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Adrenal insufficiency: Diagnostic approaches, treatments, and outcomes

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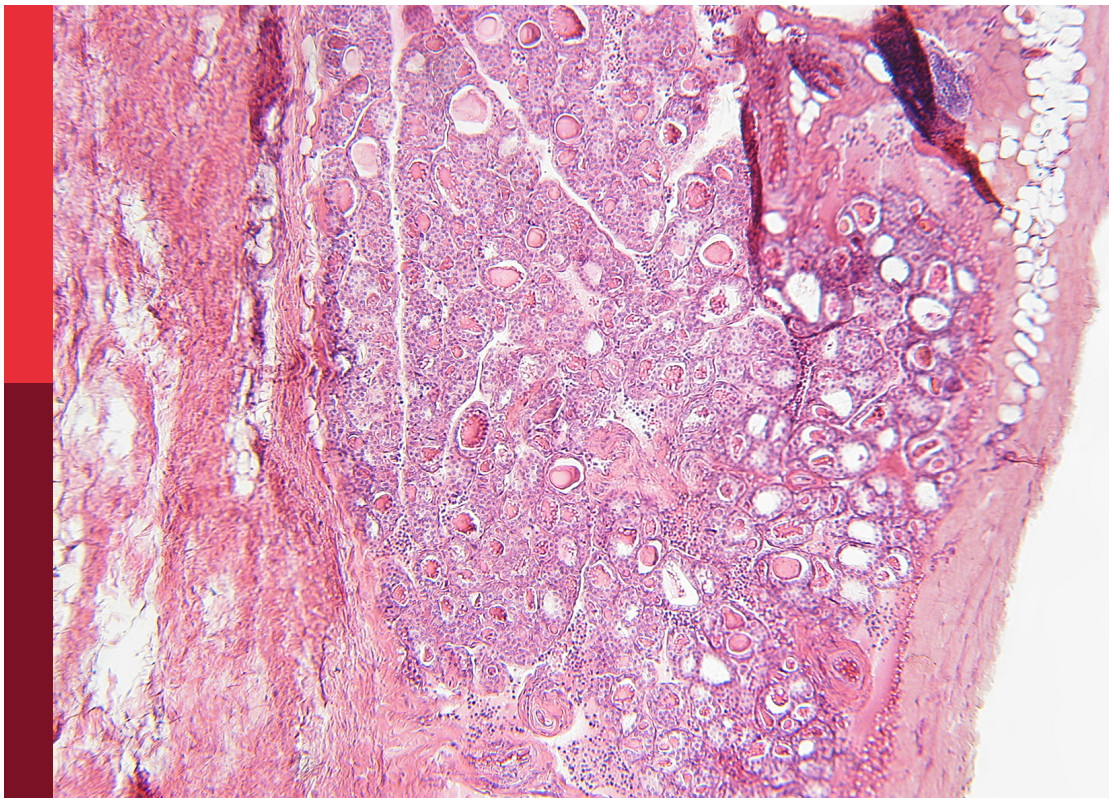

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Adrenal insufficiency: Diagnostic approaches, treatments, and outcomes

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Editorial: Adrenal insufficiency: Diagnostic approaches, treatments and outcomes

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Editorial on the Research Topic
Adrenal insufficiency: Diagnostic approaches, treatments, and outcomes

The diagnosis and treatment of adrenal insufficiency (AI) remains a challenge for both patients and clinicians. Long term studies reveal that patients with adrenal insufficiency have increased morbidity, mortality and impaired quality of life (1). Possible contributors are failure of the currently-available glucocorticoid (GC) replacement formulations to mimic the physiological diurnal secretion of cortisol, high incidence of infection, and suboptimal patient education, amongst others. It is therefore gratifying that a recent issue of Frontiers in Endocrinology is dedicated to novel research on adrenal insufficiency.

GCs are widely used for treatment of various conditions and are the most common cause of adrenal insufficiency (2). In this issue, Einarsdottir et al. report findings from a large population-based study investigating all-cause and disease-specific mortality amongst oral GC users, defined as patients receiving ≥5 mg prednisolone, or equivalent dose of other GCs, for three weeks or longer. GC users had increased all-cause mortality (HR adjusted for age, sex and comorbidities (95%CI) 2.08 (2.04-2.13), p<0.0001) compared to controls. The excess deaths were mainly due to pulmonary embolism, pneumonia and sepsis- mirroring complications observed in endogenous hypercortisolism. Whether the high mortality is caused by GC treatment, or merely reflects the severity of the underlying medical condition, remains unknown.

