Diagnostic and Therapeutic Aspects on Adrenocortical Carcinoma and Pheochromocytoma

Amir Khorram-Manesh

Göteborg 2004

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AKADEMISK AVHANDLING

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Amir Khorram-Manesh

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I. Khorram-Manesh A, Ahlman H, Jansson S, Wängberg B, Nilsson O, Jakobsson CE, Eliasson B, Lindstedt S, Tisell LE. Adrenocortical carcinoma: Surgery and mitotane for treatment and steroid profiles for follow-up. World J. Surg. 22: 605-612, 1998.

II. Ahlman H, Khorram-Manesh A, Jansson S, Wängberg B, Nilsson O, Jakobsson CE, Lindstedt S. Cytotoxic treatment of adrenocortical carcinoma. World J. Surg. 25: 927-933, 2001.

III. Khorram-Manesh A, Ahlman H, Jansson S, and Nilsson O. N-Cadherin expression in adrenal tumours: Up-regulation in malignant pheochromocytoma and down- regulation in adrenocortical carcinoma. Endocrine Pathology. 13(2): 99-110, 2002.

IV. Khorram-Manesh A, Ahlman H, Nilsson O, Friberg P, Odén A, Stenström G, Hansson G, Stenquist O, Wängberg B, Tisell L-E and, Jansson S. The outcome of 121 consecutive patients surgically treated for pheochromocytoma. Submitted.

V. Khorram-Manesh A, Ahlman H, Nilsson O, Odén A, and Jansson S. Increased risk of death and risk for additional tumour in patients diagnosed for adrenal pheochromocytoma. Eur J Surg Oncol 2004; 30: 556-559.

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Adrenocortical Carcinoma (ACC) is a rare malignant tumour with poor prognosis. Surgical removal is the only cure. Adjuvant use of mitotane, an adrenolytic drug with toxic effects, is controversial at best. We evaluated our treatment program for patients with ACC including active surgery, adjuvant mitotane treatment monitored by serum levels of the drug, and urinary steroid profiles in the follow-up to detect early recurrences. Monitoring of serum levels of mitotane made long-term treatment possible in advanced disease with moderate side effects. Repeat surgery for recurrence was valuable in patients with long disease-free intervals, and urinary steroid profiles indicated early recurrence in individual patients. The 5-year survival of 30 consecutive patients with ACC (44% low Stage and 56% high Stage tumours) in our series was 64%. Patients with high Stage ACC (Stage III-IV), treated with mitotane after surgery, seemed to have better prognosis than expected. The results of cytotoxic treatment in advanced disease, using single or multiple cytotoxic agents, have so far been disappointing. Better results were obtained when mitotane was combined with the chemotherapy. Multicenter trials are needed in order to find the best combined medical therapy for these patients.

In order to find histopathological markers that can predict the prognosis in ACC and pheochromocytoma (PC), 87 adrenal tumours were analysed for the expression of cell adhesions molecules by immunocytochemistry and Western blotting. Both cortical and medullary adrenal tumours expressed NCAD, NCAM and CD44, but all tumours were devoid of ECAD. The expression of CD44 and NCAM did not correlate with the malignant potential of the tumours. During cell transformation and tumour progression NCAD expression seemed to be up-regulated in medullary tumours, but down-regulated in cortical tumours. Loss of NCAD may thus be involved in the development of malignant adrenocortical tumours. Thus, NCAD may function as tumour suppressor and lack of its expression may be of prognostic significance in adrenocortical tumours.

PC and paraganglioma (PG) rarely metastasize, but may still be life-threatening due to excessive secretion of catecholamines (CA). The cause of death in untreated PC/PG is hypertensive complications and in rare cases metastatic disease. The overall prognosis after surgical removal of PC has not been studied in detail. In the present study with long-term follow-up (1950-1997), 121 consecutive cases of surgically treated PC/PG were reviewed in order to evaluate outcome, cause of death and histopathological features. There was no intra- or post-operative mortality. Eight patients proved to have malignant PC/PG during the period. The number of deaths in the series was higher than expected in the general population ($p < 0.001$). The main causes of death were cardiovascular and other tumour diseases. High age at primary surgery and high urinary excretion of methoxy-CA were significant risk factors for death. For comparative reasons, all cases with adrenal PC, diagnosed in Sweden 1958-1997, were investigated. Patients in the national cohort had almost 4 times higher risk for death in tumour diseases than the general population, but no elevated death risk for cardiovascular diseases. Life-long follow-up of these patients is important not only to diagnose and treat cardiovascular diseases and recurrent PC at early Stage, but also to diagnose other tumour diseases.

Key Words: Diagnosis, treatment, adrenocortical carcinoma, pheochromocytoma, cell adhesion molecules, death risks, cause of deaths, urinary steroid profiles, mitotane.

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Think not those faithful who praise all thy words and actions; but those who kindly reprove thy faults.

Socrates (469 BC-399 BC)

To My Mother, Father, Marina, Yasmin and Nicki

Contents

1. List of papers

The thesis is based on the following papers, which are referred to in the text by their Roman numerals:

I. Khorram-Manesh A, Ahlman H, Jansson S, Wängberg B, Nilsson O, Jakobsson CE, Eliasson B, Lindstedt S, and Tisell LE. Adrenocortical carcinoma: Surgery and mitotane for treatment and steroid profiles for follow-up. World J. Surg 1998; 22: 605-612.

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2. Abbreviations

A	adrenaline
ACA	adrenocortical adenoma
ACC	adrenocortical carcinoma
CA	catecholamines
CAM	cell adhesion molecule
CgA	chromogranin A
CT	computed tomography
DA	dopamine
DHEA	dehydroepiandrosterone
ECAD	E-cadherin
ICD	international classification of diseases
MEN 2	multiple endocrine neoplasia 2
MIBG	meta-iodo benzyl guanidine
MRI	magnetic resonance imaging
MTC	medullary thyroid cancer
NA	noradrenaline
NCAD	N-cadherin
NCAM	neural cell adhesion molecule
NCR	national cancer registry
NF1	neurofibromatosis type 1
o, p´-DDD	1,1- dichloro-2-(o-chlorophenyl)-2-(p-chlorophenyl) ethane
PC	pheochromocytoma
PET	positron emission tomography
PG	paraganglioma
pbz	phenoxybenzamine
RIA	radioimmunoassay
TH	tyrosine hydroxylase
VHL	von Hipple-Lindau´s disease
VMA	vanillyl mandelic acid
VRD	von Recklinghausen´s disease
WB	western blotting

3. Abstract

Adrenocortical Carcinoma (ACC) is a rare malignant tumour with poor prognosis. Surgical removal is the only cure. Adjuvant use of mitotane, an adrenolytic drug with toxic effects, is controversial at best. We evaluated our treatment program for patients with ACC including active surgery, adjuvant mitotane treatment monitored by serum levels of the drug, and urinary steroid profiles in the follow-up to detect early recurrences. Monitoring of serum levels of mitotane made long-term treatment possible in advanced disease with moderate side effects. Repeat surgery for recurrence was valuable in patients with long disease-free intervals, and urinary steroid profiles indicated early recurrence in individual patients. The 5-year survival of 30 consecutive patients with ACC (44% low Stage and 56% high Stage tumours) in our series was 64%. Patients with high Stage ACC (Stage III-IV), treated with mitotane after surgery, seemed to have better prognosis than expected. The results of cytotoxic treatment in advanced disease, using single or multiple cytotoxic agents, have so far been disappointing. Better results were obtained when mitotane was combined with the chemotherapy. Multicenter trials are needed in order to find the best combined medical therapy for these patients.

In order to find histopathological markers that can predict the prognosis in ACC and pheochromocytoma (PC), 87 adrenal tumours were analysed for the expression of cell adhesions molecules by immunocytochemistry and Western blotting. Both cortical and medullary adrenal tumours expressed NCAD, NCAM and CD44, but all tumours were devoid of ECAD. The expression of CD44 and NCAM did not correlate with the malignant potential of the tumours. During cell transformation and tumour progression NCAD expression seemed to be up-regulated in medullary tumours, but down-regulated in cortical tumours. Loss of NCAD may thus be involved in the development of malignant adrenocortical tumours. Thus, NCAD may function as tumour suppressor and lack of its expression may be of prognostic significance in adrenocortical tumours.

PC and paraganglioma (PG) rarely metastasize, but may still be life-threatening due to excessive secretion of catecholamines (CA). The cause of death in untreated PC/PG is hypertensive complications and in rare cases metastatic disease. The overall prognosis after surgical removal of PC has not been studied in detail. In the present study with long-term follow-up (1950-1997), 121 consecutive cases of surgically treated PC/PG were reviewed in order to evaluate outcome, cause of death and histopathological features. There was no intra- or post-operative mortality. Eight patients proved to have malignant PC/PG during the period. The number of deaths in the series was higher than expected in the general population ($p < 0.001$). The main causes of death were cardiovascular and other tumour diseases. High age at primary surgery and high urinary excretion of methoxy-CA were significant risk factors for death. For comparative reasons, all cases with adrenal PC, diagnosed in Sweden 1958-1997, were investigated. Patients in the national cohort had almost 4 times higher risk for death in tumour diseases than the general population, but no elevated death risk for cardiovascular diseases. Life-long follow-up of these patients is important not only to diagnose and treat cardiovascular diseases and recurrent PC at early Stage, but also to diagnose other tumour diseases.

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4. Introduction

Primary tumours in adrenal glands are derived from the cortex [adrenocortical adenomas (ACA) and adrenocortical carcinomas (ACC)] or from the medulla [benign and malignant pheochromocytomas (PC)]. Extra-adrenal PC or paraganglioma (PG), with catecholamine (CA) production are localised in the chromaffin tissue of paraganglia located outside the adrenal glands.

4.1 Adrenocortical carcinoma

4.1.1 Epidemiology. ACC is a rare and aggressive tumour with an incidence of 0.5-2.0 per million inhabitants a year and slight female preponderance. The age distribution is bimodal with a first peak early in childhood and a second larger peak around the age of 40-50 yr (*Wooten et al 1993, Schulick et al 1999, Dackiw et al 2001*).

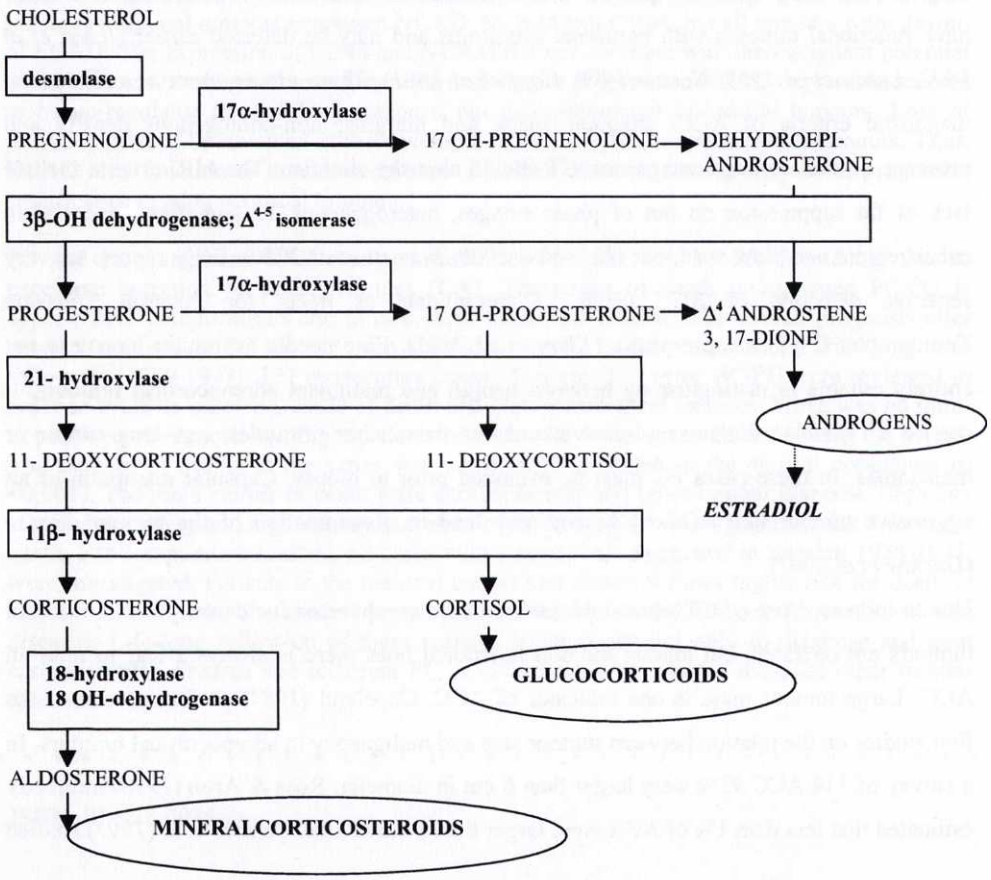
4.1.2 Clinical presentation and diagnosis. The disease is often advanced at the time of diagnosis and the patient may present with an abdominal mass. More than half of the patients have functional tumours with hormonal symptoms and may be detected earlier (*Icard et al 1992, Luton et al 1999, Norton 1999, Kopf et al 2001*). There are no absolute radiological diagnostic criteria of ACC. Irregular shape and margins; non-homogenous density and presence of haemorrhage/necrosis on CT should alert the clinician. The MRI criteria include lack of fat suppression on out of phase images, heterogeneous T2 signalling, gadolinium enhancement and slow wash-out (*Korobkin 2000, Barnett et al 2000*). Early reports on very sensitive detection of ACC using ^{11}C-metomidate as tracer for Positron Emission Tomography (PET) are promising (*Khan et al 2003*). Fine needle aspiration biopsy is not entirely reliable in distinguishing between benign and malignant adrenocortical tumours. It can be of value to diagnose adrenal metastases from other primaries, *e.g.* lung cancer or melanomas. In these cases PC must be excluded prior to biopsy. Capsular disruption of an aggressive tumour like ACC by biopsy may lead to dissemination of the tumour disease (*Dackiw et al 2001*).

