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Paediatric Cushing's disease — a literature review of epidemiology, pathogenesis, clinical symptoms, and diagnostics

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Abstract
Cushing's disease (CD) is characterised by excess production of adrenocorticotropic hormone (ACTH) by a pituitary corticotroph adenoma, which results in hypercortisolaemia. CD is extremely rare in the paediatric population, and few paediatric endocrinology centres have experience in diagnosing and treating this disease. The clinical presentation of hypercortisolaemia is variable, so proper and rapid diagnosis of CD is often challenging. The molecular pathogenesis of CD was largely unknown until recently. The latest research has revealed somatic mutations in the *USP8* gene as the most common pathogenic molecular variants of this disease. Herein, we describe the current state of knowledge of paediatric CD epidemiology, molecular pathogenesis, clinical symptoms, and diagnostics. **(Endokrynol Pol 2020; 71 (1): 87–95)**

Key words: *Cushing's disease; pituitary adenoma; hypercortisolaemia; Cushing's syndrome; growth retardation; weight gain*

Introduction

Cushing's disease (CD) is defined as a state of hypercortisolaemia caused by excessive secretion of adrenocorticotropic hormone (ACTH) by a pituitary corticotroph adenoma. Cushing's disease is characterised by an increased risk of cardiovascular, metabolic, psychiatric, and infectious diseases [1]. Because of an increased mortality ratio in the case of persistent disease, CD has become a diagnostic and therapeutic challenge for physicians [2].

Epidemiology

While the incidence rate of Cushing's syndrome (CS) is 1.89–2.3/mln/year in Danish patients [3], 2–5/mln/year in the Spanish population [4], and 39.5–48.6/mln/year in the US population aged ≤ 65 years [5], the incidence rate of CD in these populations is reported to be, respectively: 1.2–1.7/mln/year [3], 0.7–2.4/mln/year [4], and 6.2–7.6/mln/year [5]. It should be noted that only 10% of new cases occur in children [6].

Cushing's disease is the most common cause of endogenous CS, especially in children over five years of age [7–9]. It accounts for 75-80% of paediatric CS cases (for comparison, 49–71% of adult cases) [7, 10]. In children under five years of age CD occurs rarely — the most common causes of CS in this age group are adrenal lesions: adenoma, carcinoma, or bilateral adrenal hyperplasia [6, 11]. According to Kunwar et al. [12], pituitary corticotroph adenomas constitute 54.8% of all pituitary adenomas in the age group 0–11 years and 29.44% in the age group 12–17 years. The mean age of CD presentation (on the basis of large studies on the paediatric population; groups of 41–182 children) is 12.3–14.1 years [13–15].

However, CD has a female preponderance in adults (with an overall prevalence of 79%), in children a male preponderance has been documented, with an overall prevalence of 63% [8, 14, 16].

According to the study by Lubuit et al., boys may have more aggressive disease with an elevated body mass index, shorter height, and higher ACTH levels, in comparison with girls [17].

Molecular pathogenesis

The pathogenesis of CD is not fully understood. Studies on the molecular basis of pituitary adenomas have been the goal of many researchers in recent years. It is known that mutations in genes that cause syndromes

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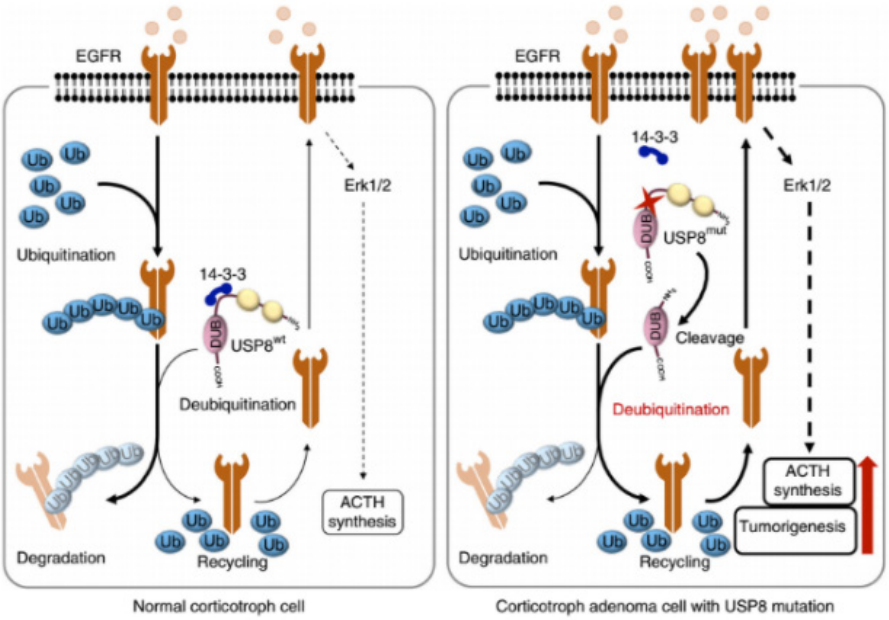