Patients with AI are at increased risk of infections, and hospitalizations due to infections (3, 4). During the COVID-19 pandemic concerns were raised about the susceptibility to, and potentially worse outcomes in SARS-CoV-2 infections, amongst AI individuals. Yedinak et al. conducted a 27-item survey amongst AI patients, recruited world-wide *via* social media, websites and advocacy groups, to determine self-reported incidence of COVID-19 infection. Overall, 3.1% of 1291 respondents with AI tested

positive for COVID-19, compared to 1.3% global cumulative incidence for the same time-period. 22.5% of COVID-19 positive AI individuals required hospitalization with a risk ratio that was 24-fold higher than in the global population. While the data is striking, like many studies on incidence of COVID infections in specific populations, self-selection bias, inability to verify diagnosis and recall bias, may have contributed.

The diagnosis of AI is based on demonstrating suboptimal cortisol response to 250 µg of synthetic ACTH. Some argue that this dose represents a supraphysiological stimulus and may lead to false-negative results, particularly in secondary AI. The insulin tolerance test, once considered a "gold standard" for the diagnosis of secondary AI, is utilized less commonly nowadays as it is labor-intense and carries risks associated with hypoglycaemia. The metyrapone test is an alternative to diagnose secondary AI. However, the use of this test is limited by access to 11-deoxycortisol measurements. Papierska et al. investigated if a single measurement of ACTH, instead of 11-deoxycortisol, during the metyrapone test, can provide acceptable diagnostic accuracy. The optimal cut-off of ACTH during the metyrapone test was estimated at 147 ng/l with a sensitivity of 71% and specificity of 84%. However, even with an ACTH diagnostic threshold of >200 ng/L, false negative results were present in 20% of secondary AI patients, highlighting that a single ACTH measurement cannot replace 11-deoxycortisol measurements.

Ali et al. report an eight-year-old boy with isolated primary glucocorticoid deficiency with hypoplastic adrenal glands possibly due to combined digenic, tri-allelic inheritance of two *STAR* (steroidogenic acute regulator protein) mutations and a *CYP11A1* (encoding the P450 side chain cleavage enzyme) variant, which is the first description of digenic, tri-allelic primary AI.

Cognitive dysfunction is common in patients with hypercortisolism which may persist after reversal of the cortisol excess. Pupier et al. evaluated patients younger than 60 years of age in remission following surgical treatment for Cushing disease (excluding relevant comorbidities and psychotropic use) and show that memory impairment is not present at long-term follow-up. However, the patients displayed impaired quality-of-life, the intensity of which was proportional to the duration of active hypercortisolism prior to the cure, underscoring the importance of early diagnosis and treatment.

Adrenal crisis affects a substantial proportion of patients with AI, with an incidence of approximately 5-10 per 100-patient-years, with many suffering from recurrent episodes (5). However, little is known about the epidemiology of adrenal crises in adolescents and young adults who may be particularly vulnerable to this complication because of the challenges associated with transition to adulthood. Chrisp et al. analyzed all hospital admissions in Australia between 2000/1 to 2019/20 for AI, including adrenal crises, in 10 -24-year-olds. The authors showed that between 2000/1 and 2019/20 admission rates for adrenal crises increased, especially in young women aged 20-24 years where it increased from 3/million to 40/million. This concerning trend is unexplained and has not been reported previously.

To date, few risk factors for adrenal crises have been identified which limits application of preventive measures. Vulto et al. investigated a biological predisposition to adrenal crisis by studying cortisol pharmacokinetics and pharmacodynamics in patients with secondary AI. Patients with history of adrenal crises demonstrated differences in cortisol and cortisone excretion, as well as metabolomic profiles suggestive of reduced glucocorticoid sensitivity compared with those who did not experience adrenal crisis.

Current guidelines for treatment of AI advocate the use of lower doses of short-acting GCs, equivalent to a daily dose of 15-25 mg of hydrocortisone(HC) (6).Higher daily GC doses have been associated with cardiovascular morbidity and mortality and infections (7). Caetano et al. argue that the currently-recommended replacement doses may lead to overtreatment in at least some patients. They present a titration method (reducing GC dose by 5 mg of HC equivalent every 2-6 months) for determination of the individual's optimal daily GC dose, based on empirical assessment of AI symptoms. Using this method, the mean daily HC dose equivalent achieved was 13.9 ± 6 mg (7.6 ± 3.4 mg/m^2), approximating the daily cortisol production rate of 7 mg/m^2, and significantly lower than the current recommendations.

Replacement GC, especially in excess doses, can affect bone quality in patients with AI (8). Zdrojowy-Wełna et al. evaluated densitometry parameters, and trabecular bone score in 29 patients with primary AI. There was no difference in T-score, Z-score, bone mineral density or trabecular bone score (TBS) in patients compared to controls. There was, however, a negative correlation between TBS and the duration of AI and age, as well as between densitometry parameters and 24-hour urinary cortisol, indicating that disease duration and higher HC doses may affect bone status negatively.

