Patricia A. Crock[a,*], Dieter K. Lüdecke[a,*], Ulrich J. Knappe and Wolfgang Saeger

A personal series of 100 children operated for Cushing's disease (CD): optimizing minimally invasive diagnosis and transnasal surgery to achieve nearly 100% remission including reoperations

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Abstract

Background: Transnasal surgery (TNS) is the first choice in the treatment of pediatric Cushing's disease. The question is how can high remission rates be achieved with minimally invasive investigations and TNS whilst avoiding radiotherapy or bilateral adrenalectomy in children.
Methods: Data from a published series 1 (n = 55) of surgeon DKL will be compared with his recent series 2 (n = 45) until 2009. All patients were operated by direct transnasal microsurgery. Over time, inferior petrosal sinus sampling (IPSS) was replaced by cavernous sinus sampling (CSS), restricted to unclear cases without increase of salivary cortisol in corticotropin-releasing hormone-test, difficult sellar anatomy or negative magnetic resonance imaging (MRI). Multiple direct intra-operative micro-cytology, micro-doppler and adequate visualization techniques are described.
Results: In series 1, IPSS was performed in 13 (24%) of whom 46% had false adenoma lateralization. All adenomas could be removed with extensive pituitary exploration. Three patients had early successful re-surgery. In series 2, with more refined MRI and endocrinology, CSS was used in only seven patients (15%) and all micro-adenomas were correctly localized. In three of four patients with persistent cortisol excess, repeat-TNS was necessary and successful. Side effects of TNS were minimal. Recurrence rates were 16% and 11% in series 1 and 2, respectively. Only four of 100 children with invasive adenomas were irradiated, significantly less than in other experienced pediatric centers.
Conclusions: Thus, 98% remission rate could be achieved with fewer invasive pre-surgical investigations, such as central catheter studies, refined TNS and early repeat-TNS. Repeat-TNS in recurrences minimized the need for irradiation.

Keywords: ACTH microadenoma; cavernous sinus sampling; corticotroph adenoma; pediatric pituitary Cushing's disease; petrosal sinus sampling; transnasal surgery.

Introduction

Transnasal surgery (TNS) and selective adenomectomy is the treatment of choice in children with pituitary-dependent Cushing's disease (CD) due to adrenocorticotropic hormone (ACTH)-secreting adenomas. Pharmacological options are not ideal, especially for children who need to re-establish normal growth and development patterns after the growth arrest induced by hypercortisolemia [1]. Minute micro-adenomas (less than 4 mm) are more common in children and need special diagnostic and surgical expertise, as published by Lüdecke et al. in 1987 [2] and in 2001 [3].

Pediatric CD is rare and management has evolved significantly over the last three decades [2, 4, 5]. We describe the extensive experience of a pituitary surgeon, Dieter K. Lüdecke (DKL) and highlight the approach and innovations that make it possible to achieve nearly 100% remission whilst minimizing the need for pituitary irradiation or bilateral adrenalectomy. New possibilities were discussed by DKL in the First International Meeting on CD that he organized in Crete, Greece in April, 1989 [6] together with specialists from Athens (Prof. George Tolis) and the NIH (National Institutes of Health, Bethesda, MD, USA) (Prof. George Chrousos). A recently published review from the NIH on pediatric CD, including excellent surgical results [7], did not discuss more reliable pre-operative diagnostic methods such as cavernous sinus sampling (CSS) to localize minute ACTH-adenomas [8]. CSS was already in use by DKL in 1986 and discussed at the meeting in Crete and published in the Proceedings [6].