Figure 1. *Schematic representation showing the proposed mechanisms how USP8 mutations lead to increased ACTH secretion and tumorigenesis in corticotroph, according to Reincke 2015, with permission [25]*

REVIEW

associated with pituitary adenomas: multiple endocrine neoplasia type 1 (*MEN1*), multiple endocrine neoplasia type 4 (*CDKN1B*), McCune-Albright syndrome (*GNAS1*), familial isolated pituitary adenoma (*AIP*), and many more (*CABLES1, DICER1, MC2R PRKAR1A, PRKACA, RAF1, TP53*) occur sporadically and account for only a small portion of CD cases [18–23]. Somatic mutations in the gene *USP8* encoding the ubiquitin-specific protease 8 have been detected recently as the most common alterations in patients with CD (found in 31–63% of corticotroph adenomas) [15, 24–27]. The ubiquitin-specific protease 8 inhibits the lysosomal degradation of the epidermal growth factor receptor (EGFR) [28]. As a result of the gain-of-function somatic mutation, the binding of the inhibitory 14-3-3 proteins to the ubiquitin-specific protease 8 is impaired, which results in increased deubiquitination of EGFR and induction of proopiomelanocortin transcription and ACTH secretion [25] (Fig. 1).

Clinical presentation

The rarity of CD occurrence in children poses a risk of being overlooked in everyday clinical practice. The principal features of CD in children are: weight gain and growth failure [13, 29–32]. Facial changes (facial rounding/moon facies), headaches, and hirsutism are also typical clinical features (Fig. 2).

Skin manifestations in CD: acne, facial plethora, violaceous striae, bruises and hyperpigmentation can be present but none of them is a key feature. The diagnostic process can reveal irregularities such as: puberty disorders, hypertension, and carbohydrate metabolism disorders.

Regarding puberty disorders, menstrual irregularities, amenorrhoea, and premature or delayed sexual development may be diagnosed. Young children may present with premature sexual development, accelerated epiphyseal maturation, and virilisation (as a result of increased adrenal androgen secretion), whereas older children and adolescents may have delayed puberty (as a result of glucocorticoid-induced hypogonadism) [8, 33]. Dupuis et al. in their analysis of 27 children with CD concluded that many patients had abnormal puberty and excessive virilisation associated with increased adrenal androgens, while pubertal patients had low gonadotropin concentrations, which suggested impaired pituitary–gonadal axis function [33].

Hypercortisolaemia, in addition to a number of other symptoms, also affects mood changes and may cause mental disorders (including emotional lability, irritability, or depression), but these problems are less frequently seen in children in comparison to adults [34]. Muscular atrophy and sleep disturbances are also rarely seen in children.

The mean length of symptoms prior to diagnosis reported in the literature is from 1.8 years (range 0.5–3.5) in the study by Yordanova et al. (21 children with CD), 2.33 years (range 0.25–7) in the study by Batista et al. (72 children with CD), 2.5 ± 1.7 years (range 0.3–6.6) in the study by Savage et al. (37 children with CD), to 3 ± 2 years (range: 3 months –7 years) in the study by