We hope that this Research Topic provides a valuable resource on the current knowledge regarding several aspects of clinically relevant topics associated with adrenal insufficiency, its pathophysiology, diagnosis and treatment.

Author contributions

All authors listed have made a substantial, direct, and intellectual contribution to the work and approved it for publication.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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CASE REPORT
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Can Digenic, Tri-Allelic Inheritance of Variants in *STAR* and *CYP11A1* Give Rise to Primary Adrenal Insufficiency? A Case Report

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An eight-year old South Asian boy presenting with progressive hyperpigmentation was found to have primary adrenal insufficiency (PAI) in the form of isolated glucocorticoid deficiency. Follow up of this boy for nine years, until the age of 17 years showed normal pubertal onset and progression. Molecular evaluation, by targeted next generation sequencing of candidate genes linked to PAI revealed changes in two genes that are intricately linked in the early stages of steroid biosynthesis: compound heterozygous variants in *STAR*, c.465+1G>A and p.(E99K), plus a heterozygous rs6161 change in *CYP11A1*. No variants in other known causal genes were detected. The proband's mother was heterozygous for the c.465+1G>A *STAR* and rs6161 *CYP11A1* variants, while the father was homozygous for the p.(E99K) alteration in STAR but wild-type for *CYP11A1*. Both parents had normal adrenal cortical function as revealed by short Synacthen tests. The *STAR* variant c.465+1G>A will lead to abnormal splicing of exon 4 in mRNA and the addition of the p.(E99K) variant, predicted damaging by SIFT and CADD, may be sufficient to cause PAI but this is by no means certain given that the unaffected father is homozygous for the latter change. The rs6161 *CYP11A1* variant [c.940G>A, p.(E314K)] has recently been demonstrated to cause PAI in conjunction with a severe rare disruptive change on the other allele, however sequencing of the coding region of *CYP11A1* revealed no further changes in this subject. We wondered whether the phenotype of isolated glucocorticoid deficiency had arisen in this child due to tri-allelic inheritance of a heterozygous *CYP11A1* change along with the two *STAR* variants each of which contribute a partial loss-of-function burden that, when combined, is sufficient to cause PAI or if the loss-of-function c.465+1G>A combined with the presumed partial loss-of-function p.(E99K) in *STAR* could be causative.

Keywords: primary adrenal insufficiency, isolated glucocorticoid deficiency, STAR, CYP11A1 (P450scc), puberty

INTRODUCTION

Congenital primary adrenal insufficiency (PAI) in children is usually inherited as one of numerous monogenic disorders, with a molecular diagnosis being made in more than 90% of individuals (1–3). Principal causes include congenital adrenal hyperplasia (CAH), X-linked congenital adrenal hypoplasia (AHC), familial glucocorticoid deficiency (FGD) caused by adrenal cortical unresponsiveness to ACTH, adrenoleukodystrophy (ALD) and triple A syndrome (Allgrove syndrome) (1, 2). More recently, next generation sequencing (NGS) of patients with PAI has identified mutations in genes linked to altered redox potential, impaired oxidoreductase activity and sphingolipid metabolism, which include minichromosome maintenance 4 (*MCM4*), nicotinamide nucleotide transhydrogenase (*NNT*), thioredoxin reductase 2 (*TXNRD2*) and sphingosine 1-phosphate lyase (*SGPL1*) (4–7). Reaching a specific diagnosis can have implications for management and counselling. The genetic causes of PAI and their spectrum of disease have been reviewed recently (2, 3, 8).

FGD usually presents with isolated glucocorticoid deficiency with minimal or no mineralocorticoid deficiency. Inactivating mutations of the type 2 melanocortin receptor gene (*MC2R*) were the first genetic cause to be associated with FGD (FGD 1) (9, 10). Later, mutations in melanocortin 2 receptor accessory protein (*MRAP*) were also found to be causative (FGD2) (10–12). Together pathogenic variants in these genes only account for less than half of all cases of FGD.