[a]Patricia A. Crock and Dieter K. Lüdecke are equal first authors.
***Corresponding authors: Patricia A. Crock,** Department Paediatric Endocrinology and Diabetes, John Hunter Children's Hospital, Locked Bag 1, Hunter Region Mail Centre, Newcastle, NSW 2310, Australia; and PRC GrowUpWell®, Hunter Medical Research Institute and Department Paediatrics, University of Newcastle, Newcastle, NSW, Australia, Phone: +61249855634, Fax: +61249213599, E-mail: patricia.crock@hnehealth.nsw.gov.au; and **Dr. Dieter K. Lüdecke:** Retired Emeritus Pituitary Surgeon, Department Neurosurgery, University Hospital Eppendorf, Hamburg, Germany, E-mail: luedecke.di@gmail.com
Ulrich J. Knappe: Department Neurosurgery, Johannes Wesling Klinikum, University Hospital of the Ruhr University Bochum, Minden, Germany
Wolfgang Saeger: Department of Neuropathology, Pituitary Pathologist, University Hospital Eppendorf, Hamburg, Germany

Limiting invasive investigations in children is important but even more so in children with suspected CD, who are obese, potentially in a pro-thrombotic state [9, 10] and in whom venous access is often painful and problematic. For these reasons late-night salivary cortisol is superior to midnight plasma cortisol levels and to urinary free cortisol [11–13] and it is easily repeatable in the home setting when there is any suspicion of cyclical CD. In contrast to adult patients, ectopic ACTH- or corticotropin-releasing hormone (CRH)-producing tumors are extremely rare in childhood [14–17] so the need for central venous sampling studies should be minimized by careful endocrine assessment and repeat testing if unclear. Techniques developed for intra-operative localization of minute adenomas will be discussed including multiple, direct intra-operative micro-cytology [18], micro-doppler and specific visualization techniques such as the use of micro-mirrors with the specially devised suction-irrigation system by DKL.

Subjects and methods

Subjects

We analyzed 100 patients with pediatric CD (aged under 18 years) who were referred to Dr. Dieter K. Lüdecke (DKL) for surgery over 30 years from 1980 to 2009. Eighty-nine patients were treated primarily at the University Hospital Eppendorf (UKE – Universitäts Klinik Eppendorf) center in Hamburg and 11 patients had been treated previously in other European centers. The first series of 55 patients from 1980 to 1995 was published in 1996 [19]. The second series of 45 patients was studied prospectively from 1996 to 2009.

One infant with a giant, suprasellar corticotroph adenoma and operated transcranially, is not included in this series and has been published separately [20]. The severely Cushingoid infant had been investigated in Zagreb and the transcranial operation by DKL was successful. Post-operatively she died from a massive pulmonary embolism due to thrombosis from a femoral vein catheter inserted for repeat endocrine testing in UKE-Children's, not requested by DKL. A retrospective review of this child's pituitary pathology did not show evidence of pituitary blastoma, the recently described entity due to DICER mutations [21, 22].

Over the course of the three decades, the endocrine investigations, diagnostic approaches and microsurgical techniques were all continuously improved and refined under the supervision of the senior author, DKL. The aim was to achieve a surgical cure in all patients with resectable pituitary corticotroph adenomas.

The authors have complied with the World Medical Association Declaration of Helsinki regarding ethical conduct of research involving human subjects. Informed written consent was given by the parents (or guardians) and the children for surgery and for the use of data for publication by the surgeon DKL and for clinical photography. Consent documents were also supplied in the family's native language. All documentation was approved by the Institutional Ethics Committee at the University Hospital Eppendorf (UKE), Hamburg.

Endocrine diagnosis of ACTH dependent hypercortisolism

Diagnostic criteria for hypercortisolism were initially: elevated 24-h urinary free cortisol and suppression of 0800 h morning plasma cortisol levels after overnight dexamethasone [23, 24].

From 1986, repeatedly elevated salivary cortisol at 2200 h was introduced by DKL and measured in his Neuroendocrine Laboratory 1 [11, 12].

The diagnostic triad for CD or pituitary-dependent hypercortisolism was and is [25]:

1. ACTH normal or elevated,
2. Dexamethasone test (low or high dose) significantly suppressed,
3. CRH-test positive [26] or desmopressin test positive [27]

Desmopressin testing was only used in special cases with extremely difficult venous access.

Pre-operative radiological investigations

Prior to 1986, patients were imaged by thin-slice computer tomography scans and post-1986 by magnetic resonance imaging (MRI) of the sellar region.

MRI is not considered helpful intra-operatively in these cases with micro-adenomas.

Central venous sampling

Our indications for central venous sampling were: equivocal endocrine tests, negative MRI, special sellar anatomy (such as a non-aerated sphenoid sinus in a younger child that would require extensive bone drilling for sellar access – see Figure 1) and/or previously failed TNS.