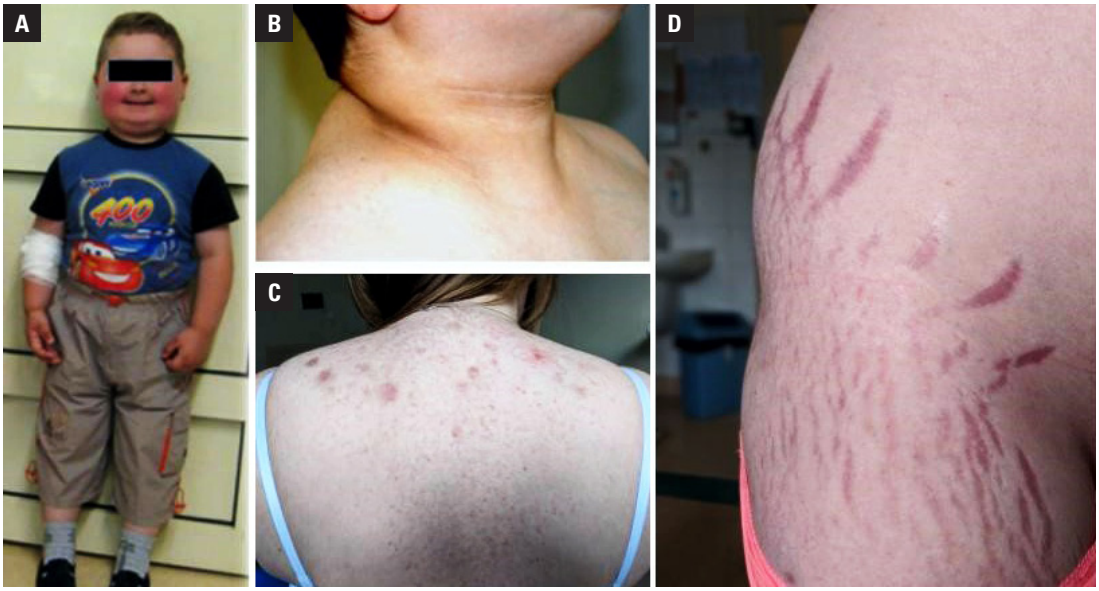

Figure 2. ***A****. Clinical presentation of a 5-year-old male with CD. Note plethora and mooning of face.* ***B****. Clinical presentation of a 10-year-old male with CD. Note acanthosis nigricans.* ***C****. Clinical presentation of a 16-year-old female with CD. Note acne.* ***D****. Clinical presentation of a 16-year-old female with CD. Note stretch marks*

Magiakou et al. (50 children with CD) [8, 29, 31, 35, 36]. Early recognition of CD key features is necessary to allow quick diagnosis and effective therapy.

The analysis of clinical features of patients from a few groups (17–200 children) presented in a few studies is shown in Table 1.

Evaluation of Cushing's syndrome

Interview and clinical evaluation, especially growth data, are key in the initial diagnosis of CD [37]. Biochemical evaluation and imaging are the basis of CD diagnosis. Before biochemical evaluation the use of exogenous corticosteroids should be excluded (glucocorticoids applied orally, intranasally, topical treatments or inhalers) as the most common reason of CS [38].

Confirmation of hypercortisolaemia

The first step of diagnostics in the direction of CS is the confirmation of hypercortisolaemia, which should take place in the ambulatory care [39].

The 1-mg overnight dexamethasone suppression test

The 1-mg overnight dexamethasone suppression test is done by administering to the patient 1 mg of dexamethasone (Dx) orally in the evening at 23:00 (or between 23:00 and midnight) [40] and measuring the serum cortisol concentration the next morning at 08:00 (or between 08:00 and 09:00) [39]. The cortisol concentration > 1.8 μg/dL (with the sensitivity 95% [41] and specificity 80% [42]) commits to further diagnostics in the direction of CS [43]. The percentage of false negatives is low (which means that false-normal suppression is rarely detected), and the percentage of false positive results is 15–20% [37].

Two studies in woman [44, 45] have potentially led to increased cortisol results by contraceptives. Although the 1-mg overnight Dx suppression test is used as a screening test for paediatric patients, there are lacking data on its interpretation or reliability in this population.

It is also important to remember about other drugs that can interfere with the evaluation tests for the diagnosis of CS: drugs that accelerate Dx metabolism by induction of CYP 3A4 (e.g. phenobarbital, carbamazepine, and others), drugs that impair Dx metabolism by inhibition of CYP34A (e.g. itraconazole, fluoxetine), drugs that increase cortisol-binding globulin (CBG) and may falsely elevate cortisol results (e.g. mitotane), and drugs that increase urinary free cortisol (UFC) results (e.g. carbamazepine, fenofibrate) [43].