Although mutations in steroidogenic acute regulator protein (*STAR*) have classically been associated with congenital lipoid adrenal hyperplasia, characterized by severe deficiency of both glucocorticoids and mineralocorticoids and no androgenization of 46,XY fetuses (13), partial loss-of-function variants in *STAR* are increasingly reported in patients diagnosed with FGD who presented with later onset, milder disease, and apparently normal gonadal function (14–16). *CYP11A1* encodes the P450 side chain cleavage enzyme (P450scc) crucial in early steroidogenesis and, similar to *STAR*, classical *CYP11A1* mutations (resulting in disruption of the enzyme, P450scc) also cause severe deficiency of both glucocorticoids and mineralocorticoids with no androgenization of 46,XY fetuses. Recently, certain mutations in *CYP11A1* have also been found in patients with milder phenotypes indistinguishable from FGD (17, 18); indeed, several studies have reported patients with the combination of a rs6161 (c.940G>A) variant that affects splicing, together with a severe loss-of-function change on the other allele as a cause of PAI presenting in childhood, but with normal or only mildly disrupted gonadal function (18). Taken together with the emerging experience from non-classic congenital lipoid adrenal hyperplasia due to *STAR* defects, these reports highlight a relation between enzyme function and phenotype and suggest that human adrenal function is more sensitive to partial disruption of *STAR* and *CYP11A1* than gonadal (testis) function.

Here we report long-term follow up of a boy with PAI presenting as isolated glucocorticoid deficiency possibly due to tri-allelic inheritance of variants in *STAR* and *CYP11A1*.

CASE REPORT

A boy born to non-consanguineous South Asian parents presented at the age of eight years with a history of progressive hyperpigmentation since early childhood. There was no prior history of electrolyte disturbances or evidence of adrenal crisis and family history was unremarkable for adrenal insufficiency. Additionally, screening for tuberculosis was negative. On examination, height was 124.5 cm (-0.6 SDS), target height 166 cm (-1.5 SDS) and weight 21 kg (-1.5 SDS). He had male external genitalia with no hypospadias, testicular volume of 2 ml bilaterally and absent pubic or axillary hair (Tanner stage 1). Blood pressure was 98 mmHg systolic and 66 mmHg diastolic. Hyperpigmentation of the skin was evident. There were no features suggestive of neurodegeneration, alacrima or achalasia, or other systemic features of note.

Serum biochemistry obtained at 8 years of age is presented in **Table 1**. His 8AM serum cortisol was within the lower normal range but was inappropriately low given the massively elevated ACTH. CT scan showed bilateral small adrenal glands. The patient was treated with oral hydrocortisone (7mcg/m^2/day) which led to significant improvement in general well-being and partial improvement in hyperpigmentation. Neither parent manifested signs or symptoms of adrenal insufficiency and both had a normal cortisol response to short standard Synacthen tests (father, 49 years of age, post Synacthen cortisol 28.4 mcg/dL; mother, 43 years of age, post Synacthen cortisol 25.9 mcg/dL; with a post Synacthen cortisol > 20 mcg/dl considered as an adequate response).

After 5 years of follow up (at age 13), the proband's testicular volume was 6 ml bilaterally, pubic hair stage Tanner P1, and his height was 142 cm (-1.85 SDS), indicating age appropriate physiological onset of puberty. On examination at the age of 16 years, he had progressed through puberty; his height was 167.5 cm (-0.80 SDS), weight 52.6 kg (-0.92 SDS), blood pressure 106/60 mmHg. His testicular volume was 20 ml on both sides, pubic hair tanner stage P3. His morning serum testosterone level was 6.98 ng/mL (normal adult range, 2.3 - 9.5 ng/mL), and serum luteinizing hormone (LH) (2.73 IU/mL), and follicle-stimulating hormone (4.71 IU/mL) were normal. Further follow up at the age of 17 years confirmed a testicular volume of 20 ml on both sides, and showed progressive hair growth with pubic hair Tanner stage 5, and axillary hair present. His height was 170.5cm (-0.66 SDS). He had adequate height gain during puberty, so that his height SDS

TABLE 1 | Biochemical investigations at initial presentation at 8 years of age.

	Value	Reference range
Serum Sodium	138 mEq/L	135-145 mEq/L
Serum Potassium	4.4 mEq/L	3.5-5 mEq/L
Serum Cortisol (8AM)	6 mcg/dL	6-23 mcg/dL
Plasma ACTH (Adrenocorticotropic hormone)	>2000 pg/mL	5-50 pg/mL
Serum DHEAS (Dehydroepiandrosterone Sulphate)	5.7 mcg/dL	23 - 209 mcg/dL
Plasma Renin activity	9.63 ng/mL/Hour	1.9- 5.2 ng/mL/Hour
Plasma Aldosterone concentration	121.8 pg/mL	12- 340 pg/mL

improved by the age of 17 years. He has a younger brother (10 years of age), who is asymptomatic and not hyperpigmented, and did not consent for hormonal or molecular testing.