Due to increased use of CT several adrenal tumours are detected incidentally. All functional tumours are operated, but among the non-functional ones there is always a fear to miss an ACC. Large tumour mass is one indicator of ACC. Copeland (*1983*) performed one of the first studies on the relation between tumour size and malignancy in adrenocortical tumours. In a survey of 114 ACC 92% were larger than 6 cm in diameter. Ross & Aron (*1990*) indirectly estimated that less than 1% of ACA were larger than 6 cm in size. Terzolo et al (*1997*) studied

a large group of patients with non-functional incidentalomas (n=210); 115 patients were operated and 15 were diagnosed as ACC. Of these only three were smaller than 6 cm in size. The 5 cm limit to identify ACC had a sensitivity of 93% and a specificity of 64%. From the same time period they studied 16 functional ACC; none was smaller than 6 cm. The proposed 5 cm limit was also tested in 38 consecutive cases of ACC from M.D. Anderson Cancer Center. Actually 5 of these tumours were smaller than 5 cm; 4 with "malignant" radiology and one functional tumour (*Barnett et al 2000*). To date more than 40 patients with ACC smaller than 5 cm have been reported (the smallest 1 cm) in the literature. ACC only rarely secrete aldosterone, but Farge et al (*1988*) described 2 cm aldosteronoma to be ACC, which means that our clinical rules and measures will never be absolute.

4.1.3 Urinary steroid profiles. In neoplasia, mutations of the adrenal cortical cells may lead to absolute or relative enzymatic deficiencies in the steroid metabolic pathway (Figure 1).

Figure 1: Pathways of adrenal steroid biosynthesis and metabolism



This in turn may lead to abnormal steroid intermediates or metabolites in urine, which can be detected by gas chromatography/mass spectrometry. Some of these steroids seem to be more common in patients with ACC than in benign adrenocortical diseases and healthy subjects, *e.g.* increased secretion of 11-deoxytetrahydroxycortisol (THS) and/or 3 β-hydroxy-5-ene steroids (*Lipsett et al 1963, Gröndal et al 1990*). Excretion of THS and metabolites of cortisol precursors have been found in both functional and non-functional ACC (*Gröndal et al 1990*). The steroid profiles may differ between primary and recurrent disease and during chemotherapy, which indicates that analysis of a single steroid as a tumour marker of recurrent disease is not sufficient. Repeated analysis of urinary steroid profiles is a tool with the potential of early identification of recurrent disease, but is limited by lack of age- and sex related reference values and strict definition of pathological intermediates (*cf. Weykamp et al 1989, Honour et al 1997*). Patients with inborn deficiencies of steroid hydroxylase and 3β-hydroxysteroid dehydrogenase/Δ^{3-4} isomerase also have aberrant steroid profiles. Likewise, women with hirsutism and polycystic ovary syndrome may have increased 3β-hydroxy-5-ene steroids. These conditions may thus be a differential diagnostic problem in patients with suspicion of ACC (*Gröndal et al 1990*).

4.1.4 Staging. The prognosis in ACC largely relates to tumour Stage (*Icard et al 2001)*, which was originally defined by McFarlane and later modified by Sullivan (*Sullivan et al 1978*) (Figure 2).

Figure 2: Tumour staging of ACC according to McFarlane, modified by Sullivan

Stage I	Tumour < 5 cm and confined to the adrenal
Stage II	Tumour > 5 cm and confined to the adrenal
Stage III	Tumour of any size with lymph node metastases or local invasive growth (i.e. tumour growth outside the adrenal but not involving adjacent organs)
Stage IV	Tumour of any size with lymph node metastases and local invasive growth, growth into adjacent organs, or distant metastases

The accuracy of diagnosis in Stage I has been questioned; in some series small adrenocortical tumours are referred to as "atypical adenomas" due to atypical histology, but with uncertain malignant potential. Inclusion of potentially benign lesions, classified as Stage I ACC has impact on the survival analysis (*Proye 1997, Norton 1999*). Icard et al (*1992*) and Lee et al (*1995*) proposed a modification of the classification so that patients with locally advanced ACC were Stage III and those with metastatic disease were Stage IV. This modification has been suggested to more accurately reflect the natural history of ACC with closer correlation to

other staging systems used for solid tumours (*Dackiw et al 2001*). Furthermore, tumours with isolated growth into the caval vein seem to have better prognosis than Stage IV tumours (*Norton 1999*).

4.1.5 Criteria for malignancy. The size of an adrenal tumour is one good predictor of malignancy with a cut-off limit of ≥4 cm for interventions (*Dackiw et al 2001*). Smaller tumours can be clinically difficult to determine in terms of malignancy (*Proye 1997*). The histopathological diagnosis of ACC can be difficult; the only definite criterion for malignancy is then occurrence of metastases and/or local invasiveness (*Pommier et al 1992, Proye 1997, Lack 1997*). Local recurrence has been proposed as a criterion for malignancy. However, this is controversial since capsular rupture of a benign tumour intraoperatively may result in local seeding, growth and invasion (*Proye 1997*). Histological indexes of malignancy to predict prognosis have been proposed (*Weiss 1984, Weiss et al 1989*) (Figure 3).

Figure 3: Histopathological scoring system for ACC

1. Diffuse architectural pattern with broad fibrous and trabecular bands	5. Vascular invasion
2. Focal necrosis	6. Nuclear grade III or IV
3. Invasion of sinusoidal structures	7. Atypical mitosis
4. Capsular invasion	8. High mitotic rate
	9. Clear cells constituting ≤25% of the tumour

None of the criteria suggested are diagnostic alone, but the possibility of malignancy increases with the presence of two, or more, criteria (*Proye 1997*). In a recent study 24 ACC with distant metastases or gross local invasion, or recurrence, were selected and matched with 25 benign ACA. Presence of ≥3 Weiss criteria was related to malignancy with 100% sensitivity and 96% specificity, further corroborating the use of the Weiss system (*Aubert et al 2002*).

4.1.6 Surgical treatment. Surgery is the standard treatment for resectable primary and secondary adrenal tumours and also for recurrent disease. For patients with low Stage (I & II) tumours and certain high Stage tumours resection is the only way to cure. With invasive tumour, or metastatic disease (III & IV), *en bloc* excision of other organs can be necessary (*Norton 1999*). The overall 5-year survival following resection varies largely between series (38-62%) (*Lee et al 1995, Schulick et al 1999*). If repeat surgery for local recurrence or metastases can be performed, it can palliate hormonal symptoms in patients with functional tumours, but the important question is whether it can prolong survival for the majority of

ACC patients. The first large series from France reported on the clinical outcome of 156 ACC patients (*Icard et al 1992*). Low Stage tumours were equally common as high Stage tumours (53 *vs.* 47%). Complete resection was performed in 81%. However, only 22 patients had resection for local recurrence with a 5-year survival of 16% after the re-operation. With reservation for selection bias somewhat better 5-year survival (28%) was achieved when complete resection of the recurrence could be done. The Italian national series of 179 ACC patients (*Bellantone et al 1997*) had a similar distribution of low- and high Stage tumours (51 *vs.* 49%) and similar rate of complete resections (80%). Recurrences were seen in 52 patients after a mean disease-free interval of 22 mos. It was three times more common to have distant metastases, or a combination of local recurrence and distant metastases, than local recurrence alone. Repeat surgery was performed in a selected group of 20 patients with a 5-year survival of 50% *vs.* 8% in 32 patients not resected. The consecutive series from one single centre [Memorial Sloan Kettering Cancer Center (MSKCC)] equals many of the national series in size (n=113). The ratio between low- and high-Stage tumours was 1:1. The resection rate was somewhat lower (60%) than in the national series, probably reflecting the status of MSKCC as nationwide referral centre. The resection rate for recurrent disease was very high. Second resection was performed in 46 patients with clear survival advantage in those with complete resection; 32 patients with complete resection had a 5-year disease-specific survival of 57% *vs.* 0% in 15 patients with incomplete resection (*Schulick et al 1999*). Complete resections were most common in patients with discrete distant metastases, *e.g.* lesions in the lungs and liver. Bulky local recurrences were one reason for incomplete resections. The active attitude for repeat surgery in this series is reflected by the fact that 47 patients had 107 resections; individual patients with complete re-resection could have several repeat interventions to remain tumour-free. When the disease-specific survival after complete repeat surgery was stratified by type of recurrence, there was a small non-significant survival advantage for those with distant metastases *vs.* those with local recurrences.

4.1.7 Medical treatment. a) Mitotane. Mitotane (o, p′- DDD) is an adrenolytic drug used for more than 40 yr as palliative and adjuvant therapy for patients with ACC. It causes selective destruction of the zona fasciculata and reticularis layers of the adrenal cortex and inhibits the production of corticosteroids (11 β -hydroxylation and cholesterol cleavage), but also the extraadrenal metabolism of steroids. ACC usually has a strong expression of the multi-drug resistance gene (*MDR-1*), which results in production of P-glycoprotein which facilitates efflux of cytotoxic agents. Mitotane potentially reverses this effect, which may favour combined treatment with mitotane and certain cytotoxic drugs (*Bates et al 1991, Flynn et al*

1992). The early high-dose (8-10 g) mitotane studies reported high tumour regression rates for patients with measurable disease at that time (*Bergenstahl et al 1960, Hutter & Keyhoe 1966, Lubitz et al 1973*). Later trials showed much less convincing responses (*Cohn et al 1986, Luton et al 1990, Wooten et al 1993, Baudin et al 2001*). Treatment with mitotane reduced steroid hypersecretion in 75% of the patients in a recent study (*Kopf et al 2001*). Furthermore, one small study with low-dose mitotane (1-2 g), given as adjuvant to surgical resection, showed a 4-fold survival advantage over non-treated patients (*Schteingart et al 1982*).

High-dose mitotane is often associated with severe side effects (gastrointestinal and neurological) and its role as adjuvant therapy after complete resection has been questioned (*Barzon et al 1999, Kasperlik-Zaluska 2000*). The response to mitotane has been suggested to be dose-related with a "therapeutic threshold" at mitotane levels of 14 µg/ml. No therapeutic effects were noted at levels below 10 µg/ml, but the "therapeutic window" was narrow (<20 µg/ml) (*van Slooten et al 1984, Haak et al 1994*). With the option to monitor mitotane levels such therapy can today be individualized, avoiding unacceptable neuromuscular toxicity. In a rare disease like ACC the use of mitotane in adjuvant or palliative settings as single agent, or as part of combined chemotherapy requires international multicenter studies. Adjuvant use of mitotane has since the first report in 1982 generated a large number of studies (all small, single centre, or retrospective) with improved survival (*van Slooten et al 1984, Venkatesh et al 1989, Icard et al 1992, Wooten et al 1993, Haak et al 1994, Kasperlik-Zaluska 2000*), or no benefit (*Luton et al 1990, Pommier et al 1992, Vassipoulou-Sellin et al 1993, Barzon et al 1997*).

b) Cytotoxic agents. Several cytotoxic agents have been used as single or combination therapy to treat advanced ACC. However, since ACC are rare and aggressive tumours associated with short survival, the disease has not allowed adequate drug trials, especially not with delayed diagnosis or previous treatment using different strategies. After reviewing published data on chemotherapy, combined with mitotane, some treatments [Etoposide + Doxorubicin + Cisplatin (EDP) + mitotane] (*Berruti et al 1998)* and [streptozocin + mitotane] (*Khan et al 2000*) had response rates exceeding 30%.

c) External radiation. This treatment modality has been used to palliate pain due to bone metastases. In limited studies, it was proven effective in the treatment of residual ACC (*Markoe et al 1991, Schulick et al 1999*). Its therapeutic potential has not been fully elucidated.

4.1.8 Prognostic factors. The overall 5-year survival for patients with ACC varies between 38-60%, but the disease is potentially curable at early Stage. However, up to 70% of ACC patients present with advanced disease (*Wooten et al 1993*). The Stage of ACC has proven to be the most important prognostic factor. Local invasion, or lymph node metastases, indicate poor prognosis, but the most severe factor is presence of distant metastases with a median survival of less than one year, not much changed over time (*Sullivan et al 1978, Henely et al 1983, Brennan 1987, Luton et al 1990, Wooten et al 1993*). In the French national series (n=253) the 5-year survival related to Stages was: Stage I (66%), II (58%), III (24%), and IV (0%) (*Icard et al 2001*). In a recent study of 105 patients the Weiss scores were also analysed: Stage I (100%), II (84%), III (33%), and IV (11%); patients with scores $\leq$3 had 100% 5-year survival, while those with scores $\geq$4 had a 62% survival strongly indicating the usefulness of a scoring system (*Lucon et al 2002*). In one large retrospective series young age, extent of the cancer, type of secretion and aspect of surgical resection seemed to favour survival, while sex, tumour size and weight had no influence (*Icard et al 1992*).

Surgery remains the mainstay of treatment in resectable ACC and repeat surgery with curative intent is of value for extended survival. New diagnostic tools to detect recurrent disease early are needed. For optimal medical treatment of advanced disease new treatment strategies must be addressed in prospective multicenter studies. The role of adjuvant mitotane after curative resection remains to be settled for both low and high Stage tumours.