24-h urinary free cortisol excretion

24-h UFC excretion is a good screening test for hypercortisolism and gives information about unbound cortisol, which is not affected by factors influencing CBG [46]. The result should be corrected for the child's body surface area [37]. 24-hour urine should be collected for three consecutive days to exclude cyclic CS with a high probability.

According to Reimondo et al., the diagnostic cut-off value of 24-h UFC (with 100% sensitivity) is > 38 μg/24 h [47]. Sensitivity of this test for CD analysed by Batista et al. in their study of 105 children with CS was 88% and

Table 1. *The analysis of clinical features at diagnosis of Cushing's disease on the basis of published data [14, 29, 31, 32, 35, 36]*

Clinical feature	Percentage of patients (%)					
	Storr 2011 (41 children)	Devoe 1994 (42 children)	Yordanova 2016 (21 children)	Batista 2009 (72 children)	Savage 2001 (17 children)	Lonser 2013 (200 children)
Weight gain	98	92	100	62[a]	100	93 (71[a])
Growth retardation		84	95	82[b]	71	63[c]
Facial changes	100	46[d]	100			63[e]
Irregular menses (females)				73		49[f]
Osteopaenia		74	30			
Fatigue	61	67	62	41	59	48
Hirsutism	59	46	52	39	53	56
Psychiatric disorders	59[g]	44[h]	71[g] (5[i]; 5[j])	28[g]	41[g]	31[g]
Headaches	51	26	52	33		38
Striae	49	36	43	40[k]	53	55[k]
Hypertension	49	63	43	46	75	36
Acne	44	46	38	46	41	47
Pubertal delay or arrest		60		7		
Easy bruising		28		22		25
Dorsal cervical or supraclavicular fat pad		28[l]				69
Acanthosis nigricans				26		32
Muscle weakness				13		
Sleep disturbances				11		
Glucose intolerance or diabetes						7
Bone fractures			5			4

[a]obesity (body mass index Z-score > 2.0); [b]short stature; [c]decreased linear growth [significantly different (Fisher's exact test, $p < .01$)]; [d]plethora; [e]moon facies; [f]amenorrhoea (primary or secondary); [g]depression, anxiety, mood swings; [h]compulsive behaviour; [i]anxiety combined with challenging behaviour; [j]acute psychosis; [k]hyperpigmented abdominal striae; [l]Buffalo hump; [m]vertebral fractures due to severe osteoporosis

specificity was 90%, which means that 24-h UFC alone is not an ideal test in screening for CS [39].

Falsely high UFC (pseudo-Cushing syndrome) can be obtained in the following states: physical activity, stress, anxiety, depression, anorexia, pregnancy, severe obesity, alcoholism, malnutrition, and high water intake [37].

Normal results in both 1-mg overnight Dx suppression test and 24-h UFC exclude CS in 90–95% of cases (in 5–10% the disease has intermittent or cyclic course or does not manifest in any test) [37].

Cortisol circadian rhythm

It is recommended that cortisol circadian rhythm is performed in a hospital setting. A venous sampling catheter should be inserted at least two hours before the test. Time points in which cortisol should be measured vary depending on the centre. Batista et al. (the National Institute of Health, Bethesda, USA) suggest measuring cortisol levels at 23:00 and 24:00 and at 07:30 and 08:00 [38]. Storr et al. recommends measuring serum cortisol level at three time points: 09:00, 18:00, and midnight (sleeping: 24:00) [48]. Late night salivary cortisol has the highest sensitivity and specificity (also in the obese population) — according to Batista analysis: 99% and 100% [39]. The diagnostic cut-off value of midnight cortisol is > 1.8 μg/dL (50 nmol/L) [49]. If a patient is awakened at night, blood should be collected within five minutes of awakening [50]. Then, the sensitivity and specificity of a midnight serum cortisol level > 7.5 μg/dL in an awake patient is reported to be > 96% [51].

Low-dose dexamethasone suppression test

A low-dose dexamethasone suppression test (LDDST) is performed with the dose regimen for adults (unless the child weighs < 40 kg): 0.5 mg every 6 h (at 09:00, 15:00, 21:00, and 03:00) for 48 h. If the child weighs < 40 kg, the dose is 30 μg/kg per dose, max. 0.5 mg/dose [8]. The National Institute of Health in Bethesda sug-

gests adjusting the dose according to the weight for children weighing < 70 kg by dividing the dose by 70 and multiplying by the weight of the child [6]. Urine is collected for 24-h UFC for two consecutive days before Dx administration and on the second day of Dx administration [34]. Serum cortisol is measured at 0, 24, and 48 h. UFC < 10 μg/d 48 h after Dx administration and serum cortisol level < 1.8 μg/dL are a normal response [34]. Serum cortisol values < 1.8 μg/dL (measured after 48 h) exclude CS with 97–100% sensitivity [39, 49].