From 1986 to 1998 inferior petrosal sinus sampling (IPSS) according to Oldfield et al. [28] was performed detecting one ectopic ACTH syndrome, but localizing minute adenomas (<4 mm) correctly at the pituitary in only 54% [19]. Additional pituitary hormone levels did not lead to a solution [29].

Since 1986, parallel intra-operative cavernous sinus sampling (OPCSS) was introduced by Lüdecke [30]. It was only used in cases where MRI was negative and the adenoma not immediately found on exploration. This technique involves direct puncture of the cavernous sinuses bilaterally by the pituitary surgeon and simultaneous sampling for intra-operative measurement of ACTH. The availability of rapid ACTH determinations is implicit in this approach and was done in the co-located Neuroendocrinology Laboratory 1 of DKL at UKE before commercial assays were even available [30]. The ACTH and other steroid immunoassays in the laboratory were also essential for *in vitro* studies that first showed that Cushing adenomas remain micro due to growth suppression by steroids [31].

As even severe complications of IPSS had been published in a child [32], we decided in 1999 to change for preoperative CSS according to Teramoto et al., whose group developed very fine catheters to enter different areas within the cavernous sinus [33].

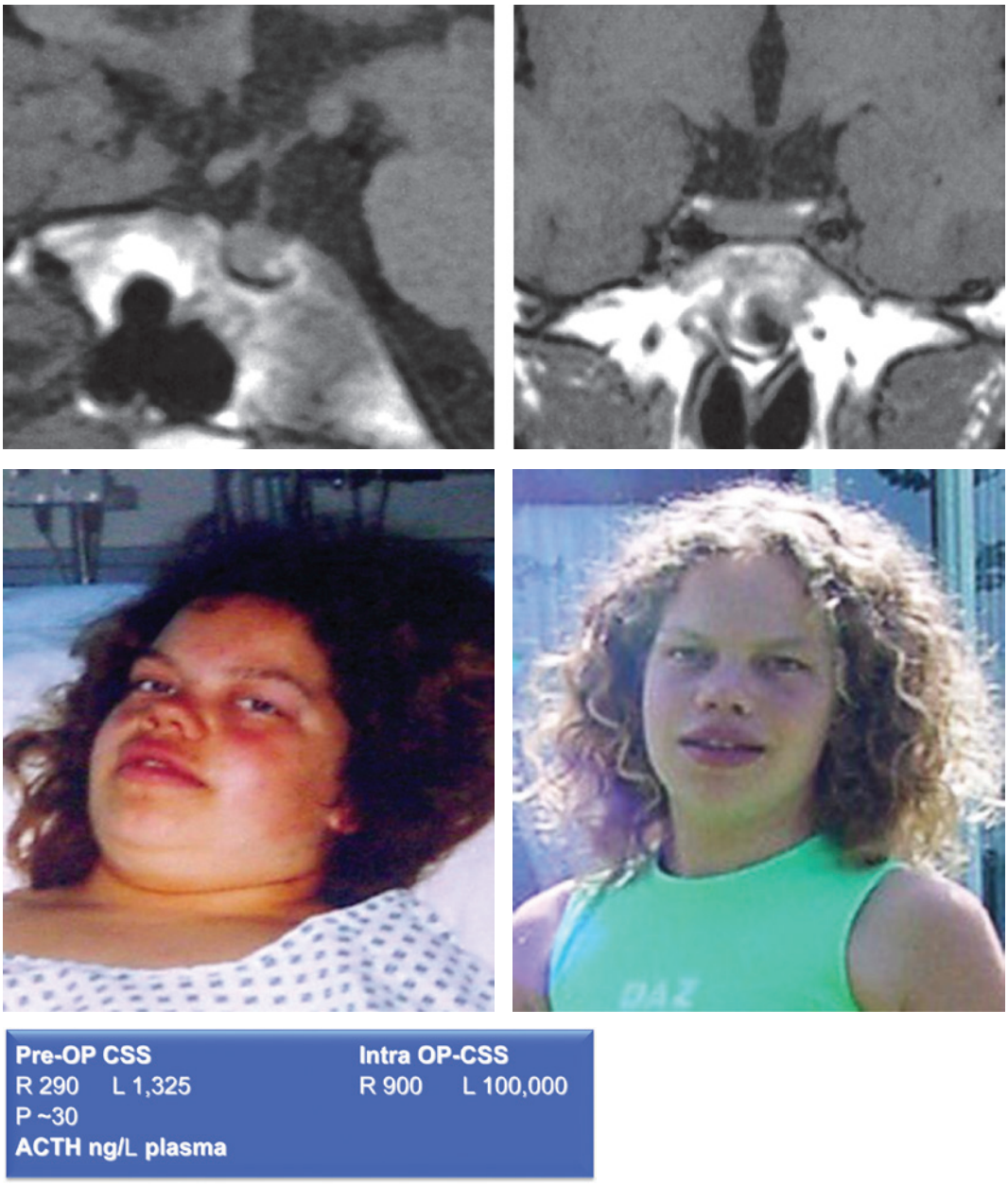

Figure 1: A 14-year old girl with the classical Cushing triad and negative MRI (sagittal and coronal sections in upper left and right panels, respectively).
Lower left panel: 1 h post-operation with as usual, no nasal packing. Lower right panel: in remission 10 months post-operatively. Cavernous sinus sampling with correct adenoma localization with 4× fold higher ACTH in the left CSS pre-OP and 100× fold in the left, directly intra-OP (lower panel). The patient and her parents consented to clinical photographs that showed the classical facial features of Cushing's disease. P, peripheral ACTH level.

Direct TNS

