is aangeraden mitotaanbehandling te monitoren door dalspiegels af te nemen, dat wil zeggen vóór inname van een nieuwe dosis. De vroege ochtend lijkt het meest geschikte moment voor deze afname gezien het variabele verloop van de spiegel gedurende de dag.

Hoofdstuk 11 bevat een algemene beschouwing van deze bevindingen met conclusies en aanbevelingen die volgen uit dit proefschrift.

VALORIZATION

VALORIZATION

It is important to translate academic results to societal or economic benefit and also to suggest routes of implementation to achieve this. This chapter discusses the potential societal or economic benefit of the presented findings.

PART I: EPIDEMIOLOGY

The population-based studies on the epidemiology of adrenocortical carcinoma in children and in adults confirmed the rarity of the disease (**chapter 1 and 2**). With an incidence of one patient per one million inhabitants and an estimated prevalence of 0.5 to 2 patients per hundred thousand inhabitants, ACC is not very interesting from a commercial point of view. After all, investments in new treatment options are difficult to redeem. For this reason, increasing international collaboration and globalization are welcome developments. The European Network for the Study of Adrenal Tumours (ENSAT), founded in 2002, is an expanding collaboration formed by researchers and clinicians. It holds a database with clinical data of >2200 ACC patients (as of June 2015), which is of great potential for future studies. It is expected that this international platform of collaboration will spark future large-scale research projects. The FIRM-ACT trial, launched in 2004 and published in New England Journal of Medicine in 2012, was the first proof that a large, international, multicenter trial could succeed, even if initiated by a non-commercial sponsor. Building on existing networks and infrastructure laid out by the FIRM-ACT trial, the GALACCTIC trial from 2009 demonstrated that a large commercial study on ACC is feasible. In this trial, the inclusion goal of 135 patients was met within the pre-defined enrollment period of 2 years. These undertakings prove that rare diseases are rare, but rare disease patients are numerous.

PART II: DIAGNOSTICS

A much discussed matter in management of patients with a non-functioning adrenal adenoma is the frequency and duration of follow-up evaluation. According to current guidelines, it is recommended to repeat adrenal imaging by CT-scan in patients with non-functioning adenomas smaller than 4 cm within 6-12 months after the initial discovery to detect size changes. In addition, annual repetition of hormonal work-up is recommended during 4 years of follow-up. The rationale behind this is mainly to recognize lesions with malignant potential that have escaped detection on primary analysis, and also to identify changes in size or functionality of the adrenal adenoma.

Based on a critical appraisal of the literature as well as on clinical experience of healthcare professionals involved in analysis and follow-up of patients with adrenal incidentaloma, there is a strong impression that adherence to the guidelines results in a substantial amount of unnecessary additional investigations. However, this impression has not yet been underpinned by prospective data that are needed to revise current guidelines. In September 2013, physicians from the University of Groningen conducted a survey among all members of the Dutch Society

of Endocrinology (NVE) in order to examine the current clinical practice with respect to the management of adrenal incidentaloma. With a response rate of 52%, it was shown that a large majority of the respondents follows the guidelines with regard to follow-up investigations including medical imaging with repeat CT-scans.¹ A minority of the internist-endocrinologists ordered even more repeat CT-scans than advised by the guidelines.

Our study on urinary steroid profiling (**chapter 6**) suggests that the diagnostic value of this single laboratory examination is at least similar to repeat imaging studies with CT-scans. These findings will have to be confirmed in a prospective trial, which is already launched (Structured Evaluation of adRENal Tumors Discovered Incidentally - Prospectively Investigating the Testing Yield [SERENDIPITY-trial], NCT02324647). If successful, this is expected to change clinical management of patient with adrenal incidentaloma. USP has several potential advantages over repeat CT-scanning. Collection of a 24-hour urine sample is part of standard care. Consequently, USP does not require an extra effort of the patient. Obviously, USP is much more patient friendly than repeat CT-scanning with its associated extra hospital visits, waiting and procedure time and administration of intravenous contrast. In addition, the patient would no longer be exposed to the potential health risks of CT-scanning (i.e. ionising radiation, contrast nephropathy, contrast allergy). Also, costs of USP (€70,-) are much lower than of CT-scanning (single CT-scan € 200,-). Implementation of urinary steroid profiling after prospective confirmation of our findings is expected to result in an 80% decrease in CT-scan orders.