Molecular Analysis

The proband was further evaluated for putative pathogenic variants in causal FGD genes and no variants were found in *MC2R* and *MRAP*. Further sequencing was performed on a HaloPlex targeted NGS panel (19) enabling concomitant detection of any variants in genes associated with PAI. Analysis showed that the proband had two heterozygous variants in STAR: chromosome 8:38003806C>T; c.465+1G>A and 8:38005729C>T; c.295G>A (rs903915397); p.(E99K) (GRCh37) (**Figures 1A, B**). Neither change has been previously reported in association with PAI and both are extremely rare. The c.465+1G>A change is not present in the gnomAD variant database, and the p.(E99K) change is very rare with only one heterozygous individual identified in gnomAD (minor allele frequency of 0.00003267 in South Asians). The c.465+1G>A variant was inherited from the boy's mother, and is a canonical splice site mutation at the junction of exon 4 and intron 4. This variant alters the canonical donor splice site of intron 4 and is likely to lead to the skipping of exon 4 with the predicted consequence for the protein being an in-frame deletion of 56 amino acids (p.103_155del). The other *STAR* variant, c.295G>A; p.(E99K) is predicted to be damaging by SIFT (score 0.01) and CADD (score 27.5). His father who is homozygous for this variant, did not manifest an adrenal phenotype suggesting at least some residual function. The patient also inherited a heterozygous change, rs6161, in *CYP11A1* [c.940G>A, p.(E314K)] from his mother (**Figures 1A, B**), which was recently shown to result in partially defective splicing thereby producing a non-functional protein (18, 20). A second variant in *CYP11A1* was not identified. No other variants in genes on the HaloPlex array and known to cause adrenal insufficiency were uncovered by this analysis. This combination of findings raises the possibility that a non-classical congenital (lipoid) adrenal hyperplasia is the cause of isolated glucocorticoid deficiency in

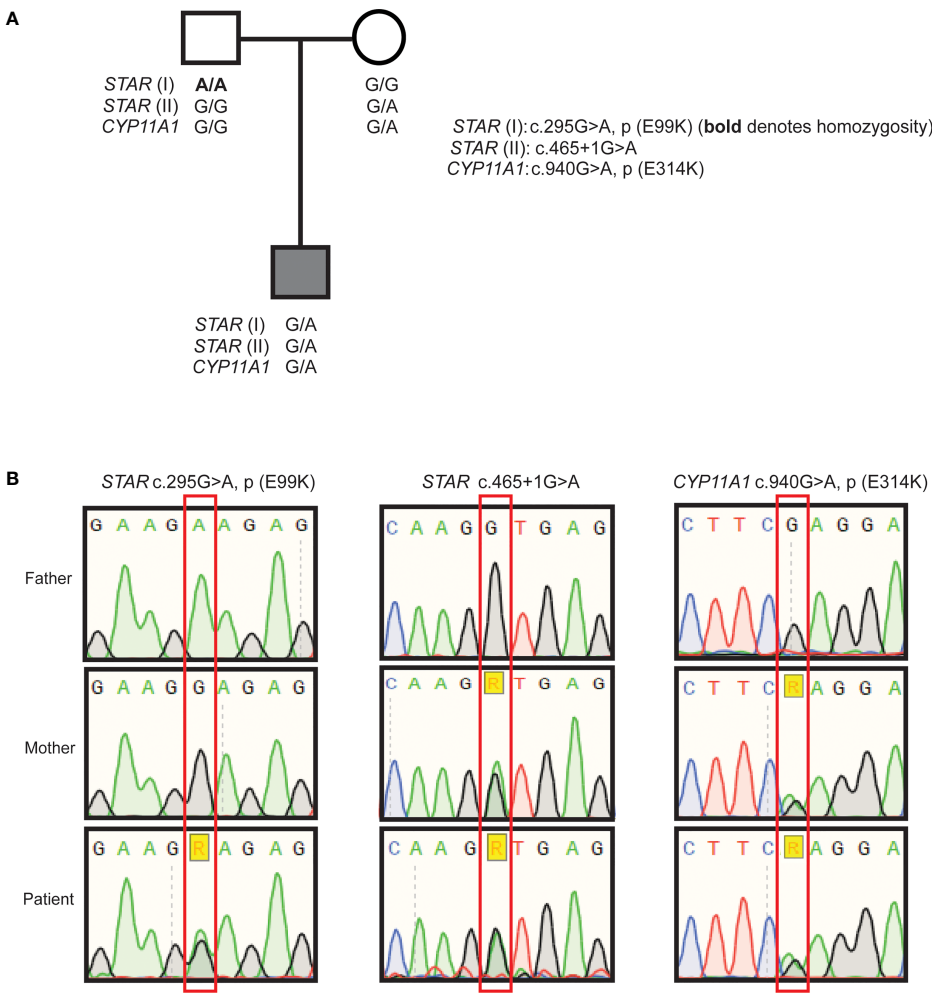

FIGURE 1 | Pedigree of the patient and Sanger sequencing results. Family pedigree indicating inheritance of the variants **(A)**, partial chromatograms from Sanger sequencing of *STAR* variants p.(E99K), c.465+1G>A, and *CYP11A1* rs6161; c.940G>A **(B)**.

this patient due to combined digenic, tri-allelic inheritance of two *STAR* mutations and the rs6161 *CYP11A1* variant.