Patients were placed in a semi-seated position to adapt cavernous sinus pressure and thus, the surgeon could operate comfortably in the sitting position and was able to concentrate for long periods (see Figure 2). The patients' eyes were prepared pre-operatively so that during surgery the anesthetist or an assistant could open the eyes and the surgeon could directly observe any pupillary changes indicating oculomotor problems in the cavernous sinus. This was important when there was evidence of adenoma invasion in the cavernous sinus.

DKL performed direct TNS mostly via a single nostril (depending on the individual anatomy) with a very fine nasal speculum. Using his suction-irrigation system [34] with fine pediatric cannulas (refined first in rhesus monkey pituitary research in 1983 with Prof. E. Nieschlag, Max Planck Institute, Münster) and specially designed angle-tipped bipolar forceps for complete hemostasis, blood loss was minimal during access to the sella. In contrast

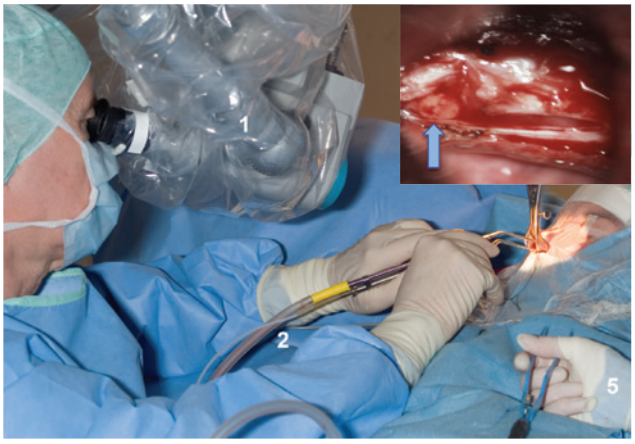

Figure 2: Operative set-up of the direct transnasal micro-surgical approach (TNS) according to DKL.
Surgeon seated comfortably at the microscope (1) with the Lüdecke micro-irrigation-suction system in the surgeon's left hand (2).
A small nasal speculum is used. Inset shows the view on the monitor for the scrub nurse and learners. Arrow is at the sellar floor with an ACTH-adenoma above. Micro-doppler, micro-mirror techniques and final heating of the cavity with bipolar forceps (5) under irrigation are used. Note the tape on the eyes that can be removed to monitor for oculomotor function when operating and/or heating tumor rests in or near the cavernous sinus. Closure is done with sphenoid bone fragments from the approach and crafted by DKL to fit the sellar hole. Muscle or fat are only used if there is a risk of CSF leak.

to some other colleagues he visited, DKL never required blood transfusions for his patients and very rarely a nasal tamponade. An operating microscope was used with the operation field visible on a computer screen for scrub nurses and the many colleagues as visitors (see insert, Figure 2). The floor of the sella was opened with a special drill and the whole front of the pituitary gland was exposed. In children, the sphenoid sinus is often not yet pneumatized and this makes sellar access more difficult due to the need for extensive bone drilling with the aid of a short intra-operative X-ray.