PART III: TREATMENT

Concentration of care in a limited number of specialized hospitals aimed at improving outcomes is an important concept in general healthcare, but particularly in oncological care, high risk surgical care and rare diseases.² Regarding ACC, increasing evidence suggests that establishment of (inter)national collaborative networks of expert centres has a favourable effect on survival (**chapter 7**).^{3, 4} The concept that rare diseases should be treated in a limited number of specialized hospitals seems intuitively logical. Interestingly, a better outcome of oncological treatment in specialized centres is not necessarily correlated with volume requirements.⁵ Similar observations were reported in high-risk surgical treatment.⁶ Moreover, common minimum volume requirements as instituted for other cancers are hardly feasible for ACC due to the low incidence. For example, the number of new patients with ACC per year in the Netherlands is about 20 (median 21, range 13-26 between 1993 and 2010, **chapter 2**). In reality, the number of surgical procedures for suspect adrenal malignancy will be higher as some pathologies might mimic ACC pre-operatively (**chapter 5**). Nonetheless, the grand total is expected to be too low to institute meaningful minimum volume requirements. In bigger countries this number will be higher, but with 1 patient per 1 million inhabitants it is questionable whether geographical spread would allow for concentration of care on this scale. Quality criteria other than volume are expected to be of greater importance in rare diseases. It seems more important that centres adhere to current state-of-art treatment concepts, which in turn seems best feasible in specialized centres with dedicated physicians. Experience with multidisciplinary oncologic surgery and preferably adrenal/endocrine surgery is a strong recommendation and maybe even

a prerequisite.² In addition, participation in international networks and clinical trials should be encouraged to facilitate research on a larger scale, as discussed above in part I.

Chapters 8 to 10 focused on the application of the drug mitotane in clinical management of patients with ACC. Primary aim of this research was to investigate how mitotane dosing can be optimized in order to reach therapeutic plasma levels as early as possible. Also, individually tailored dosing regimens prevent excessive dosing and an overshoot to potentially toxic plasma levels. This is expected to reduce the need for symptom-alleviating drugs and even hospital admissions due to drug toxicity and improve quality of life for patients on mitotane.

Future research aimed at pharmacogenetics of mitotane is expected to further facilitate personalized medicine. For example, patients with genotype 'A' might benefit from a high-dose regimen, whereas in patients with type 'B' a low-dose regimen might result in similar mitotane plasma levels in the same time. By including results on treatment efficacy, it might become clear that some patients do not benefit from mitotane at all and other treatment options should be instituted.

In conclusion, potential societal and economic benefits of this research are numerous and varied, both through direct and indirect effects. The most important items of societal and economic benefit in this thesis are:

- Arguments in favour of increasing international collaboration in clinical trials and basal research due to the rarity of the disease in both adults and children.
- Arguments in favour of less expensive and less invasive follow-up of patients with adrenal incidentaloma.
- Arguments in favour of specialized care in adrenocortical carcinoma, resulting in better treatment outcome.
- First steps towards personalized medicine in mitotane treatment, aimed at more accurate dosing regimens, less drug toxicity and improved quality of life.

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CURRICULUM VITAE

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The author of this thesis was born 2 June 1986 in Maastricht. After finishing secondary school at Trichter College in 2004, he attended Medical School at Maastricht University. In 2005 he also started studying Dutch Law, from which the propaedeutic exam was passed in 2006. He worked as undergraduate teaching assistant at the Department of Criminal Law during one year. Eventually it was decided to fully focus on studying medicine and the study in Dutch law was terminated. The internship Internal Medicine was conducted at Máxima Medical Center, where he was acquainted with Harm Haak, at that time supervising attending endocrinologist and later promotor. The internship included an introduction to the illness adrenocortical carcinoma. This resulted in a return to Máxima MC to conduct the senior internship in the final year of medical school (2009-2010). During this period, the foundation was established for a PhD project on adrenocortical carcinoma, from which the present thesis is the final result. The medical degree was obtained in 2010. After Medical School, the PhD project was combined with outpatient care for patients with adrenocortical carcinoma and a part-time appointment as resident-not-in-training. Residency in Internal Medicine was started in July 2014 at Máxima MC, to be continued per February 2016 in Maastricht University Medical Center.