DISCUSSION

This case describes the follow up of a boy with PAI, presenting as isolated glucocorticoid deficiency with small adrenals, possibly due to tri-allelic inheritance of two *STAR* mutations and the rs6161 *CYP11A1* variant. Although classically individuals with *STAR* mutations reportedly have large lipid laden adrenal glands in early life, there are reports of individuals with small adrenals, which may sometimes be due to the longer term effects of adrenal damage (21–23). In non-classic congenital lipoid adrenal hyperplasia, due to partial loss-of-function of *STAR*, the picture is mixed with both normal sized and hypoplastic adrenals described (14, 16, 24). For classic, loss-of-function mutations in *CYP11A1* large adrenal glands have not been described, but more often small or absent adrenals are noted (25) and - similar to *STAR* - partial loss-of-function variants of P450scc are associated with normal or small but never enlarged adrenal glands (18, 26, 27). Hence, whilst enlarged adrenals could have indicated a complete loss-of-function of *STAR*, normal or even small sized adrenals cannot rule out *STAR* or *CYP11A1* defects and give us no clue here to the underlying cause.

STAR mutations usually give rise to congenital lipoid adrenal hyperplasia but partial loss-of-function variants can present with an FGD-like phenotype of isolated glucocorticoid deficiency. Indeed, Baker et al. (16) described three FGD phenotype patients with STAR p.(V187M) and p.(R188C) mutations. Structural and functional analysis of these mutants revealed that they retained greater than 20% cholesterol binding activity. This residual activity may be the reason for the mild phenotype in these patients. Other studies also demonstrated that certain mutations in STAR [p.(R192C) and p.(R188C)] can present with a phenotype similar to that of FGD (13). In our patient, the *STAR* variant c.465+1G>A is predicted to lead to the skipping of exon 4 of the transcript with consequent in-frame deletion of 56 amino acids (p.103_155del) in the protein. This variant is likely to be loss-of-function. The second STAR variant p.(E99K) is predicted to be damaging, however the father of the patient was homozygous for this variant and did not manifest disease, suggesting only partial loss-of-function. Classical *CYP11A1* mutations also cause severe deficiency of both glucocorticoids and mineralocorticoids and no androgenization of 46,XY fetuses. Partial loss-of-function variants in *CYP11A1* can also give rise to a mild phenotype of a predominant FGD phenotype, with variable mineralocorticoid deficiency and often preserved testicular function (17). Indeed, the *CYP11A1* rs6161 variant found in our patient, which was initially considered to be benign and has a minor allele frequency in gnomAD of 0.002561 (0.001592 in South Asians), has recently been found to cause isolated glucocorticoid deficiency when combined with a severe loss-of-function *CYP11A1* defect, especially in patients from a European lineage (18, 20). This variant has been shown to have partial loss-of-function through mis-splicing and a reduced half-life of the protein P450scc (18, 28). We were unable to identify a second *CYP11A1* variant in the patient, suggesting a P450scc defect alone is not causal. We hypothesize that the combination of a null allele (p.103_155del) with a partial loss-of-function allele [p.(E99K)] in STAR along with reduced function of P450scc due to the *CYP11A1* variant results in the phenotype; in effect, that sub-optimal function of the STAR protein, with biallelic inheritance of the two variants, alongside haploinsufficiency of p450scc side chain cleavage (*CYP11A1*) can be enough to reduce steroidogenic output such that the patient has late onset glucocorticoid deficiency but preserved mineralocorticoid and gonadal steroid production. This would be akin to the situation seen with non-classical congenital lipoid adrenal hyperplasia due to *STAR* variants p.(R188C) & p.(R192C) (14, 16) and non-classic P450scc deficiency due to mutations such as p.(R451W), p.(L222P), c.940G>A (rs6161) & p.(A269V) (26, 27, 29).

This young man appeared to progress through puberty. Individuals with partial defects in P450scc can sometimes later develop abnormal germ cell function associated with FSH elevation and oligozoospermia and impaired sperm motility and development of testicular adrenal rest tumours (30). Similarly, men with partial loss-of-function variants in *STAR* often progress through normal puberty, but can later show signs of gonadal dysfunction (14). Even though our patient has progressed through puberty normally, he needs to be monitored for later development of gonadal dysfunction and possible reduction in other adrenocortical hormones including mineralocorticoids, highlighting the importance of making a specific diagnosis in individuals with PAI (31).