When MRI was unclear, and CSS not done pre-operatively, DKL prepared for intra-OPCSS to localize the ACTH adenoma.

The ACTH adenoma was found by micro-dissection, starting on the expected side and performing micro-biopsies of the abnormal tissue for direct cytology. The suction system could be manually stopped as soon as the tissue was inside the cannula to keep it for histology. Micro-mirrors were used with the suction-irrigation system that kept them clean and enabled wide visualization of the field. Bipolar forceps were used to heat saline locally at the site of residual invasive tumor tissue. Micro-doppler was always used to localize the adjacent carotid arteries.

In DKL's experience, intra-operative ultrasound in this period was not of high enough resolution [35].

Intra-operative pituitary pathology methods

Intra-operative cytology [18] micro-biopsies were taken with very fine forceps and material transferred directly onto sterile glass slides (Figure 3, left panel), so no material was lost. It was then processed by the double-gloved surgeon (DKL), stained with methylene blue and checked under the microscope in the operating theatre. The aim of this method was to confirm the presence of adenoma tissue (Figure 3, right panel), as opposed to normal anterior pituitary containing Crooke's cells and to exclude other co-incident pathology [36].

The intra-operative slides were also reviewed by the specialized pituitary pathologist (WS). If adequate tissue was available, it was preserved for definitive, specific histopathology. In series 2, U. Knappe suggested measuring the ACTH concentration in the adenoma smear itself, which showed extremely high ACTH levels. DKL and his senior technician, G. Glagla designed the method of comparing very small biopsies taken with special, fine forceps from adjacent

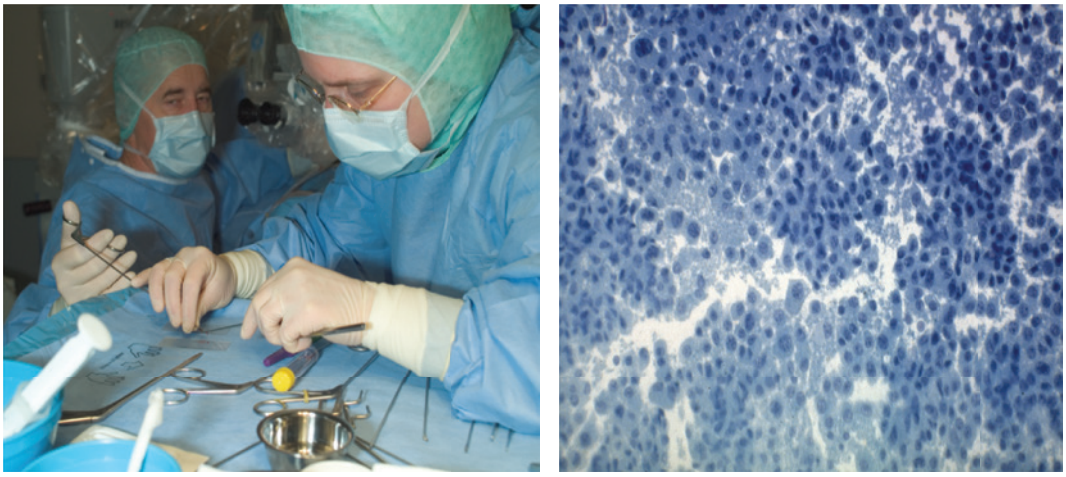

Figure 3: Intra-operative direct cytology.
DKL (seated) transferring a micro-biopsy of the adenoma directly onto a glass slide for staining (left panel) and direct intra-operative cytological examination by the double-gloved surgeon. The right panel shows a typical adenoma with no normal pituitary tissue which would contain Crooke's cells.

pituitary tissue (with suppressed corticotrophs) and tumor tissue [37]. In cases with a well-defined pseudo-capsule [38], the technique outlined above may not be necessary.