4.2 Cell adhesion molecules in adrenal tumours

The biological behaviour of adrenal tumours is highly variable and the clinical outcome of an individual tumour can be difficult to predict. New markers that can distinguish between benign and malignant adrenal tumours are clearly needed. During the last decades several endocrine tumour markers have been discovered. Immunocytochemical studies have been performed to identify the distribution of markers in various tumour types. Tumours with different degree of differentiation have been studied to reveal possible molecular mechanisms behind progression. Cell adhesion molecules (CAM) are important for cell and tissue structure and integrity and play an important role in the tumorigenesis of many tumour types; dysregulation of CAM correlates with tumour de-differentiation, metastatic spread, and infiltrative tumour growth (Table 1).

CAM are specific surface proteins, distributed throughout the cell membrane, which mediate cell-cell adhesion or cell-matrix interaction. Some of these proteins make connections with

the cytoskeleton, while others are involved in signal transduction pathways (*Ikeguchi et al 00*). CAM can be divided into 4 major groups: Cadherins (calcium-dependent CAM), Ig-like CAM (non-calcium dependent), integrins, and selectins. The calcium-dependent cadherins (*e.g.* ECAD, NCAD) and non-calcium dependent cadherins (*e.g.* neural-CAM, CD44) are directly involved in cell-cell interactions (Table 1).

Table 1: Characteristics of CAM analysed in this thesis

CAM	Type	Examples of changed expression and possible clinical relevance
Calcium dependent	ECAD	Loss of ECAD (*Perl et al 1998, Christofori et al 1999, Ikeguchi et al 2000*). ➢ Transition from adenoma to carcinoma; a factor behind invasiveness and metastasis formation in pancreatic cancer ➢ A marker for poor prognosis in colorectal cancer
	NCAD	NCAD expression (*Kim et al 2000, Kovacs et al 2003*). ➢ Up-regulation of NCAD (and down-regulation of ECAD) in squamous cell carcinoma; up-regulation of NCAD (but no down-regulation of ECAD) in breast cancer ➢ Inappropriate expression leads to conversion of epithelial tumour cells into a fibroblast-like phenotype with increased motility & invasiveness
Non-calcium dependent	NCAM	Loss of NCAM (*Perl et al 1999, Esni et al 1999).* ➢ Important for tumorigenesis and metastatic spread of endocrine pancreatic tumours
	CD44	CD44 expression (*Lesley et al 1993, Peck et al 1998, Wallach-Dayan et al 2001*). ➢ Malignant tumours, *e.g.* colon, gastric and breast cancers. ➢ Associated with tumour progression and metastasis formation; lymphocyte homing to the inflammatory area and promotion of cell migration

The role of CAM in adrenal tumours is not well understood. CD44 was studied in adrenal tumours; its cytoplasmic expression in ACC contrasted with its membranous staining in PC. Consequently, CD44 might be of value in distinguishing between these tumour types (*Barshack et al 1998*). The expression of ECAD seemed to have no diagnostic or prognostic value in adrenal medullary tumours (*Gupta et al 2000*).

The expression of CAM and its pathophysiological role in adrenal tumours has not been fully evaluated. Some molecules may be helpful to distinguish between benign and malignant tumours.

4.3 Pheochromocytoma and paraganglioma

4.3.1 Epidemiology. PC/PG is a rare tumour disease with an incidence of 1.5-1.9 cases per million inhabitants a year, most common between the ages of 30-50 yr (*Kebebew et al 1998*). PC/PG rarely metastasize, but may still be life-threatening due to excessive secretion of CA (*Russell et al 1998*).

4.3.2 Clinical presentation and diagnosis. The diagnosis of PC is usually based on biochemical determination of CA, vanillylmandelic acid (VMA), and metanephrines in 24 h urinary collections. Recently it was shown that CA were metabolized to free metanephrines by tumour cells, independent of CA release, which makes free metanephrines in plasma the most sensitive biochemical test for PC/PG (*Lenders et al 2002*).

Classical signs and symptoms related to high circulating levels of CA are hypertension, headache and excessive sweating (*Goldstein et al 1999*), which can occur attack-wise. Hypertension can also be sustained in about half of the patients (*Plouin et al 1997, McClellan 2002, Williams et al 2003*). Cerebrovascular and cardiac accidents may occur during hypertensive crises (*Russell et al 1998, Williams et al 2003*). Since the disease is rare and the symptoms may be intermittent, typical symptoms can easily be neglected and the diagnosis delayed. In older patient series up to 40% of PC/PG were not diagnosed until autopsy (*Stenström et al 1986*).

4.3.3 Genetics. PC/PG usually occur as sporadic tumours. Until recently 10% were supposed to be part of rare syndromes with autosomal dominant heredity (Table 2). Very few families with hereditary PC only have been reported. Ten years ago the relevance of the "10% rule" in PC was questioned (*Proye et al 1994*), since long follow-up resulted in detection of more cases with hereditary PC/PG.

Table 2: Syndromes associated with familial PC/PG

Syndrome	***Gene***	***Function***	***Chromosome***	***Overall incidence of PC (%)***
MEN 2	*RET*	*Tumour proto-oncogene*	10q11	30-50
VHL	*VHL*	*Tumour suppressor*	3p25	15-20
VRD	*NF1*	*Tumour suppressor*	17q11	1-5
Hereditary PG	*SDHD*	*Tumour suppressor*	11q21-23	---

Recently it was reported that almost one fourth of the patients with apparently sporadic PC in fact had mutations in *RET, VHL, NF1* or *SDHD* at screening (Table 2) (*Neumann et al 2002, Bryant et al 2003*). Genetic testing as a routine was therefore proposed for patients with sporadic PC.

4.3.4 Criteria for malignancy. Several investigators have reported that longer follow-up of patients with PC/PG result in higher number of evidently malignant tumours, which exceed the historically cited "10%" (*Pommier et al 1993, Proye et al 1994*). Metastases may occur more than 10 yr after diagnosis and are common to regional lymph nodes, bones, liver, lung and brain (*Vassilopoulou-Sellin 1998, Williams et al 2003*). In recent series high numbers of malignant PC (>20%) have been reported (*Kebebew et al 1998, John et al 1999*). Many authors consider that the only reliable evidence of malignancy is the presence of metastases in an organ not normally containing chromaffin tissue, since the histopathological evaluation cannot reliably distinguish between malignant and benign tumours (*Pommier et al 1993, Clarke et al 1998, Kubota et al 1998, Goldstein et al 1999, Edström et al 2003*). Criteria for malignant PC/PG have been suggested by the Armed Forces Institute of Pathology; presence of metastases and/or extensive local invasion (*Lack 1997*). Microscopic features like local invasiveness of blood vessels or tumour capsule, and increased mitotic rate, are usually signs of malignancy in other tumour types, but may occur in both malignant and benign PC (*van Heerden et al 1990*).

4.3.5 Preoperative management. Induction of anaesthesia and surgical resection of PC without prior α-adrenoceptor blockade has been associated with high mortality (24-50%). During surgery these patients can have circulating CA levels up to 500 times higher than normal (*Russell et al 1998*). Thus, patients with PC are recommended α-adrenoceptor blockade prior to any intervention to decrease the risk for hypertensive crisis (*Russell et al 1998, McClellan 2002*). The most commonly used drug is phenoxybenzamine (pbz), which is a non-selective and non-competitive α-receptor antagonist with long-lasting effect (*McClellan 2002*). Use of pbz has decreased the intra-operative mortality to very low figures (2%) (*Grant 1997*). It usually takes up to 2 wk to obtain adequate blockade and control of hypertension (*Russell et al 1998, McClellan 2002*). The non-selective action of pbz affects α_2-adrenoceptors in addition to α_1-adrenoceptors and may cause loss of feedback inhibition via cardiac sympathetic nerves in turn leading to tachycardia, which may require additional blockade of β-adrenoceptors. The use of β-blockade before α-receptor blockade can worsen hypertension secondary to unopposed vasoconstriction (*McClellan 2002*). Other treatment alternatives include selective α_1-blockade, calcium channel antagonists and metyrosin (*Proye et al 1989, McClellan 2002*).

4.3.6 Surgical treatment. Surgery is a standard treatment of benign PC with low operative mortality (<3%), if the patients are properly prepared (*NCI 2003, Williams et al 2003*). Laparoscopic removal has become the first option at most centres today with low morbidity

and short hospital stay. In patients with familial, or bilateral, PC cortical-sparing adrenal resection has been recommended to avoid long-term steroid substitution (*Williams et al 2003*). Prophylactic contralateral adrenalectomy is not recommended in familial PC with diagnosed unilateral tumour (*Inabnet et al 2000, NCI 2003*). In malignant PC with regional lymph node metastases, or in systemic tumour disease, an active surgical approach has been recommended as an attempt to increase survival (*Mahoney et al 1977, NCI 2003*).

4.3.7 Cytotoxic agents and MIBG radiotherapy in malignant PC. In non-resectable PC radiotherapy with ^{131}I-MIBG and chemotherapy can be considered in addition to medical treatment of CA hypersecretion/hypertension (*Grant 1997, NCI 2003*). MIBG radiotherapy has had limited success due to low uptake of radioisotope in the tumour, but may increase survival in selected cases (*Grant 1997, Loh et al 1997, NCI 2003*). Chemotherapy with the triplet CVD [Cyclophosphamide + Vincristine + Dacarbazine] (*cf. Averbuch et al 1988*) has resulted in objective tumour regression (61%) and biochemical response (74%), but there is no evidence that it will improve survival (*NCI 2003*). Combination of MIBG radiotherapy and CVD treatment may have additive effects (*Sisson et al 1999*). External radiation can be used as palliative treatment of bone metastases (*Grant 1997*).

4.3.8 Clinical outcome and cause of death. Several studies have reported an increased risk for unexpected death in patients with PC due to high CA secretion (*Kebebew et al 1998, Russell et al 1998*). Cardiovascular arrest was the main cause of death in patients with non-diagnosed PC (*Kebebew et al 1998, Lo et al 2000*). Few series have analysed the late cause of death in patients with diagnosed and treated PC (*Williams et al 2003*); the causes were considered to be cardiovascular disease and recurrent tumour (*Kebebew et al 1998, Lo et al 2000*). In these series, the follow-up was too short and often limited by small numbers of patients.

There is no large consecutive series with long follow-up that has evaluated the total risk and the cause of death in patients with surgically treated PC/PG. To avoid selection biases the results in regional series should be compared with national cohorts.

5. Aims

1. To evaluate our treatment strategy for ACC including active surgical treatment of primary/recurrent disease monitored treatment with mitotane in an adjuvant setting and determination of urinary steroid profiles to detect early recurrence (I).

2. To review the literature in order to find the best medical treatment for recurrent/non-resectable ACC, which can serve as background for discussion of future randomized studies (II)

3. To find new markers of malignancy in adrenal medullary and cortical tumours by evaluating immunocytochemical expression of CAM in relation to clinical behaviour (III).

4. To identify risk factors and causes of death in patients with PC/PG by studying the long-term survival and outcome after surgery in a large regional consecutive series of patients (1950-1997) (IV).

5. To evaluate the death risk and occurrence of second tumour disease in a national cohort of clinically diagnosed PC (1958-1997) (V).

6. Patients and methods

6.1 Adrenocortical carcinoma (I, II)

Patients. Eighteen consecutive patients with a mean age of 53 ± 3 yr and a female/male ratio of 0.8 were studied up to 1997. Complete resection was performed in 17 out of 18 patients. Palliative debulking surgery was performed in one patient. Seventeen patients were primary cases and one was referred after primary surgery elsewhere. Fourteen patients were offered monitored mitotane as adjuvant therapy; 12 patients (6 Stage II, 2 Stage III and 4 Stage IV) accepted such treatment. Two years later 4 more patients were included (12 Stage II, 2 Stage III, 8 Stage IV), all treated with mitotane. In 2002 the series included 30 patients (13 Stage II, 5 Stage III and 12 Stage IV). Tumour staging at the time of primary surgery was based on the McFarlane/Sullivan classification. The survival was calculated with the Kaplan–Meier method. Correlation between disease-free interval and observed survival after repeat surgery was calculated as Pearson's coefficient. All p-values were two-tailed.

Methods (I). Postoperatively, all patients were followed at 3 m intervals during the first year with clinical examination and urinary steroid profiles. CT/MRI was performed at 6 mos. intervals. Patients with no evidence of disease were thereafter followed every 6 mos. The mitotane dose was titrated by monitoring of serum levels every 1-2 wk during the first 2-3 mos. of treatment and later every 3 mos., or earlier on suspicion of side effects. The serum concentration of mitotane was measured by capillary gas chromatography with an electron capture detector (ECD) (*Moolenaar et al 1977, Benecke et al 1987*) and was adjusted to the so-called "therapeutic interval" (14-20 μg/ml) (*van Slooten et al 1984*). Patients with advanced disease received mitotane life-long, while patients without evident disease were planned for a treatment period of 3 yr. Since 1990 urinary steroid profiles were regularly performed at our hospital; 12 patients had postoperative samples and five patients had preoperative samples as well. The steroids were extracted before enzyme hydrolysis of steroid conjugates as methoxine-trimethylsilyl derivatives and analysed by gas chromatography/mass spectrometry (*Axelsson et al 1981, Schmidt et al 1985*).

Methods (II). A detailed review of the world literature on available medical treatment for advanced ACC (metastatic or recurrent) was performed including symptomatic endocrine therapy, mitotane, single/multiple agent chemotherapy as basis for future trials.