Limitations of the Study

In vitro functional studies to determine whether the combination of the three variants together reduce function to a level where steroidogenesis is not supported were not possible. To try and recapitulate even the effects of one variant in a transient or short-term cellular system only really gives a snapshot – hence trying to combine the effects of these variants in a biologically relevant system would be very challenging.

We cannot rule out the possibility that an, as yet, unidentified genetic cause of adrenal insufficiency is causative in this case but the finding of three variants in two known causal genes is persuasive that this could represent the first description of digenic, tri-allelic PAI.

DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article. Further inquiries can be directed to the corresponding authors.

AUTHOR CONTRIBUTIONS

NA clinically and biochemically evaluated the patient and wrote the first draft of the article. LM conceptualized the article. LM and AM did the molecular analysis. FB and JA contributed to the molecular evaluation and concept of the article. All authors contributed to manuscript revision, read and approved the submitted version.

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Susceptibility to Adrenal Crisis Is Associated With Differences in Cortisol Excretion in Patients With Secondary Adrenal Insufficiency

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Objective: To compare cortisol pharmacokinetics and pharmacodynamics mapped through several glucocorticoid sensitive pathways in patients on hydrocortisone substitution with or without an adrenal crisis.

Design: A *post-hoc* analysis of a previously conducted randomized controlled trial in patients with secondary adrenal insufficiency examining the effects of 2 weight-adjusted hydrocortisone doses.

Methods: Comparisons were primarily made on a hydrocortisone dose of 0.2-0.3 mg/kg/day for plasma cortisol and cortisone, 24-hour urinary steroid profile, the glucocorticoid sensitive tryptophan-kynurenine pathway, the renin-angiotensin-aldosterone system and aspects of quality of life. Variables of interest were also analyzed on the hydrocortisone dose of 0.4-0.6 mg/kg/day.

Results: Out of 52 patients, 9 (17%) experienced at least one adrenal crisis (AC+ group) and 43 did not develop an adrenal crisis (AC- group) during an observation period of 10 years. 24-hour urinary excretion of cortisol and cortisone were lower in the AC+ group (0.05 [IQR 0.03; 0.05] vs. 0.09 [0.05; 0.12] µmol/24h, P=0.01and 0.13 [0.10; 0.23] vs. 0.24 [0.19; 0.38] µmol/24h, P=0.04, respectively). No differences in pharmacokinetics of cortisol were observed. Kynurenine concentrations were higher in the AC+ group (2.64 [2.43; 3.28] vs. 2.23 [1.82; 2.38] µmol/L, P=0.03) as was general fatigue (Z-scores 1.02 [-0.11; 1.42] vs. -0.16 [- 0.80; 0.28], P=0.04). On the higher hydrocortisone dose urinary excretion of cortisol and cortisone was still significantly lower between the AC- and AC + group. The differences in glucocorticoid sensitive variables disappeared.

Conclusion: Patients susceptible to an adrenal crisis demonstrated differences in cortisol and cortisone excretion as well as in pharmacodynamics when compared to patients who did not experience an adrenal crisis, suggesting a biological predisposition in certain patients for the development of an adrenal crisis.

Keywords: biomarkers, adrenal crisis, pharmacokinetics, cortisol, hydrocortisone, kynurenine, susceptibility to adrenal crisis

1 INTRODUCTION

Adrenal insufficiency (AI) is characterized by loss of endogenous cortisol production. The pathophysiology is designated according to the level at which the hypothalamus-pituitary-adrenal gland (HPA)-axis is affected, i.e. tertiary (TAI), secondary (SAI) or primary insufficiency (PAI) for insufficiency at the level of the hypothalamus (i.e., CRH deficiency), pituitary gland (i.e., ACTH deficiency) or adrenal cortex, respectively (1).. Patients suffering from AI are at risk to develop an adrenal crisis (AC).

AC is an acute life- threatening medical situation which is caused by an absolute or relative cortisol deficiency (2, 3). With an incidence of approximately 5-10 per 100 patient-years it is not an infrequent medical emergency (2, 4–7). The risk of AC is higher in PAI than in SAI, probably because of some residual cortisol secretion in some patients with SAI and the lack of mineralocortioid secretion in PAI (6). Patients with TAI, which is most commonly due to glucocorticoid treatment, are at low risk to develop an AC, but precise data are not available (8). AC is a potentially life-threatening clinical condition and an important cause of death in patients with AI, with a mortality rate of 0.5 per 100 patient-years (9).