Confirmation of Cushing's syndrome

Once CS is diagnosed, the next step is to distinguish ACTH-dependent disease from the ACTH-independent syndrome.

Basal plasma ACTH

As hypercortisolaemia leads to corticotropin (CRH) and ACTH suppression from healthy corticotroph cells, measurement of plasma ACTH differences in ACTH-dependent and ACTH-independent CS: in CD ACTH is always detectable; in ACTH-independent CS ACTH is low and usually undetectable [11, 34, 51]. According to Batista et al., the diagnostic cut-off value for CD is ACTH level >29 ng/L — the sensitivity and specificity above this value are documented as, respectively, 70 and 100% [39].

Pecori Giraldi et al. emphasise that many commercially available ACTH assays are imprecise in the low ranges, and the result should be interpreted with caution [52]. Moreover, single measurements of cortisol and ACTH are not of great value in diagnosis because of their circadian nature [37].

High-dose dexamethasone suppression test

Classic high-dose dexamethasone suppression test (HDDST, Liddle test) is used to differentiate CD from ectopic ACTH secretion and adrenal causes of CS [37]. In brief, after LDDST as described above, a high dose of Dx (120 μg/kg/dose; max 2 mg/dose) is given per os every six hours for two days [6]. A single high dose of Dx (in children < 70 kg adjusted for weight as described above, max 8 mg) can be also given at 23:00, and the plasma cortisol level is measured the following morning (sensitivity and specificity are similar to classic Liddle test). Urine is collected for 24-h UFC as described in LDDST and on the second day of high dose of Dx administration. Cortisol is measured at 09:00 the day after Dx administration. A 50% suppression of serum cortisol levels from baseline differentiates CD (> 50% suppression) from other causes of CS (adrenal or ectopic ACTH production) (< 50% suppression) [6].

Approximately 85% of patients with CD will have suppression of serum cortisol, UFC, and 17-hydroxysteroid values, and less than 10% of patients with ectopic ACTH secretion will have suppression [37].

Stratakis C.A. [37] in his article indicates situations when the classic Liddle test is preferred rather than a modified overnight HDDST: 1) non-suppression of serum cortisol levels during the HDDST; 2) negative imaging studies; 3) suspected adrenal disease.

CRH test

The ovine (o)CRH test has equal or even greater diagnostic value than HDDST in differentiating between CD and ectopic ACTH secretion [38, 53, 54]. The patient should be fasting and lying in bed. A venous catheter should be inserted the night before the test. The ovine CRH is administered at a dosage of 1 μg/kg (max. 100 μg) of body weight at 08:00. Samples for cortisol and ACTH are taken 5′ before administration, at 0′, and 15′, 30′, and 45′ after the administration of oCRH.

The criterion for diagnosis of CD is a mean increase of 20% above baseline for cortisol values 30′ and 45′ and an increase in the mean ACTH concentrations of at least 35% over basal value 15′ and 30′ after oCRH administration [6, 35].

In CRH test, 85% of patients with CD respond to oCRH with increased plasma ACTH and cortisol production. The response to oCRH is not observed in 95% of patients with ectopic ACTH production [6,38].

Results of oCRH test and HDDST (Liddle or overnight) taken together give the diagnostic accuracy of 98% [6].

LDDS-CRH test

The diagnostic power of the LDDST and the oCRH test is enhanced when both tests are combined (the test enables also differentiation between CS and pseudo-Cushing syndrome more accurately).

The test is performed by intravenous CRH administration (1 μg/kg, max 100 μg) 2 h after the last dose of Dx in LDDST (LDDST described above) [55]. Adrenocorticotropic hormone and cortisol serum concentrations are measured at baseline (–15′, –5′, and 0′) and 15′ after the administration of oCRH. In the case of pseudo-Cushing syndrome, basal plasma cortisol is low or undetectable and there is no response to oCRH.