6.2 Expression of cell adhesion molecules in adrenal tumours (III)

Tumours. Eighty-seven adrenal tumours were investigated by immunocytochemistry and/or Western blotting (WB). Fifty-seven benign adrenal tumours (27 PC and 30 ACA) were randomly selected among specimens registered at the Department of Pathology, Sahlgrenska University Hospital (1965-1999). All malignant adrenal tumours (8 sporadic malignant PC and 22 ACC) (1979-1999) were also included. Fresh tumour tissues (10 sporadic benign PC, 10 ACA and 10 ACC) were obtained from 30 of the patients and were analysed by WB.

Methods. a) Immunocytochemistry. Sections of formalin-fixed and paraffin embedded material were mounted on positively charged glass slides, deparaffinized, rehydrated and subjected to antigen retrieval by microwave treatment. Primary antibody was applied overnight at 4°C. Bound antibodies were visualized by indirect immunoperoxidase techniques (DAKO EnVision +). All slides were coded and evaluated by three independent observers. The following monoclonal antibodies were used; anti-tyrosine hydroxylase (TH) (2/40/15), anti-CD44 (A3D8), anti-ECAD (HECD-1), anti-NCAD (3B9), anti-NCAM (ERIC-1), and anti-Ki-67 (MIB-1). As positive controls served for TH: normal adrenal medulla, CD44: haematopoietic tissue, ECAD: intestinal mucosa, NCAM, NCAD: normal adrenal gland, Ki67: lymph node. In negative controls the primary antibody was omitted. The number of Ki-67 positive nuclei and mitotic figures were counted in 10 high power fields from each tumour. **b) Western blotting.** Frozen tumour tissues were homogenized in 10 mM potassium-phosphate buffer pH 6.8 containing 1 mM EDTA, 10 mM 3-(3-cholamidopropyl) dimethylamonio-1-propane sulphate and protease inhibitors. Homogenates were sonicated twice for 15 sec followed by centrifugation for 10 min at 10,000 *g*. The clear supernatant was withdrawn, assayed for protein content according to Bradford, and stored at -80 °C. Aliquots of proteins (20-35 µg protein) were diluted in sample buffer. Reducing agents was added followed by denaturation at 70 °C for 10 min and electrophoresis on precast polyacrylamide gels (10% NuPAGE Bis-Tris-gels; Novex) using NuPAGE MOPS SDS as running buffer. Proteins were transferred to polyvinyldifluoride membranes using a Novex blotting system. Membranes were incubated with primary antibodies at 4 °C over night followed by alkaline-phosphatase-conjugated goat-anti-rabbit/mouse antibody and CDP-Star (Tropix) as substrate. Membranes were exposed to ECL film at room temperature for 10-120 sec. Molecular weight markers (See-Blue or Mark 12, Novex) were used to calculate the apparent size of immunoreactive proteins.

6.3 Pheochromocytoma and paraganglioma (IV, V)

Patients. Regional series (IV). A total of 121 consecutive patients with PC/PG (68 females and 53 males, median age at surgery: 47 yr, range 15-82 yr were operated at our unit (1950-1997). Hypertension was defined according to the WHO recommendation >140/90 mm Hg (*Chalmers 1999*).

Patients. National cohort (V). All patients (n=660) with diagnosed adrenal PC during 1958-1997 in Sweden, registered in the National Cancer Registry (NCR) were identified. Autopsy-based diagnoses (n=179) were excluded and a cohort of 481 patients with clinically diagnosed tumours was included in the analysis.

Methods. Regional series (IV). a) Archival data. Clinical and biochemical data, tumour characteristics and localization, as well as anaesthesiological and surgical procedures were all obtained from the medical records. The mean value of the 3-5 highest blood pressure recordings before and within one year after surgery was calculated in available patients (n=116). The concentrations of adrenaline (A), noradrenaline (NA), methoxy-CA and VMA were analysed in 24 h urine collections; the mean of the 3-5 highest preoperative levels was recorded. All patients alive in 1980, and all successive patients, were biochemically screened for multiple endocrine neoplasia type 2 (MEN 2) with analyses of basal and pentagastrin-stimulated serum calcitonin concentrations after adrenal surgery. Genetic testing has been routine for all patients with medullary thyroid cancer (MTC) since 1995.

Early in the series, the tumours were localized by angiography and in individual patients by pneumoperitoneum. These techniques were after 1981 replaced by CT. Scintigraphy, using radioiodinated MIBG, was used alone or in combination with CT after 1985. One hundred twelve patients were treated with pbz preoperatively; an initial dose of 10-40 mg/d was given orally, increased by 10-20 mg/d at intervals of 1-3 d until symptomatic relief and control of hypertension was achieved. The individual final dosage varied largely (20-400 mg/d). The anaesthetic records of 77 patients (1960-1997) were carefully evaluated concerning intra-operative blood pressure variations and other complications.

Histopathologically malignant diagnosis was based on presence of extensive local invasion, or on detection of metastases to one or more sites, where chromaffin tissue is not usually present (*cf. Lack 1997*). Tumours with features as irregular growth pattern, areas of necrosis, local invasiveness of blood vessels or tumour capsule, cellular polymorphism, and increased mitotic rate, were recorded separately as a "border-line risk" group. In 4 patients diagnosis of adrenal medullary hyperplasia data was confirmed by morphometric analysis to assess adrenal

medullary weight and volume *(Jansson et al 1988).* All patients alive in 1997 (n=79) were subjected to follow-up. For deceased patients, the cause of death was obtained from death certificates, autopsy reports, or medical records.

b) Statistics. Statistical analyses were performed to evaluate clinically relevant factors for survival and malignant disease. The relationship between age at surgery, sex, recognized hereditary disease, associated cardiovascular and tumour diseases, presenting symptoms and their duration, blood pressure pre- and postoperatively, urinary levels of CA and metabolites, tumour weight and the risk for death were tested by a non-parametric test (*Breslow et al 1987*). The expected number of deaths was calculated, assuming that the risk coincided with that of the general Swedish population taking age, sex and calendar year into account and compared with the observed numbers of deaths (Poisson distribution) using two-tailed tests.

Methods. National cohort (V). a) Registry data. Since 1958 the Swedish National Board of Health and Welfare requires that all clinicians and pathologists/cytologists report all clinically and autopsy-diagnosed malignant and several benign tumours. Consequently, the cases included in NCR are reported from two sources, but the registry does not contain any information on tumour Stage or treatment. The completeness of the registry is approximately 98% (*Lundegårdh et al 1990, Hansson et al 1999*). To maintain confidentiality each individual is given a code number, which is also used in the National Registry of Deaths & Main Causes of Death. This registry reports the total number of deaths and the number of deaths from different causes separately for men and women in 5-year age cohorts. The data in these registries can be cross-matched, so no one can be included in a series more than once. The following information was obtained from the registries; diagnosis based on the diagnose number for PC (195.0) listed according to ICD-7 (*WHO 1957*), sex and age, date of diagnosis, diagnostic modality (histopathology/cytology), histopathologically verified malignancy, cause of death based on the ICD-7 death code, but also each new tumour disease after diagnosis of PC. The control population was the entire Swedish population (~ 8.2 millions). The observed numbers of death and causes of death in patients with PC were compared with the expected numbers in the control population. The incidence of additional tumour diseases (metastases not included) in patients after diagnosis of adrenal PC were analysed in NCR and compared with that of the control group.

b) Statistics. The expected numbers of deaths and new tumour diseases after diagnosis of adrenal PC were calculated taking current age, sex and calendar year into account. The patient years related to different age intervals, sex and time were multiplied by the corresponding risk

figures of the normal population and the products were added. The observed and expected numbers were compared by use of the Poisson distribution. Confidence intervals for risk ratios were calculated exactly. Analyses were performed for men and women separately. Two-tailed tests were used.

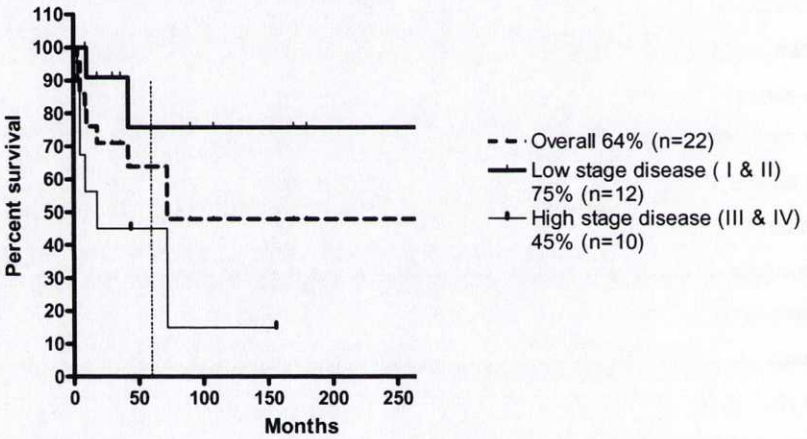
7. Results and Discussion

7.1 Adrenocortical carcinoma (I, II)

7.1.1 Clinical features. The most common clinical manifestations of ACC in our patients were fatigue, abdominal pain, and abdominal mass. One-third of patients had hormonal symptoms.

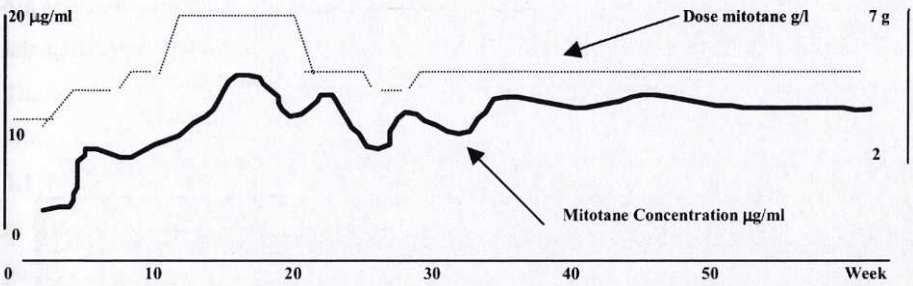
7.1.2 Surgery. All 22 patients (12 Stage II, 2 Stage III, 8 Stage IV) underwent macroscopically radical surgery by adrenalectomy alone or combined with nephrectomy, or more extensive surgery (Figure 4). There was no postoperative mortality. Re-exploration was performed in 9 patients; in 5 patients due to biochemical suspicion of recurrence after a mean disease-free interval of 59 mos. Four patients underwent second-look operation without signs of recurrent disease. The outcome for the 5 re-operated patients was as following: Two died of ACC (Stage II & IV), one died of other causes (Stage II), one was alive with disease (Stage II), and one patient had no evidence of disease (Stage II). The mean observed survival after repeat surgery was 55 mos.

Figure 4: 5-year survival of 22 consecutive patients with ACC (1971-1999)



7.1.3 Mitotane therapy. After titrating mitotane to a median serum concentration of 14.5 µg/ml (range 13-20 µg/ml) the median daily dose was 4 g (range 2.0-6.5 g). Significant side effects were reported in 5 out of 12 patients; one (Stage II) refused further mitotane treatment after negative second-look operation. Four patients had nausea and one had visual disturbances; all could continue after dose reduction. Two elderly patients refused mitotane initially due to potential side effects. Figure 5 shows dose adjustment in a patient with nausea during induction with mitotane.

Figure 5: Dose and serum concentration of mitotane in a patient with nausea during induction of mitotane therapy.



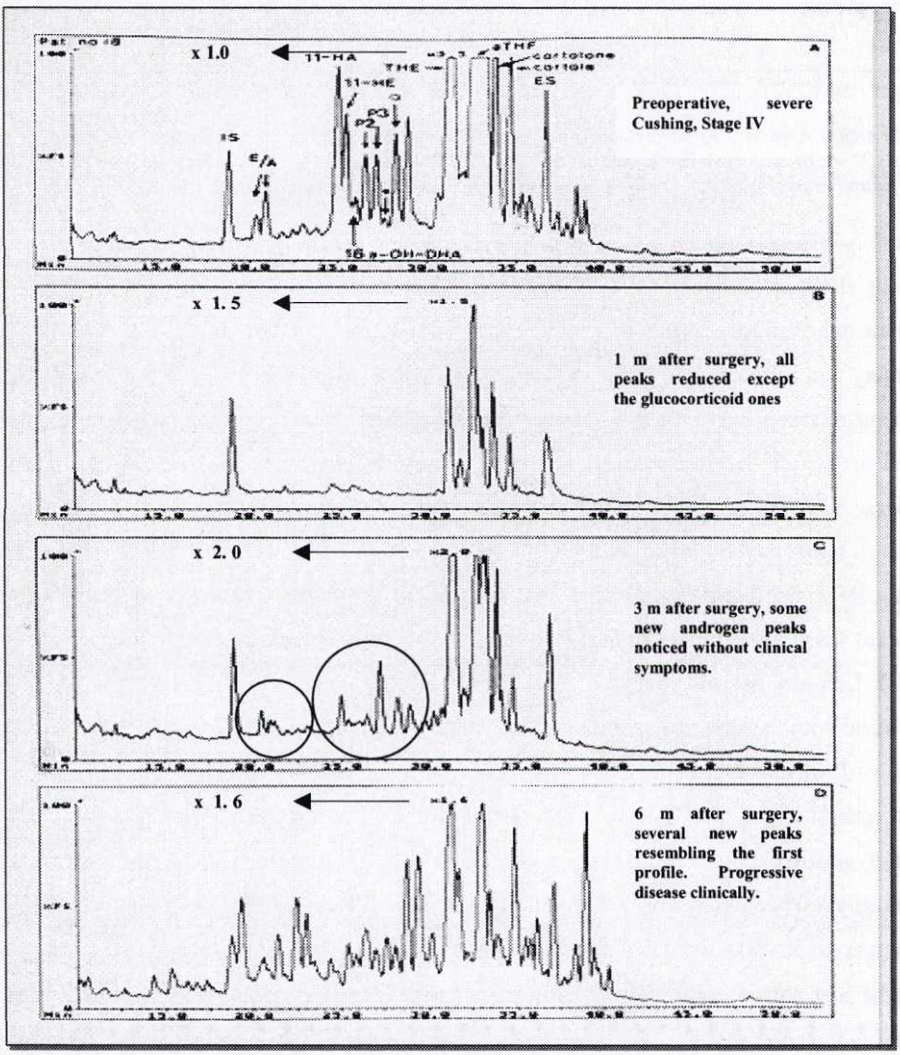
With a daily dose of 6.5 g of mitotane nausea appeared and the dose had to be reduced. In this patient it took about 20 wk to reach a stable serum concentration of mitotane with a dose not causing any side effects (around 14 µg/ml). In most patients 2-3 mos. of treatment was needed to reach such individual dosage.