Peri-operative management

Plasma for cortisol and ACTH was taken again at the start of anesthesia and after surgery every 2 h for measurement the following morning in the Neuroendocrine Laboratory of DKL. When awake, patients also had salivary cortisol taken. In both series 1 and 2, no perioperative cortisol replacement was given unless hypocortisolemia had been confirmed or if signs of cortisol insufficiency such as hypotension developed. Great caution needs to be taken in children as subnormal cortisol levels have been observed in a median of 8 h after selective adenomectomy. This decline in children is significantly faster than in adults.

Results

Results of CSS in series 2 from 1996 to 2009

An example of correct localization with OPCSS compared with preoperative CSS is given in Figure 1. Samples were immediately measured for ACTH in the Neuroendocrine Laboratory of DKL [30].

Pre-operative CSS correctly localized minute adenomas in seven of eight from a total of 45 children. In four children with special problems, pre-operative CSS and OPCSS were performed as well. In two boys with CD, TNS was successfully performed after obtaining CSS results in the same anesthesia: one a previous failure from DKL and one primary surgery in a very anxious boy. Both are now adults without recurrences.

Pituitary pathology results

In all children in series 2, cytology was finally positive, at least in the second operation, for an ACTH adenoma.

The numbers of intraoperative micro-biopsies per case were evaluated in two parallel series of 26 children and 30 adults with CD by PAC and DKL. The original data was supplied by a pituitary pathologist, W. Saeger. This showed that more than three biopsies were performed in 70% of the children and only in 13% of adults.

Surgical results

Remission was defined by subnormal cortisol initially or normal diurnal cortisol for more than 6 months [25]. Overall results are outlined in Table 1.

Table 1: Summary of remission rate and recurrences in both series.

Years	n	Remission	Re-TNS	Remission	Recurrence
1980–1995[a]	55	53 (96%)	2	(100%)	9 (16%)
1996–2009	45	41 (91%)	3	(98%)	5 (11%)

[a]Series 1 published by Knappe and Lüdecke in 1996 [19].

In the case of a residual adenoma with persistent hypercortisolemia, early repeat surgery (ReTNS) was well tolerated and successful; two patients in series 1 and three patients in series 2. The patients and parents readily agreed as nasal tamponades were not used (see Figures 1 and 4) with the Lüdecke suction-irrigation system and special bipolars, which stopped any nasal bleeding.

Complications and recurrences

We specifically asked referring pediatric pituitary centers for information about complications and/or recurrences in DKL patients (see Tables 1 and 2 and Figure 5).

There was no mortality in either series and no neurological deficits.

There were no severe nasal problems with the use of finer, pediatric instruments, especially the nasal speculum (see Figure 2). In particular, there were no maxillary fractures and no sub-labial access as reported by the NIH-group [7, 39]. There were no complications related to withholding peri-operative administration of glucocorticoids.

The percentage of children who had radiation was very low, 4% as compared to London, 39% [40] and Mumbai, 16% [41]. In the patients with recurrences, one patient was seen at the NIH for a second opinion, and was recommended for radiation rather than repeat surgery, as DKL had proposed.

Discussion

This second largest series of pediatric CD operated by a very experienced pituitary surgeon demonstrates the improvements in less invasive diagnosis and transnasal surgical techniques over a 30-year period. Children comprised 10% of all patients with CD operated by DKL.

Refined endocrine diagnostic investigations, especially salivary cortisol in the late evening (2200 h) [12] and in CRH-tests, make the diagnosis of CD less invasive. This is important for children with CD whose obesity makes venous access more traumatic [3, 11, 42]. The role of

Figure 4: The last young patient in the series operated with Cushing's disease with surgeon DKL and PAC.
Photograph taken on the first post-operative day at Marienkrankenhaus, Hamburg with no nasal tamponade used. Hydrocortisone replacement already started after a rapid fall in serum cortisol. The patient and her parents consented to the clinical photograph.

Table 2: Comparison of % complications between series 1 (55 patients) and series 2 (45 patients).