Since 24 May 2014, the author is happily married to the love of his life Meike Welkenhuizen.

De schrijver van dit proefschrift werd op 2 juni 1986 geboren te Maastricht. Het eindexamen Gymnasium B werd behaald in 2004 aan het Trichter College te Maastricht, waarna werd begonnen met de studie geneeskunde aan de Universiteit Maastricht. In 2005 werd ook begonnen met de studie Nederlands recht, waarvan het propedeutisch examen in 2006 werd behaald. In 2006 was hij werkzaam als student-assistent aan de rechtenfaculteit bij de vakgroep strafrecht. Uiteindelijk werd besloten de aandacht volledig op de studie geneeskunde te richten en de studie Nederlands recht niet verder te vervolgen. Het co-schap Interne Geneeskunde werd in 2008 uitgevoerd in het Máxima Medisch Centrum, alwaar een eerste kennismaking plaatsvond met Harm Haak, toenmalig opleider en latere promotor. Dit ging gepaard met een introductie in het ziektebeeld bijnierschorscarcinoom. Een en ander heeft geleid tot een terugkeer naar het Máxima MC voor de semi-arts stage in het zesde studiejaar (2009 -2010). Tijdens deze stage werd de basis gelegd voor het promotie-onderzoek bijnierschorscarcinoom, waarvan dit proefschrift het eindresultaat is. Het artsexamen werd in juni 2010 behaald. Na het afstuderen werden de onderzoekswerkzaamheden gecombineerd met de poliklinische zorg voor patiënten met bijnierschorscarcinoom en een aanstelling als escalatie-arts en arts-assistent Interne Geneeskunde bij het Máxima MC. In juli 2014 werd begonnen met de opleiding tot internist, eveneens in het Máxima MC. De opleiding zal per februari 2015 worden voortgezet in het Maastricht Universitair Medisch Centrum.

Sinds 24 mei 2014 is de auteur getrouwd met zijn grote liefde Meike Welkenhuizen.

LIST OF PUBLICATIONS

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This thesis:

1. Brouns SHA, **Kerkhofs TM**, Hermesen IG and Haak HR. Adrenal Incidentaloma and Adrenocortical Carcinoma: A Clinical Guideline on Treating the Unexpected and a Plea for Specialized Care Contemporary Aspects of Endocrinology, Dr. Evanthia Diamanti-Kandarakis (Ed.), 2011, ISBN: 978-953-307-357-6, InTech.
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carnaval, het inmiddels traditionele Ardennenweekend of gewoon een zaterdagavond waarop niets bijzonders te vieren is. Dat er nog maar vele gelegenheden mogen volgen!

Thijn, Roel en Mathieu, onze vakantie in Barcelona (1.0) markeerde de overgang van student naar dokter en was een versteviging van onze bestaande vriendschap. We hebben nu allemaal onze eigen weg gevonden maar zien elkaar gelukkig nog regelmatig. Ik vind het geweldig dat we zo'n leuke groep hebben en kijk uit naar nog vele mooie momenten, natuurlijk met Sanne, Tirza, Corinne en de kleintjes, waarbij de jongste aanwas nog op komst is bij het ter perse gaan van dit boekje ;)

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De komende tijd gaat er veel veranderen met de verhuizing naar Maastricht en bovenal met het volgende hoogtepunt dat jij nu nog in je buik draagt, maar zich begin maart 2016 zal aandienen ☺ Ik geniet van elke dag met jou en hoop dat nog de rest van ons leven te doen. Iech haw vaan diech!