7.1.4 Additional therapies. Five patients received additional treatment; radiation therapy (n=1), streptozocin alone (n=4) or combined with doxorubicin (n=1), or interferon (n=1), or chemoembolization of the liver with doxorubicin (n=1), when the initial treatment with surgery and mitotane had failed. Two patients with Stage II disease received streptozocin at tumour recurrence; one died 24 yr after surgery with no evidence of disease, the other patient died of ACC 6 yr after surgery. The third patient, treated with streptozocin, had Stage IV disease and died of ACC within 8 mos. The fourth patient also had Stage IV disease treated with streptozocin combined with interferon died of ACC within 4 mos. The fifth patient also with Stage IV disease received a combination of doxorubicin and radiation and died of disease 3.5 yr after initial surgery.

7.1.5 Urinary steroid profiles. Patients without recurrent disease had steroid profiles identical with the first postoperative urine collection. One female patient with Stage II tumour and virilism developed a selective increase of the secretion of pregnanediol, pregnanetriol, and androstentriol, which led to second-look operation with normal findings. In subsequent urinary profiles these steroids were again normalized. To date this patient (11 yr) is still alive with no evidence of disease. In two out of 5 patients with pre and postoperative urine samples the steroid profiles indicated recurrent disease despite normal radiological findings. One patient had normal radiological findings, but high secretion of 3-β-hydroxy-5-ene steroids, which increased rapidly. The patient died of disseminated disease 4 mos. later. Another patient rapidly recurred with a steroid pattern typical for the original tumour. She was re-operated 6 mos. later, when she had developed radiologically proven liver metastases.

Another patient clearly had a pathological steroid profile preoperatively (Figure 6). The primary tumour was excised, but she had residual tumour in the liver. The steroid profile after surgery showed diminished secretion of androgens (androsterone, etiocholanolone, DHEA and 11-OH androsterone), but persistent secretion of pregnanediol & -triol, pregnenediol & -triol and glucocorticoids (tetrahydrocortisone/cortisol, cortolon and cortoles), suggesting that the primary tumour was the source of androgen production.

Figure 6: Pre- and postoperative urinary steroid profiles in a patient with ACC, Stage IV.



αTHF = allo-Tetrahydrocortisol
11 – HA = 11-Hydroxyandrosterone
11– HE = 11-Hydroxyetiocholanolone
E = Etiocholanolone
THE = Tetrahydrocortisone
THS = 11-Deoxytetrahydrocortisol

7.1.6 Survival. The 5-year survival according to the Kaplan-Meier method was for the 18 patients in paper I was 58%. The median observation time for the 8 patients, who died of ACC was 0.5 yr (range 2-72 mos) and for the other 10 patients 7.2 yr (range 7-294 mos.). The 5 patients with repeat surgery for recurrent disease after a mean disease-free interval of 59 mos. (7-168 mos.) survived for a mean of 55 mos. (2-120 mos.) after the second operation. There was a significant correlation between disease-free interval before and observed survival after repeat surgery ($p = 0.014$).

7.1.7 Treatment alternatives in advanced disease (II)

a) Inhibitors of steroid biosynthesis. Control of hormonal symptoms can cause good palliation. ***Metyrapone*** blocks the 11β-hydroxylation of the cortisol synthesis (*cf.* Figure 1), but can enhance androgen synthesis (hirsutism and virilism). It is an effective second-line drug for all types of Cushing's syndrome with reversible effects (*Trainer et al 1994*). ***Ketoconazole*** inhibits both gonadal and adrenal steroid synthesis by its action on 11β-hydroxylase and 18-hydroxylase and the cholesterol synthesis (*cf.* Figure 1). Hepatotoxicity is a serious side effect. It is particularly effective in the treatment of hypercortisolism caused by ACC and may have antiproliferative effects (*Feldman 1986*). ***Aminoglutethimide*** blocks the conversion of cholesterol to pregnenolone by inhibition of aromatases in all tissues (*cf.* Figure 1) and therefore reduces the synthesis of cortisol, aldosterone and estrogens (*Trainer et al 1994*).

b) Glucocorticoid receptor antagonist. ***RU 486*** (mifepristone) is a potent glucocorticoid receptor antagonist, which can be effective in the treatment of Cushing's syndrome. One obvious limitation for monitoring of clinical response is that cortisol levels are not lowered and hypocortisolism not easily identified (*Bertagna et al 1988*).

c) Chemotherapy with single agent. ***Suramin*** had low response rate (14%) and serious side effects, so this treatment has been abandoned (*Arlt et al 1994*). ***Gossypol*** could induce partial remission, but only in a minority of patients with metastatic ACC (*Flack et al 1993*). ***Doxorubicin:*** In a prospective non-randomized study of advanced ACC patients with functional, well- or poorly differentiated tumours (n=36), who first received mitotane (6g/d) for 3 mos. had better response rate (22%) than those, who only received doxorubicin (19%) (*Decker et al 1991*). Patients with progressive disease on mitotane were crossed-over to doxorubicin without any objective response. Doxorubicin thus seemed to be ineffective as second-line chemotherapy for patients with functional ACC. ***Cisplatin:*** Initially individual transient complete responses with cisplatin (*Chun et al 1983*) and an improved response rate

with cisplatin and mitotane were reported (*Bukowski et al 1993*). These results were not corroborated by later studies (*Williamson et al 2000*).

d) Chemotherapy with multiple agents. Before 1998 the results with chemotherapy using multiple agents were not encouraging, but this year a combination of cisplatin, etoposide and mitotane (mEP) was reported to offer a response rate of 33% (*Bonacci et al 1998*) (Table 4).

Table 4: Combined chemotherapy in ACC

Series	Number	Regimen	Year	Response rate
van Slooten	**11**	**PDC**	**1983**	**18%**
Schlumberger	**13**	**DP5-FU**	**1991**	**23%**
Bukowski	**37**	**mP**	**1993**	**30%**
Bonacci	**18**	**mEP**	**1998**	**33%**
Berruti	**28**	**mEDP**	**1998**	**54%**
Williamson	**45**	**mEC**	**2000**	**11%**
Khan	**22**	**mS**	**2000**	**36%**
Abraham	**36**	**mEDV**	**2002**	**22%**

B = bleomycin C = cyclophosphamide D = doxorubicin E = etoposide m = mitotane
P = cisplatin, S = streptozotocin V = vincristine 5-FU = 5-fluorouracil

In a phase II multicenter trial EDP treatment was combined with mitotane (mEDP) (*Berruti et al 1998*). The overall response rate among the 28 patients (both functioning and non-functioning advanced ACC) with measurable disease was high (54%). The time to progression in responding patients was two years. EDP treatment was reasonably well tolerated and only few patients needed reduced doses, or discontinued their therapy. The addition of mitotane increased the side effects leading to reduction of the planned mitotane dose (4 g daily) in a majority of patients. All patients were substituted with hydrocortisone combined with mineral corticoids.

7.1.8 Comments. In our region treatment of ACC is centralized to our centre and all patients in this limited series were consecutive cases from the Western Region of Sweden; the selection bias may include non-resectable Stage IV disease and ACC not clinically diagnosed. Complete tumour removal was possible in 17 out of 18 patients (50% Stage II and 50% Stage III-IV). Patients with Stage II disease had good prognosis. Second-look operations in such patients without any biochemical or radiological evidence of recurrence can today be avoided with more reliable radiological investigations. At the end of our study the 8 deceased patients only survived for a mean of 0.5 yr after surgery, whereas the survivors (9 Stage II, 1 Stage III

and 2 Stage IV) were observed for a mean of 7.2 yr. The mean survival time before repeat surgery for recurrence in the 5 reoperated patients was almost 5 yr and so was the observed survival after the second operation. In accordance with previous studies the significant correlation between disease-free interval and long survival after the repeat surgery suggests a survival advantage for these patients.

Analysis of urinary steroid profiles in our series enabled early detection of recurrence in two patients, but both patients were reluctant to re-exploration without radiological evidence of disease. After completion of this study another two patients developed steroid profiles compatible with tumour recurrence, which led to rapid medical treatment. One patient had a false positive finding and was explored with negative results. Thus, the urinary steroid profile may be a tool for early detection of recurrent disease in individual patients. Previous studies have proposed that patients with ACC may have specific patterns in their urinary steroid profiles, which could discriminate malignant from benign cortical tumours (*Minowada et al 1985*, *Gröndal et al 1990*). It has recently reported that patients with ACC had a dominance of 11-deoxycortisol or 3β-hydroxy-5-ene steroids *vs.* cortisol, 18-hydroxycortisol, or aldosterone in patients with ACA (*Kikuchi et al 2000*). Since the individual secretory pattern can vary depending on age, sex and hormonal status (*e.g.* pregnancy, menstrual cycle), a steroid profile (determined by gas chromatography/mass spectrometry) diagnostic for ACC, based on numerical figures of certain key steroids, cannot be given at present time (*cf. Weykamp et al 1989*).

All our patients treated with mitotane had their treatment adjusted after the serum concentrations of the drug. This strategy enabled long-term treatment in the majority of our patients with rather few side effects. Due to the small number of patients and a treatment period without mitotane monitoring, no conclusion about the efficacy of mitotane treatment can be drawn. However, in a large retrospective series (n=253) from France mitotane treatment seemed to offer a survival advantage in patients with metastatic ACC or with residual tumours, *i.e.* high Stage or non-resectable disease (*Icard et al 2001*). In our series 8 Stage IV patients were included; 4 with and 4 without mitotane treatment with a 5-year survival of 50% *vs.* 0%.

We reviewed the literature to find better medical treatment for patients with advanced/non-resectable ACC, which also could serve as a background for future randomized studies. With our present knowledge single agent chemotherapy is not effective; the best results were obtained by multiple agent therapy, but such therapy without addition of mitotane was not very successful (Table 5). The patients in these series were few and the response rates low

(*van Slooten et al 1983, Schlumberger et al 1991*). Since mitotane partially reverses the "*MDR-1*" multi-drug resistance gene by reducing drug efflux, combined treatment with mitotane and cytotoxic agents seems to be theoretically favourable (*Bates et al 1991*). Bonacci et al (*1998*) treated 18 patients with cisplatin and etoposide combined with mitotane (mEP) and reported 33% response rate. In a phase II multicenter trial EDP treatment combined with mitotane (mEDP) had a response rate of 54% (*Berruti et al 1998*). EDP has been widely used in gastric carcinoma and has been accompanied by significant side effects and toxicity-related deaths. However, in ACC patients it was better tolerated, which can be due to lower dosage of etoposide and better performance status of the ACC patients. The combination of mitotane and streptozocin (mS) has been used with variable results (*Gröndal et al 1990, Haak et al 1994*). However, a recent study showed a response rate of 36% with significant effect on disease-free interval and survival (*Khan et al 2000*). Thus, mitotane-based chemotherapy with mEDP and mS both had response rates higher than 30%. A randomized, multicenter trial comparing these treatment arms in advanced ACC will soon be launched using monitored mitotane therapy (*Schteingart, pers. comm.*).

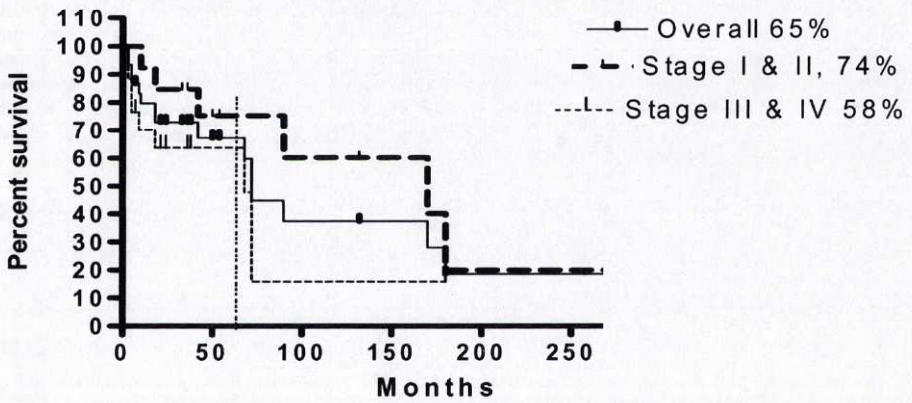
Table 5: The results of reoperation in some recent series of patients with ACC

Author	Patients No.	Stage I & II	Stage III & IV	Primary Complete resection	Recurrence No.	5-y Survival after complete re-resection	5-y Survival after non-complete re-resection
Icard (1992)	156	53%	47%	81%	22	28% (n=12)	8%
Bellantone (1997)	170	51%	49%	80%	52	50% (n=20)	8%
Schulick (1999)	113	50%	50%	60%	47	57% (n=32)	0%

ACC is a heterogeneous disease; some patients with resected metastatic disease survive for long periods, while others die within months from rapidly progressive disease, poorly responsive to medical therapy. The tumour biology in individual patients can be difficult to predict (*Vassilopoulou-Sellin et al 2001*). Intentionally curative surgery increases the 5-year survival in Stage I-III disease (*Kendrick et al 2001*), but even after complete resection a majority of ACC patients will develop recurrences (*Icard et al 1992, Pommier et al 1992, Bellatone et al 1997*). The surgical attitude to treat recurrent tumour varies largely between different series. Table 5 shows the results of repeat surgery in recurrent disease.