TNS series of DKL	I 1980–1995	II 1996–2009
No. of patients	55	45
CSF-fistula, managed, %	3.6	2.2
Some/total pituitary deficits, %	14/4	10/2
Diabetes insipidus > 2 weeks, %	7	0

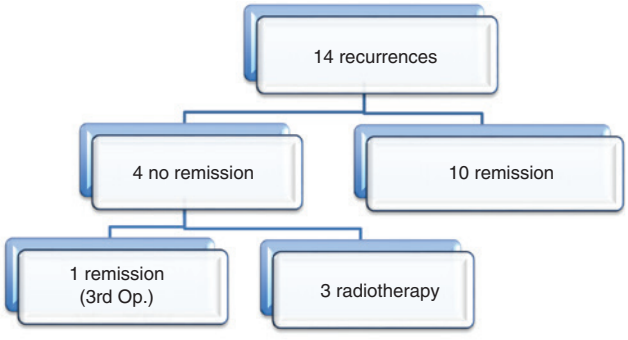

Figure 5: Summary of re-operations in 14 recurrences from 100 patients.

genetic testing in the management of CD is not yet defined [43–46].

Invasive and risky, IPSS was found to be unreliable for the localization of minute micro-adenomas [3, 7, 47]. CD due to ectopic ACTH and/or CRH is extremely rare in children [14], thus IPSS is not a necessary part of the investigation of CD in children. Both Oldfield and Lüdecke had already agreed in 1996 at a pituitary workshop in Hamburg, that IPSS is not reliable enough for the localization of minute adenomas to perform hemi-hypophysectomy. As predicted by DKL in 1989, this was confirmed in an NIH publication from 2006 in pediatric Cushing where only 58% localized correctly with IPSS [48]. In contrast intraoperative CSS as introduced by DKL was very helpful in highly selected cases and in our recent series, if necessary, it was done preoperatively for very minute adenomas.

Microsurgical and pathological techniques as outlined already [3] led to long-term remission in most of DKL's primary and recurrent pediatric CD patients. The significant difference between the number of intra-operative micro-biopsies in children vs. adults highlights the difficulties in pediatric CD with minute adenomas and the mandatory need to preserve normal pituitary function in children, yet completely remove the adenoma. The use of saline, heated locally by bipolar forceps and suction-irrigation at the adenoma bed was also a factor in improving the remission rate in invasive adenomas. The monitoring of oculomotor function intra-operatively made the use of heated saline a safe option.

The largest pediatric CD series of E. Oldfield with 200 and D.K. Lüdecke with 100 children, had remissions (including re-surgeries) >95% [7, 19]. Other pediatric series

with less than 50 patients [49–53] reported cure rates ranging from 45 to 69%.

In the two DKL series combined, only 4% of patients received radiation therapy (RT) (one primary and three with recurrent, invasive tumors). In pediatric series from London, 39% [40] and from Mumbai, 17% [41] had RT after transsphenoidal surgery and many developed pituitary-deficiencies, especially GH deficiency (in up to 46%). Even when a child or adolescent is in remission without GH deficiency, it can take time to recover normal growth and bone density but body composition remains abnormal, with an increased ratio of visceral to subcutaneous fat that suggests these patients are at increased risk of metabolic syndrome [54].

Summary

In summary, significant improvements in non-invasive diagnosis, salivary cortisol at 2200 h and salivary cortisol in CRH testing, have been very reliable for differentiating obese from CD children. In difficult cases, DKL was the first to use bilateral CSS intra-operatively [30] before the advent of CSS catheters by Teramoto [55, 56]. IPSS was shown to not be reliable for ACTH adenoma localization.

Routine, intraoperative direct micro-cytology proved to be essential for detecting and selectively removing minute ACTH-adenomas using specially designed Lüdecke suction-irrigation instruments and preserving normal pituitary function [38]. Minimally invasive, unilateral, microsurgical TNS is important functionally for both the nose and pituitary. This allows for early re-surgery when ACTH and cortisol levels remain high. Including early re-operations, a 98% remission rate could be achieved and the high risk of pituitary function loss with radiotherapy could be avoided.

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