According to analysis by Martin et al., the sensitivity of the LDDST-CRH test is 100% and the specificity is 67% [56]. Obesity can confound the results of this test [57].

Imaging

Magnetic resonance imaging (MRI) is considered to be the imaging method of choice for detecting ACTH-secreting adenomas [58]. It should be done only after biochemical confirmation of ACTH-dependent CS [59].

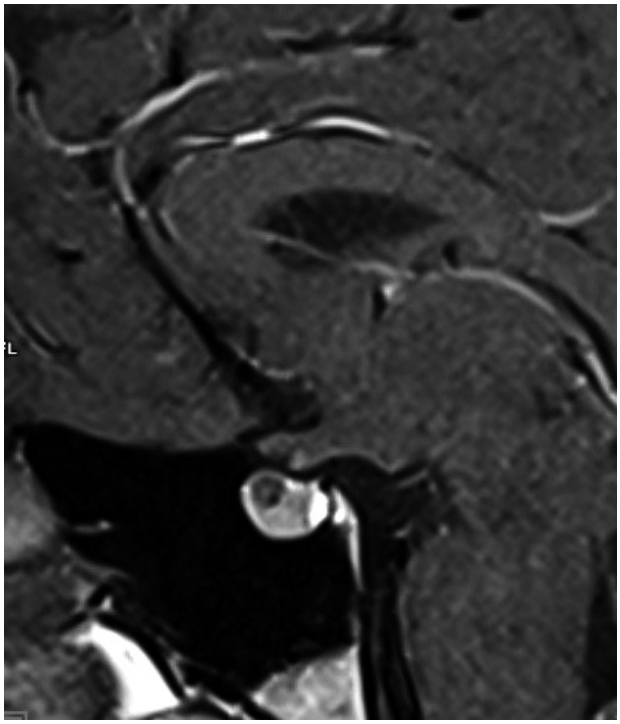

Figure 3. *MRI scan showing pituitary adenoma. Sagittal plane. SE T1-weighted image after contrast injection*

Detection of pituitary adenoma, on the basis of four large paediatric studies (groups from 21 to 72 children with CD), is 43–72% [14, 29, 32, 35]. Similarly, the detection rate of pituitary corticotroph adenomas in adults is documented to be 50–70% [60–62].

Pituitary adenomas are generally hypointense compared with the adjacent gland and take up contrast less avidly and in a more delayed fashion, and therefore fail to enhance with gadolinium [30] (Fig. 3). The poor visualisation rate can be explained by the limited spatial resolution of MRI, i.e. small lesions within a small pituitary gland are less conspicuous [30]. Spoiled gradient-recalled acquisition in the steady-state (SPGR) with improved spatial resolution is a newer technique in MRI considered better than the conventional T-1-weighted spin echo (SE) technique in identifying pituitary tumours [59].

Studies on the adult population reported that localisation of a tumour in preoperative imaging significantly raises the cure rate for CD: by up to 90% (if MRI detects pituitary tumor) *vs.* 50–70% when there is no tumour in MRI [63–65]. According to the Consensus Statement on Diagnosis and Complications of Cushing's Syndrome, finding a pituitary lesion > 6 mm is regarded as a cut-off that can be considered as a corticotroph adenoma [34]. In the presence of dynamic biochemical studies compatible with CD the size of 6 mm provides a definitive diagnosis and does not require further evaluation [34, 58]. However, the size of pituitary adenoma detected in MRI in children is usually less than 6 mm [36], and their size at surgery turns out to be even less (≤ 2 mm) [12]. Pituitary macroadenomas are extremely rare in children [66]. Furthermore, distinguishing a corticotroph adenoma and an incidentaloma on the basis of the lesion size in MRI has not been confirmed in the studies [67, 68]

Bilateral simultaneous inferior petrosal sinus sampling

Bilateral simultaneous inferior petrosal sinus sampling (BSIPSS) enables distinction between CD and ectopic ACTH syndrome and helps to localise the microadenoma within the pituitary gland. If the test is performed in an experienced centre, the distinction between CD and ectopic ACTH syndrome is nearly 100% [37]. Storr et al., in their analysis of 41 children with CD, reported that the cure rate in patients who underwent BSIPSS preoperatively was higher (73%, 24/33) than in those who did not undergo the procedure, although this was not statistically significant ($p < 0.5$) [14].