Due to the high risk for recurrent disease in ACC adjuvant medical treatment of both low and high Stage tumours has been tested. Adjuvant use of mitotane is still controversial, mainly due to the lack of randomized prospective studies of sufficient size. Another obstacle for treatment is the severe side effects of mitotane. Retrospective studies of mitotane serum levels *vs.* clinical outcome have indicated that drug concentrations ≥14 µg/ml are required for therapeutic responses (*van Slooten et al 1984).* Mitotane is a lipophilic substance with large inter-individual variation between dosage and serum levels of the drug. Side effects are frequent with drug levels close to 20 µg/ml. Monitoring of mitotane levels at institution of this therapy improves the quality of life for the patients and prevents toxic drug levels (*van Slooten et al 1984, Terzolo et al 2000*). There is no consensus about the duration of adjuvant mitotane treatment, but 2-3 yr may be reasonable time with a toxic drug in patients without recurrence. The effect on sex steroid secretion and its reversibility has not been sufficiently studied. The overall survival in our consecutive series updated to 30 patients in 2002 (44% low Stage and 56% high Stage tumours) compares favourably with the best reported; the overall 5-year survival was 65%, Stage II 74% and Stage III & IV 58% (Figure 7).

Figure 7: 5-year survival of 30 consecutive patients with ACC (1975-2002).



If the one, or several, components in our treatment strategy (active surgery, adjuvant monitored mitotane therapy and urinary steroid profiles during follow-up) contributed to the treatment results cannot be assessed.

7.2 Cell adhesion molecules in adrenal tumours (III)

7.2.1 Results. a) NCAD. Immunocytochemical expression of NCAD was shown in the normal adrenal cortex and medulla with labelling confined to cell membranes of cortical

parenchymal cells and medullary chromaffin cells. It was strong in all layers of the adrenal cortex, but much weaker in the medulla. All adrenal tumours expressed NCAD, but to different degrees (Table 6). The labelling was confined strictly to the cell membranes of tumour cells with no staining over stroma, blood vessels or lymphocytes (Figure 8). WB demonstrated a single immunoreactive band of approximately 100 kDa in almost all benign PC and ACA, but only in 5 out of 10 ACC in accordance with the immunocytochemical results (Figure 9).

Table 6: Summary of immunocytochemical findings in adrenal tumours

	TH	CD 44	NCAD	ECAD	NCAM	Ki-67 index
BPC	**27/27**	**23/27**	**12/27**	**0/27**	**26/27**	8±12 **(1)**
(n = 27)	(0/0/0/27)	(4/6/1/16)	(15/4/6/2)	(27/0/0/0)	(1/2/8/16)	
MPC	**8/8**	**6/8**	**8/8**	**0/8**	**7/8**	14±24 **(1)**
(n = 8)	(0/0/0/8)	(2/0/1/5)	(0/3/1/4)	(8/0/0/0)	(1/0/1/6)	
ACA	**0/30**	**7/30**	**28/30**	**0/30**	**21/30**	11±13 **(1)**
(n = 30)	(30/0/0/0)	(23/6/1/0)	(2/5/6/17)	(30/0/0/0)	(9/4/8/9)	
ACC	**0/22**	**4/22**	**9/22**	**2/22**	**17/22**	370±780 **(4)**
(n = 22)	(22/0/0/0)	(18/1/2/1)	(13/5/3/1)	(20/2/0/0)	(5/4/7/6)	

The results of analysis for TH, CD44, NCAD, ECAD and NCAM expression are given as the number of positive tumours/total number of tumours. The number of positive cells was graded as follows: 0 = no labelled tumour cells, + = 1-24% positive cells, ++ = 25-74% positive cells, +++ = >75% positive cells. The estimated percentage of labelled cells in positive tumours is given within brackets (0, +, ++, +++). For Ki67, the number of positive nuclei in 10 high power fields (HPF) is given as mean ± SD. The number of mitotic figures per 10 high power fields is given within brackets. (BPC: Benign PC, MPC: Malignant PC, ACA: Adrenocortical adenoma, ACC: Adrenocortical carcinoma).

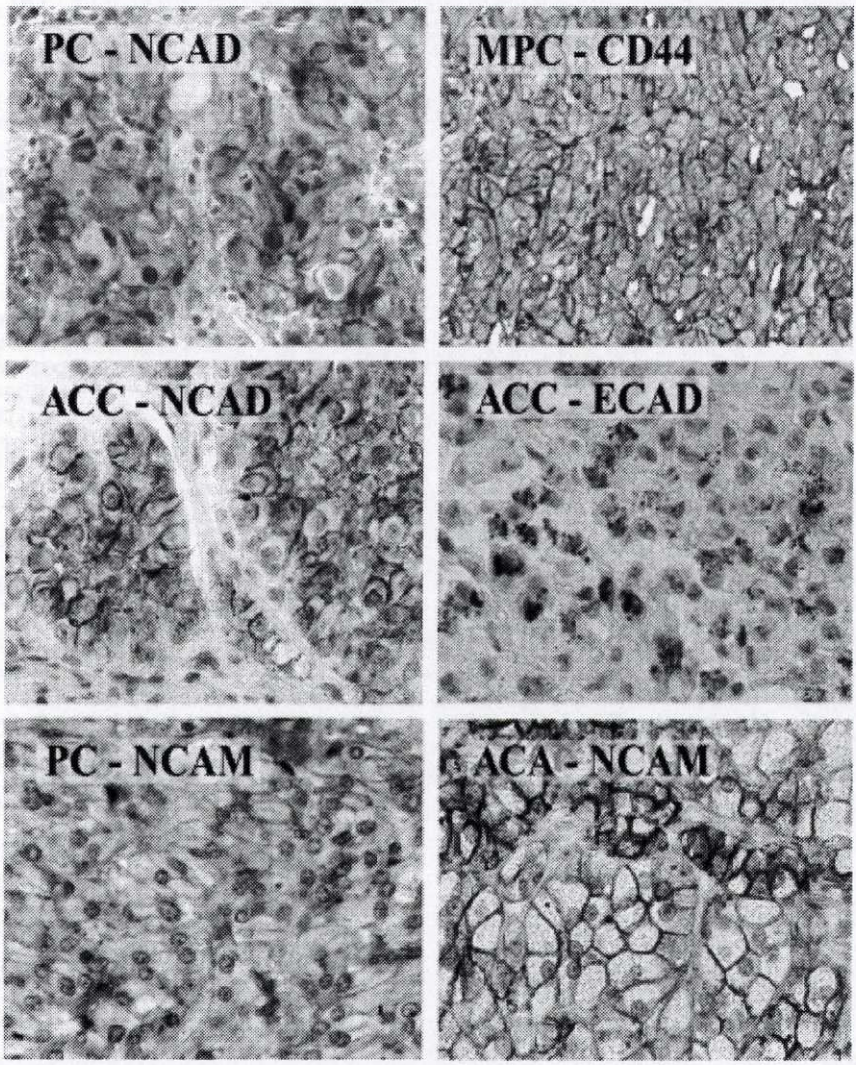
b) ECAD. There was no ECAD staining in the normal adrenal cortex or medulla, neither was it expressed in the adrenal tumours (Figure 8). WB did not reveal ECAD in any of the investigated cortical/medullary tumours (Figure 9).

c) NCAM. NCAM was demonstrated immunocytochemically in the normal adrenal cortex and medulla with labelling confined to cell membranes of cortical parenchymal cells and chromaffin cells; it was stronger in the medulla. All adrenal tumours expressed NCAM, but to different degrees (Table 6). The labelling was mainly distributed over cell membranes of tumour cells (Figure 8). WB demonstrated two immunoreactive bands at 180 kDa and 150 kDa, respectively, in the majority of benign PC, ACA, and ACC (Figure 9) in accordance with the immunocytochemical results.

d) CD44. Focal and weak labelling of CD44 was demonstrated in cell membranes of cortical cells (zona glomerulosa) and strong labelling in the cell membrane of chromaffin cells of the

medulla. All adrenal tumours expressed CD44 (Table 6). All PC had strong membranous staining and the cortical tumours very weak cytoplasmic staining (Figure 8). WB demonstrated a single immunoreactive band of approximately 65 kDa in 10/10 benign PC, 9/10 ACA, and 6/10 ACC (Figure 9).

Figure 8: Expression of CAM in adrenal tumours demonstrated by immunocytochemistry

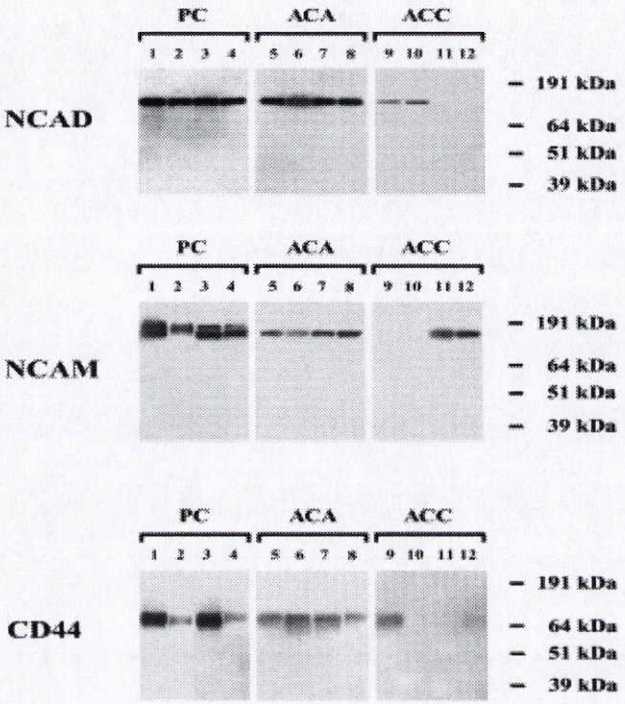


Immunocytochemical demonstration of CAM in primary adrenal tumours. NCAD, NCAM and CD44 were expressed in all types of adrenal tumours, usually with a distinct membrane labelling. ECAD was only detected in very few ACC with a patchy, cytoplasmic labelling. (PC: Pheochromocytoma, MPC: Malignant PC, ACC: Adrenocortical carcinoma, ACA: Adrenocortical adenoma)

e) Ki-67. The number of Ki-67 positive tumour cells was low for PC and ACA, whereas ACC had very high Ki-67 index (Table 6).

f) TH. In the cytoplasm of all chromaffin cells of the medulla TH was demonstrated, but not in cortical parenchymal cells. All PC (benign and malignant) demonstrated strong cytoplasmic staining for TH, but no staining for TH was seen in cortical tumours.

Figure 9: Expression of CAM in adrenal tumours demonstrated by Western blotting (WB)



Western blotting demonstrating CAM in adrenal tumours. A single NCAD-band of approximately 100 kDa was detected in all sporadic BPC, a majority of ACA and in half of the ACC. NCAM was demonstrated as two immunoreactive bands of approximately 150 kDa and 180 kDa, respectively, in all benign PC, and the majority of ACA and ACC. A single CD44 band of approximately 65 kDa was demonstrated in all sporadic BPC, a majority of ACA and in half of the ACC.

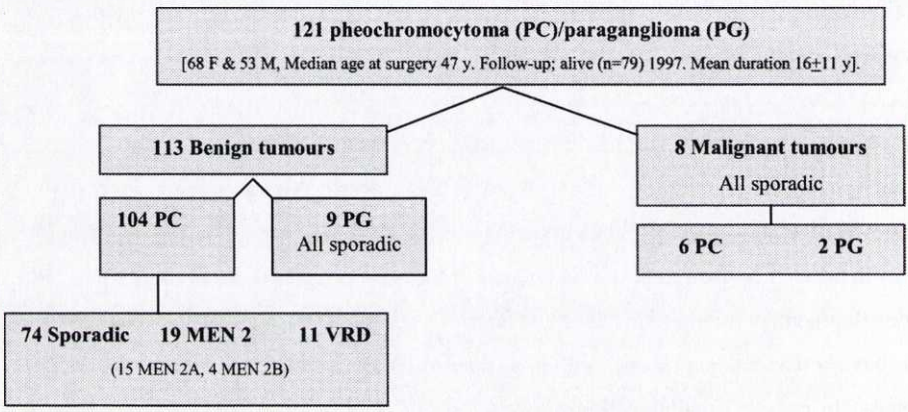
7.2.2 Comments: The expression of CAM has been evaluated extensively in many tumour types, but adrenal tumours have not been studied in detail (*Gupta et al 2000*). The expression of adhesion molecules in different tumour types can be studied immunocytochemically on formalin-fixed paraffin sections. By using material from such archives with access to clinical data, information on prognosis can be obtained. The majority of PC in our series expressed CD44 (75-85%), but only 15-25% of the cortical tumours examined. CD44 is thus expressed in both tumour types, but with higher expression in medullary tumours. Barschack et al (*1998*) had suggested that CD44 might differentiate between medullary and cortical tumours,

but its importance as prognostic marker was not clear. Our data suggest that CD44 expression in adrenal tumours is not related to malignant course. NCAM was demonstrated in the normal adrenal gland and in the majority of cortical and medullary tumours. All layers of adrenal cortex express NCAM, but to different degrees. ACA and cortical hyperplasia had a membranous and ACC a cytoplasmatic staining (*Zeromski et al 1998*). Despite these differences, there was no evidence for loss of NCAM during adrenal tumorigenesis and no relation between its expression and the biological behaviour of cortical tumours. Loss of ECAD expression has been reported to be important for development of different tumours, *e.g.* breast cancer and endocrine pancreatic tumours (*Perl et al 1998, Christofori et al 1999, Chen et al 2003, Kowalski et al 2003*). ECAD was neither demonstrated in normal adrenal cortex or medulla, nor in adrenal tumours. Hence, there is no proof that ECAD is important for development of adrenal tumours and has no use as prognostic marker in adrenal tumours. NCAD expression varies in different tumours, but has been reported to be up-regulated in squamous cell carcinoma and breast cancer (expressed in one third of invasive breast carcinomas) (*Kovacs et al 2003, Chen et al 2003, Ordonez 2003, Krengel et al 2004).* NCAD was expressed in both cortical and medullary tumours regardless of their malignant potential. During cell transformation and tumour progression, NCAD expression was maintained in PC, but was down-regulated in ACC. The results suggest that NCAD may function as a tumour suppressor in adrenal cortical tumours. The reason why NCAD was not down-regulated in PC during development of malignant PC is unclear. We may assume that different malignant tumours have different expression patterns due to their biological characteristics and invasiveness. In order to maintain tissue dynamics, cell proliferation and programmed cell death (apoptosis) balance each other. It is important to study this balance to understand differences between normal, hyperplastic and neoplastic adrenal tumours (*Sasano et al 1995*). A higher cell proliferation, indicated by higher number of Ki-67 positive tumour cells, was reported for ACC than for ACA and normal cortex. A Ki-67 index >5% is suggestive for ACC (*Nakazumi et al 1998, Wachenfeld et al 2001*). For medullary tumours a Ki-67 index >3% had 50% sensitivity, but 100% specificity, to identify malignant PC (*Clarke et al 1997*). The Ki-67 index in our study discriminated between ACA and ACC. Studies using semiautomatic image analysis programs and large tumour cell populations from primary tumours have generated individual prognostic information for other endocrine tumour types, *e.g.* MTC (*Tisell et al 2003*).

7.3 Pheochromocytoma and paraganglioma (IV & V)

7.3.1 Clinical features. Our consecutive series (1950-1997) consisted of 110 PC and 11 PG. Ninety-one patients had sporadic PC, 19 had PC as part of a MEN 2 syndrome and 11 patients had von Recklinghausen's disease (VRD). All PG were sporadic (Figure 10). The most frequent presenting symptom in patients with PC/PG was hypertension, equally common in benign and malignant tumours. The mean age at surgery for PC and PG was similar (47 $\pm$ 22 and 47 $\pm$ 16 yr, respectively). There was a median delay of 12 m (range 0-360 m) between the occurrence of symptoms related to PC/PG and tumour diagnosis. The diagnostic delay was somewhat shorter (20 $\pm$ 14 mos.) for malignant tumours *vs.* benign tumours (37 $\pm$ 66 mos.).

Figure 10: Review of 121 patients with PC/PG



7.3.2 Hypertension. Hypertension was found in 98 out of 116 patients, in whom blood pressure was recorded at diagnosis. Less than half of the hypertensive patients had antihypertensive treatment before diagnosis. One year after surgery 100 patients had blood pressure recordings; two of the preoperatively normotensive patients (n=18) had developed hypertension and 57/100 patients with preoperative hypertension were still hypertensive. Thus, more than half of the hypertensive patients remained hypertensive after surgery. Notably, only one third of the hypertensive patients had received antihypertensive treatment postoperatively (Table 7). At latest follow-up in 1997, 66 out of 98 patients with initial hypertension were alive and 25 remained hypertensive (one third were on medical treatment).

The survival analysis showed that pre- or postoperative hypertension did not significantly influence the risk of death (p>0.30) *vs.* controls.

Table 7; Blood pressure at diagnosis and during follow-up in patients with PC/PG

121 PC/PG patients								
Blood pressure available (n=116)						Blood pressure not available (n=5)		
Sporadic n= 87		VRD n=11		MEN 2 n= 18		Sporadic n= 4	VRD n= 0	MEN 2 n= 1
At diagnosis								
HP n=79	NP n=8	HP n=8	NP n=3	HP n=11	NP n=7			
One year postoperatively								
HP, n=46 16 mt		HP, n=3 2 mt		HP, n=8 1 mt		Dead n=1 No results available, n=10		

Blood pressure recordings before and within one year after PC/PG surgery. Out of 87 patients with sporadic tumours 79 were hypertensive at diagnosis. One year postoperatively 46 remained hypertensive; one normotensive had developed hypertension. Out of 11 VRD patients 8 were hypertensive at diagnosis; one year later 3 remained hypertensive. Out of 18 MEN 2 patients 11 were hypertensive at diagnosis; one year later 8 patients were still hypertensive. (mt = medically treated).

7.3.3 Hormonal diagnosis. Urinary analysis of VMA, NA, A, and methoxy-CA were performed in 104, 107, 97, and 70 patients, respectively. All malignant PC investigated (n=5) had elevated levels of methoxy-CA. At follow-up in 1997 one of the 60 tested patients had elevated urinary excretion of CA and metabolites. This patient had recurrent metastatic disease.

7.3.4 Tumour imaging. One hundred and fourteen patients had imaging studies and in 4 patients the tumours were not localized until surgery. No information was available for three patients. Between 1950 -1981, the most common imaging study was angiography with a positive diagnosis in 41 out of 46 investigated patients. In 7 patients pneumoperitoneum gave correct localization. From 1982 the tumours were localized by CT with a positive finding in 55 out of 61 investigated patients. MRI was used as complementary study in 6 cases, in which CT had indicated tumour. In 42 patients MIBG-scintigraphy was performed with a diagnostic sensitivity of 79%.

7.3.5 Preoperative α-adrenoceptor blockade. In 103 out of 112 patients treated with pbz the daily dosage was recorded. The final dosage for the entire group was 139 $\pm$ 74 mg (mean $\pm$ SD). Hypertensive patients received higher dose, 147 $\pm$ 74 mg (n=94) than normotensive

ones, 92 ± 52 mg (n=9). All patients received treatment until side-effects (ortostatism or nasal congestion) appeared.

7.3.6 Surgery. A transabdominal approach was used in a vast majority of patients. Laparoscopic adrenalectomy was only performed in three patients, but is since 1997 the dominating technique for removal of benign PC at our hospital. Major bleeding, or intra-operative arrhythmias, elicited by surgical tumour manipulation, was seen in 8 patients. Postoperative complications of clinical importance (re-bleeding, infection, or bowel obstruction) were only seen in 18 patients. There was no intra or postoperative mortality (within 30 d) within the entire series. One hundred twelve patients (104 PC, 8 PG) underwent unilateral adrenalectomy and 6 patients with bilateral tumours had bilateral procedures. Eight patients with juxta-adrenal PG underwent ipsi-lateral adrenalectomy; one patient underwent liver resection and two distal pancreatic resections due to the localization of PG. All MEN 2B patients had bilateral operations; in one case the cortical part of the adrenal was preserved unilaterally and in another patient bilaterally. Also one patient with VRD had bilateral operations with preservation of cortical tissue. Four patients underwent unilateral adrenalectomy due to typical symptoms, biochemical findings, or positive imaging MIBG studies compatible with PC. All had microscopic proof of adrenal medullary hyperplasia, confirmed by a point-counting morphometric analysis to assess adrenal medullary weight and volume. Estimated medullary weights were 1, 1, 2.4 and 3.4 g *vs.* glands from age and sex-matched controls with a normal medullary weight of 0.3-0.5 g (*Jansson et al 1988*). These four patients with adrenal medullary hyperplasia were followed postoperatively for 16 ± 3 yr with normal CA-levels and no symptoms.

7.3.7 Histopathology. Four primary tumours had advanced local growth and were thus classified as malignant. These patients and 4 other patients with tumours histopathologically classified as benign developed metastases during follow-up. Eleven other patients had histopathological features suggestive of malignancy ("borderline risk"), but none of these patients developed metastases during an observation time of 11.5 ± 6.5 yr. The mean weight of 113 tumours with recorded weight was 88 ± 16 g.

7.3.8 Outcome. The average follow-up for the 121 patients was 15 ± 6 yr. Clinically malignant tumours were found among 8 patients with sporadic tumours, who developed metastases at a median of 6.5 yr (range 1-17 yr) after primary surgery [6/110 patients with PC (5.5%) and 2/11 patients with PG (18%)] (Table 8). None of the patients with malignant tumours had metastases at diagnosis. Four of these patients had repeat surgery with tumour removal/reduction. One female patient with PG (no. 35) recurred 15 yr after primary surgery

with para-aortic lymph node metastases, so she underwent nephrectomy and lymph nodes clearance and is alive with no evidence of disease 11 yr after repeat surgery. Two patients are alive with disease and one died of other causes. Four patients died of malignant PC 8, 9, 23 and 30 yr after diagnosis, respectively.

Table 8; Characteristics of 8 sporadic cases with malignant PC/PG

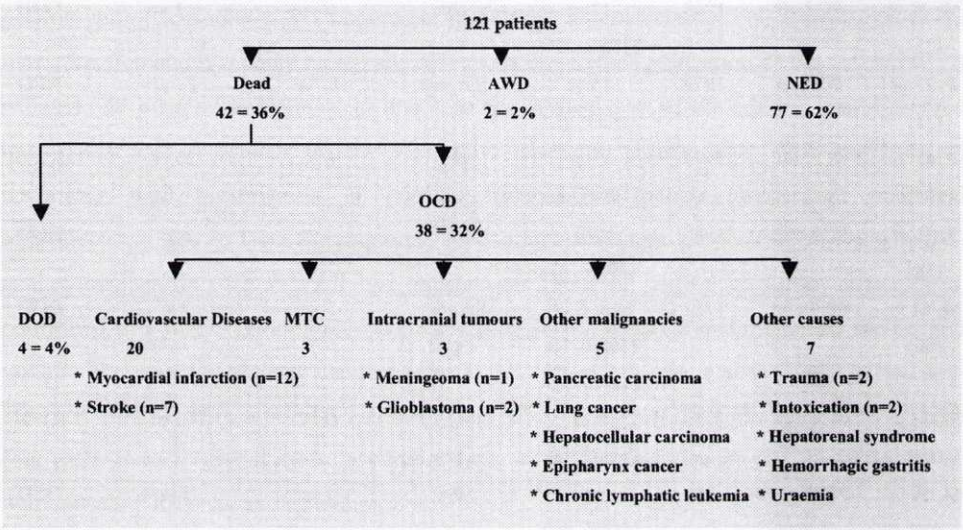
Sex & age (Pat. no)	Tumour type* & site	Unilat. adx	Latency to recurrence	Additional Treatment	Obs time after 1:st recurrence	Obs time after primary surgery	Outcome
F, 29 yr (17)	PC/Left *	1967	7 yr (1974), dist	Surgery + pbz	23 yr	30 yr	DOD
M, 48 yr (25)	PC/Left	1968	17 yr (1985), dist	pbz	6 yr	23 yr	DOD
F, 22 yr (35)	PG/Left	1972	15 yr (1987), loc	Surgery	11 yr	27 yr	NED
F, 41 yr (54)	PC/Left	1979	13 yr (1992), dist	pbz	6 yr	20 yr	AWD
F, 76 yr (58)	PC/Left*	1980	6 yr (1986), dist	MIBG	2 yr	8 yr	DOD
M, 61 yr (66)	PG/Left*	1982	4 & 6 yr (1986), loc (1988), dist	Surgery + pbz	5 yr	9 yr	DOD
M, 54 yr (85)	PC/Left*	1986	1 yr (1987), loc	Surgery + pbz	11 yr	12 yr	AWD
M, 59 yr (95)	PC/Right	1989	5 yr (1994), dist	pbz	1 yr	5 yr	OCD

DOD= Dead of disease
NED= No evidence of disease
AWD= Alive with disease
OCD= Other cause of death
adx= adrenalectomy
pbz= phenoxybenzamine
loc= local recurrence
dist= distant metastases at diagnosis
* Primary tumours with malignant histology

Three patients with cortical tissue preservation have been observed without any need for corticosteroid substitution for 2, 4, and 18 yr, respectively. Two MEN 2 patients with bilateral PC were only operated on one side; one of these had a small second PC diagnosed without symptoms at high age, so surgery was withheld. In the other case a small contra-lateral PC was incidentally diagnosed at autopsy 9 yr after unilateral adrenalectomy. All patients with bilateral PC had hereditary disease and associated neuroectodermal abnormalities. The 5 deceased patients with bilateral PC died of MTC/associated neuroectodermal diseases with a mean follow-up of 13.5 ± 11.0 yr.