Bilateral simultaneous inferior petrosal sinus sampling is routinely performed in adult practice when the MRI does not show unequivocally a pituitary adenoma [30]. Bilateral simultaneous inferior petrosal sinus sampling is a highly specialised technique and should be performed by experienced interventional neuroradiologists with a team of trained operators [30, 69]. In the majority of cases, general anaesthesia (GA) is not required; however, in young children GA may be necessary [30, 70]. In the study by Dias et al. children with GA were aged 5.6 and 6.6 years, and BSIPSS, despite GA, gave valid results [70].

The test is done by blood sampling from each inferior petrosal sinus for measurement of ACTH concentration simultaneously with peripheral venous sampling. The sampling is performed through catheters inserted into the femoral vein and advanced to the petrosal sinuses under fluoroscopic guidance. ACTH is measured at baseline and at 3′, 5′, and 10′ after oCRH administration (1 μg/kg, max 100 μg). Because it has been shown that each patient could have in one petrosal sinus (left or right) a much higher percentage of total ACTH [71], blood samples must be taken from both inferior petrosal sinuses to avoid making an erroneous diagnosis of ectopic ACTH secretion [69].

Interpretation of the test consists of calculating of the ratio of ACTH concentration in the sinus (central) and the peripheral sample [37]. Patients with CD have central-to-peripheral maximal gradient ≥ 2 at baseline or central-to-peripheral gradient ≥ 3 at any time point after stimulation with oCRH, while no gradient between the sinus and peripheral sample is observed in ectopic ACTH secretion [37]. Lateralisation of ACTH

secretion is defined as an interpetrosal sinus ACTH gradient (IPSG) of > 1.4. IPSG is calculated by dividing the highest IPS ACTH value (right or left) by the value at the same time in the contralateral IPS. An IPSG < 1.4 suggests a midline lesion [72].

The use of BSIPSS for the localisation of pituitary microadenoma to the right or left side of the pituitary gland is controversial [72]. The accuracy of lateralisation results after CRH stimulation in a large meta-analysis was 78% [73]. The published data of the results in children are limited. The available data show that BSIPSS has been used to predict the side of microadenoma with varying results. Batista et al. have recently reported in their large analysis of 94 children that localisation of a microadenoma by BSIPSS agreed with surgical location in only 58% of cases [74]. Having excluded 22 lesions that were either centrally located or bilateral, the percentage of predictive lateralisation increased to 70% [74]. In contrast to this result, four other analyses of smaller groups [30, 69, 75, 76]: 41, 27, 11, and 17 children, stated that ACTH sampling gives a better prediction of the site of the microadenoma than that by pituitary MR imaging. The first one [30] showed that BSIPSS correctly predicted the tumour position in all (4) cases; in the second [75] and third [76] research there was concordance between the BSIPSS finding and the position of the microadenoma at surgery in, respectively, 81% (17/21) and 91% (10/11) patients. The last study reported that in 91% of patients BSIPSS showed the correct position of pituitary adenoma [70].

The accuracy of the test may be limited by some factors: 1. duration of hypercortisolaemia (the suppression of normal corticotrophs by longstanding hypercortisolaemia confirms that any ACTH measured is secreted by tumour tissue [pituitary or ectopic]) [68]; 2. different anatomical variants (when venous drainage of the pituitary gland does not follow the expected normal anatomy, the results are inaccurate) [37]; and 3. an ectopic CRH-producing tumour [37].

Differences between Cushing's syndrome in children and adults

Standards of procedure for diagnosis and treatment are mainly based on experience from the adult population, whereas it should be noted that there are some differences in presentation of the disease and in response to treatment [14]. CD in developmental age is distinguished by: increased frequency of prepubertal CD in males, the different clinical presentation (as shown above), the decreased presence of macroadenomas, and the frequent absence of radiological evidence of an adenoma on MRI [14].

Conclusions and perspectives for the future

Recent advances in the medical diagnosis of CD and close cooperation with adult endocrinologists have improved the medical care of this challenging disease. More and more is known about the pathogenesis of CD; however, further broadening of current knowledge about molecular pathogenesis of CD is of foremost importance because it could lead to new treatment targets in the future.

Compliance with ethical standards

1. Authors declare no conflict of interest.
2. This article does not contain any studies with animals performed by any of the authors.
3. All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards. Informed consent was obtained from all individual participants included in the study.

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