7.3.9 Survival analysis and causes of death. a) Regional series. Forty-two patients died during the observation period *vs.* 23.6 expected in the control population. The observed number of deaths was significantly higher than that in the control population ($p< 0.001$). Besides high age at primary surgery ($p< 0.001$), the only significant risk factor for death in this series was high urinary excretion of methoxy-CA ($p= 0.018$). Four patients died of malignant PC. Of 38 patients, who died from other causes than PC, 20 died of cardiovascular diseases and 7 died of causes not related to PC. In the remaining 11 patients, three MEN 2B patients died of MTC, three of intracranial tumours and 5 of other tumour diseases (Figure 11).

Figure 11: Clinical outcome of 121 patients with PC/PG (1950-1997)



AWD = Alive with disease DOD= Dead of disease MTC= Medullary thyroid carcinoma

NED= No evidence of disease OCD= Other causes of death

At follow-up in 1997 42 patients had deceased, 2 patients were alive with disease and 77 patients had no evidence of disease. Four patients died of malignant PC/PG, but the main causes of death were cardiovascular diseases (n=20), PC/PG associated tumours (n=6) and other malignancies (n=5). Two patients who died of trauma had intracranial tumours at autopsy.

The number of malignant tumours was relatively high and also the second most common cause of death; three patients had no tumour-related clinical symptoms and were first diagnosed at autopsy. Two patients, who died of other causes, had intracranial tumours at autopsy. Seventy-nine patients were alive at latest follow-up (16 ± 11 yr); two with recurrent PC/PG and 77 with no evidence of disease.

b) National series: Due to the findings of high tumour mortality and several other tumour diseases in Paper IV a large national series presumably without screening bias was collected

in order to study the validity of our findings. Using the Swedish NCR we found 481 clinically diagnosed adrenal PC between 1957-1997. The mean age at diagnosis of these patients was 50.4 ± 16.8 yr (range 2-89 yr). There were 222 men and 259 women in the series. The number of deaths in this cohort was 196, which was higher than the expected number of 153.4 (p=0.001) (Table 9).

Table 9: Expected and observed causes of death in patients with PC in Sweden (1958 – 97).

Cause of death	Sex	Observed	Expected	Risk ratio	95% CI (confidence interval)
All causes	Total	196	153.4	1.28	1.10-1.47
	Men	102	108.8	0.94	0.76-1.14
	Women	94	44.6	2.11	1.70-2.60
Tumour-related causes	Total	95	26.2	3.63	2.94-4.44
	Men	48	14.1	3.41	2.51-4.52
	Women	47	12.1	3.90	2.86-5.18
Cardiovascular causes	Total	44	60.5	0.73	0.53-0.98
	Men	22	38.0	0.58	0.36-0.88
	Women	22	22.5	0.98	0.61-1.48

Analyses of death causes showed that patients with PC had almost four times higher risk dying from a tumour disease *vs.* the general population. Similar risks were observed both for men and women (Risk ratio=3.4 and 3.9, respectively). There was ***no*** excess risk for death in cardiovascular diseases among patients with PC. In fact, there were only 22 deaths of cardiovascular disease in men *vs.* 38 expected. In women, the observed number was similar in the patient and control population.

A second tumour disease subsequent to the diagnosis of PC was reported in 68 patients in the PC cohort *vs.* 31 expected in the control population (RR=2.20; CI=1.7-2.8). In men a second tumour disease was reported in 28 patients *vs.* 13.6 expected (p=0.0008) and in women 40 *vs.* 17.5 (p=0.000004). In men tumours of the liver/biliary tract (p=0.0072) and CNS tumours (p=0.00003) were most frequent. Among women malignant melanomas were highly over-represented (p=0.007), likewise carcinomas of the uterine cervix (p=0.002). On the other hand, in the general population breast cancer is common among women; the expected incidence was 5.2, but only four were reported in patients with PC (RR=0.75; CI=0.2-2.0). In men prostate cancer was expected in 3.6 *vs.* two found in patients with PC (RR=0.55; CI=0.1-2.0) (Table 10).

Table 10: Expected and observed number of a second tumour disease in patients with aPC.

		All tumours	Tumours of Liver & biliary system	CNS	Malignant melanoma	Carcinoma of Uterine cervix	Breast	Prostate
Male	**Observed**	28	3	6	2			2
	Expected	13.6	0.3	0.5	0.6			3.6
	Risk ratio	2.05	8.62	13.1	3.14			0.6
	CI	1.4-3.0	1.8-25.2	4.8-28.5				
Female	**Observed**	40		0	4	5	4	
	Expected	17.5		0.5	0.6	0.7	5.2	
	Risk ratio	2.29		0.00	6.61	7.7	0.8	
	CI	1.6-3.1			1.8-16.9	2.5-18.0		

CI= Confidence interval **CNS= Central Nervous System**

7.3.10 Comments. In our regional consecutive series of patients with PC/PG with long follow-up we demonstrated that patients after successful treatment of PC/PG still had an increased mortality risk; twice as high as the matched Swedish population. The main causes of death in these patients were cardiovascular and tumour diseases. The relation between cardiovascular and tumour deaths was 1.3. The most common causes of death reported by the Swedish National Board of Health and Welfare are cardiovascular diseases (47-50%) followed by tumour diseases (23-25%), the relation was 2.0. The much lower ratio in our series of patients with PC/PG indicated that these patients more often died of tumour diseases. About half of the deceased patients with PC in our series died from other malignancies, not commonly found in the general population. Prostate cancer in men and breast cancer in females did not cause death of any patients with PC/PG in our series.

In the national cohort, studied over a similar time period, we found that all patients treated for PC had a 4-fold increased tumour mortality risk compared with the general Swedish population. On the other hand, the risk of death in cardiovascular diseases was ***not*** elevated. The incidence of a second tumour disease, subsequent to the diagnosis of PC, in both men and women was more than twice higher than expected from control population. For men tumours of the liver/biliary tract and CNS tumours were most frequent. For women malignant melanomas and carcinomas of the uterine cervix were highly over-represented. Thus, the results from the national series of adrenal PC patients supported our findings from the regional series.

Analysis of risk factors in the regional series showed that high age at primary surgery and elevated urinary levels of methoxy-CA were correlated with an increased risk for death during

long-term follow up. It is not surprising that high age is a risk factor, however we cannot explain why one separate CA-metabolite is also a risk factor. In other studies high secretion of DA, indicative of PG, has been associated with malignancy (*John et al 1999*). Surprisingly, pre- and postoperative hypertension was not correlated with increased death risk. Increased concentrations of circulating CA in patients with PC can contribute to vascular damage and arteriosclerosis (*Pettersson et al 1990*), which in association with stress can induce changes in lipoproteins, which further aggravate vascular changes and promote the arteriosclerosis process (*Pettersson et al 1990, Bondjers et al 1991, Kaplan et al 1991*). The result indicates that structurally and functionally altered blood vessels can contribute to higher risk for cardiovascular events, *e.g.* cerebral haemorrhage (*Pacak 2001, Williams et al 2003*). Thus, long exposure to CA excess prior to diagnosis of PC can cause irreversible vascular changes (*Pettersson et al 1990*) and explain the increased risk for cardiovascular death in patients with PC/PG without evident hypertension pre or postoperatively. In an interesting study from Göteborg (1970-1993) the death risk among 6810 normotensive *vs.* 686 hypertensive men on anti-hypertensive treatment was evaluated. There was no increased risk for death in the hypertensive group during the first decade of follow-up, but during the second decade this risk increased markedly, despite adequate blood pressure control. Significant risk factors for death in that study were elevated level of serum cholesterol, smoking and signs of target organ damage, but ***not*** hypertension before and after medical treatment (*Andersson et al 1998*).

The number of patients with persistent hypertension after successful surgery in PC/PG varies between different series (*Sapienza et al 1999, Pacak 2001, McClellan 2002*). Hypertension was cured by surgical removal of PC/PG in about half of our patients. Since all of these patients, except one, had normalized CA levels after surgery, vascular changes induced by CA excess prolonged hypertension prior to surgery is one possible explanation for persisting hypertension. Only one third of our hypertensive PC patients had adequate medical treatment after surgery, which illustrates the need of a hypertension clinic for these patients.

In the present study no tumours with so called "borderline risk" histological features recurred after surgery. On the other hand, 4 of the patients who developed recurrent tumours during follow-up had none of these histological signs and were initially classified as benign. The number of histological benign PC/PG with malignant clinical course postoperatively was 3.5% in the present series *vs.* 9% in other series (*van Heerden et al 1990, Goldstein et al 1999*).

By creating large cohorts of patients with a rare disease with nearly complete follow-up and valid incidence figures, the NCR offers unique possibilities to analyse the risk for a second tumour disease. It also allows evaluation of the cause of death in a selected population *vs.* the general population. Autopsy-based death causes are reported separately, which allows separation of these cases from those clinically diagnosed. Thereby the risk for accidentally detected, clinically non-significant PC could be excluded. By definition the normal risk for death is the risk within the entire Swedish population with minimal risk for selection bias. The observed risks to develop other tumour diseases emphasize the importance of life-long follow-up of all PC/PG patients.

Overrepresentation of neuroectodermal disorders among PC patient has previously been reported (*Jansson et al 1988*). A possible mechanism for such an association could be that genetic traits were more common than previously thought *(Pacak 2001)*. Recent publications have indicated that the prevalence of germ-line mutations in cancer susceptibility genes in PC patients exceeded the earlier cited 10% (*Neumann et al 2002, Bryant et al 2003*). In hospital-based studies, almost 20% of sporadic PC were associated with mutations in VHL (6%), SDHD (succinyl dehydrogenase subtype-D) (8%), SDHB (4%), or RET (<1%) (*Bryant et al 2003*). In large population-based series, germ-line mutations in one of these 4 genes were found in 24% of patients with apparently sporadic PC (VHL=11%, RET=5%, SDHD=4% and SDHB=4%) (*Neumann et al 2002*). These data confirm that the number of hereditary PC has been underestimated (*cf. Proye et al 1994*). One explanation for such underestimation can be genetic imprinting, which means that individuals with genetic susceptibility for mutations in SDHD may not have a positive family history of PC/PG. One previous underestimation related to patients with MTC, of whom up to 25% had mutations of the *RET* proto-oncogene (*Bryant et al 2003*). Genetic testing is today strongly recommended for patients with MTC to identify MEN 2 patients at early Stage. As outlined by Neumann et al (*2002*) genetic testing of patients with "sporadic PC" to identify hereditary disease would be desirable for appropriate treatment (cortex-sparing surgery) and adequate long-term follow-up.

PC was reported as a curable cause of hypertension (*McClellan 2002*), but even after successful surgery hypertension may persist (*Plouin et al 1997*). The age of patients at the time of diagnosis and the duration of hypertension before surgery will influence the results in different series. Young age and rapid normalization of blood pressure after surgery are factors favouring cure of hypertension, but sometimes it can take more than one year to normalize blood pressure after surgery for PC (*Sapienza et al 1999*). Since patients with short history of hypertension (< 5 yr) are more likely to become normotensive after surgery, the success of

surgical treatment relies on early diagnosis (*Simon et al 1993*). The results from our series indicate that postoperative hypertension should be better managed and we think the best surveillance for these patients would be referral to a hypertension clinic.

There are still no reliable predictors of malignancy other than occurrence of metastases and wide local invasion (*Kebebew et al 1998, Goldstein et al 1999*). Age, sex, symptoms, diagnostic delay, associated hereditary syndromes and hormonal excretion did not relate to malignancy (*John et al 1999*). Some large tumours may never metastasize, while some patients with small, presumably benign tumour die of distant metastasis (*Pommier et al 1993*). The role of DNA ploidy in diagnosis of malignant PC has given conflicting results (*Pommier et al 1993, Shah et al 2003*). Tumours with histological features normally predicting malignancy are often clinically benign as shown in our series and by others (*van Heerden et al 1990, Lack 1997, Goldstein et al 1999*). The reported rates of malignancy vary largely due to the rarity of PC/PG, small series, and short follow-up (*Proye et al 1994*). Series with high numbers of malignant PC are usually from referral centres with accumulation of malignant PC in a non-consecutive manner (*Goldstein et al 1999*). In this respect our series with one centre responsible for the adrenal surgery in one geographical region is probably unique and may better represent the true biological nature of PC/PG.

8. Conclusions

- Our treatment strategy for ACC combined active surgical treatment, monitored adjuvant mitotane therapy and attempts to early detect recurrence by urinary steroid profiles. In a consecutive series with an almost equal ratio between low and high Stage tumours, the 5-year survival clearly exceeded 50%. The use of adjuvant mitotane requires multicenter randomized studies for evaluation.

- For patients with advanced, or non-resectable, ACC there is no evidence that single agent chemotherapy is useful. Multiple agent chemotherapy with addition of mitotane seems to be associated with better survival. Protocols to find the best multiple agent chemotherapy combined with mitotane in a multicenter trial are in preparation.

- Medullary and cortical adrenal tumours express specific patterns of CAM. Down-regulation of NCAD indicates malignant behaviour of cortical tumours. High expressions of Ki-67 and high mitotic index in ACC are useful in distinguishing between malignant and benign adrenocortical tumours.

- Despite successful surgical removal of PC these patients run an increased risk for death during long-term follow-up. Less than half of the patients were relieved of hypertension after surgery. Cardiovascular diseases and other malignancies were the main causes of death.

- Certain tumour diseases were overrepresented after PC had been diagnosed. Therefore, life-long follow-up of patients with PC cannot only reveal recurrence and cardiovascular diseases, but can also be helpful for detection of other malignancies.

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