Previous studies have shown that patients with adrenal insufficiency have a 2-fold greater mortality rate compared to the background population. Main causes of death were cancer, cardiovascular and infectious disease, the latter being possibly driven by inadequate glucocorticoid exposure, especially at times of intercurrent illness (10). With only limited studies available, the mortality rate from AC is estimated to be 0.3-0.5/100 patient years (2, 5, 6, 11, 12).

Little is known which factors or vulnerabilities contribute to the development of an adrenal crisis. It is likely that the occurrence of an AC revolves around three distinctive pillars. Firstly, the type and severity of the event (e.g. infection, surgery, injury or mental stress) in relation to the patient's vulnerability as determined by general characteristics such as age and comorbidities (3, 6, 13). It has been shown that the risk of an AC was associated with older age and (bacterial) infections, especially gastro-intestinal infections in addition to comorbidities such as diabetes and asthma (2, 5, 6, 14). Furthermore, the etiology of the hypoadrenalism is of relevance, considering that patients with SAI experience less AC compared to patients with PAI. Secondly, patient education on sick day rules is widely believed to be of importance, although this is not without controversy because in a study by Hahner and colleagues perceived patient education on crisis prevention did not correlate with the frequency of an AC or emergency glucocorticoid administration (6). Nevertheless, patient information is widely implemented, and extensive education is nowadays recommended both by professional and patient organizations. Lastly, a biological predisposition related to the hypothalamus –pituitary-adrenal (HPA) axis may be of importance. This predisposition could at least in part explain why 20–44% of patients with adrenal insufficiency develop one or more episodes of AC during their life, while the remainder of patients will never experience such a crisis (4, 9, 15, 16). This could be related to variability in hydrocortisone pharmacokinetics or pharmacodynamics or potential residual (endogenous) cortisol production. Previously, we showed that half of the patients with secondary adrenal insufficiency (SAI) had detectable amounts of 11-deoxycortisol, a marker of residual adrenal cortisol production (17). In addition, slow and fast metabolizers were identified, with a 10-fold difference in plasma cortisol exposure between groups who received a similar weight adjusted hydrocortisone dose (18). However, because cortisol pharmacokinetics may not accurately reflect the effects of glucocorticoid receptor activation, it is of interest to investigate cortisol pharmacodynamics as well. This can be done, for instance, by evaluating the effect of hydrocortisone treatment on certain glucocorticoid sensitive pathways such as the renin-angiotensin-aldosterone system and the tryptophan-kynurenine pathway.

In order to gain more insight into the potential biological determinants of an adrenal crisis, we conducted an exploratory study comparing several aspects of cortisol pharmacokinetics and pharmacodynamics in patients with and without a history of an adrenal crisis.

2 MATERIALS AND METHODS

2.1 Study Population

We performed a *post-hoc* analysis of a previously conducted randomized, double-blind crossover study conducted at the University Medical Center Groningen, The Netherlands (ClinicalTrials.gov NCT01546922). An extensive description of the study design is available elsewhere (18). In short, patients with secondary adrenal insufficiency were selected from the outpatient clinic of the University Medical Centre Groningen. All patients suffered from SAI as a consequence of (treatment of) underlying pituitary disease. Inclusion criteria were subjects aged between 18 and 70 years, on stable hydrocortisone substitution or if applicable additional hormone substitutions for at least 6 months. Exclusion criteria were use of drugs interacting with hydrocortisone, diabetes mellitus, and any other major medical condition. The initial cohort of patients participating in the RCT comprised of 60 patients. For this exploratory analysis laboratory measurements were available in a total number of 52 patients. Comorbidities including medication were documented.

2.2 Protocol

Patients on cortisone acetate were converted to hydrocortisone 4 weeks prior to baseline measurements. After this run-in phase of 4 weeks, patients randomly received a lower (0.2-0.3 mg hydrocortisone/kg/day) followed by a higher (0.4-0.6 mg/kg/day) hydrocortisone dose or vice versa, both for 10 weeks. Hydrocortisone was given in three divided doses before meals (breakfast, lunch and dinner). In case of intercurrent illness or fever patients were advised to double or triple their HC dose according to a fixed protocol for a maximum of seven days.

On the visit days, patients were instructed to take their morning dose of hydrocortisone at 0700 h. At 0800 h they attended to the hospital and fasting blood samples were drawn in sitting position after a short period of rest for the measurement

of plasma total cortisol and plasma free cortisol, which was repeated approximately five hours later. These samples were used for pharmacokinetic analysis. One day before the hospital visit, they collected 24h urine used for steroid profiling and determination of cortisol and cortisone excretion.

Health-related quality of life measures were collected by daily diaries. We used the patient health questionnaire -15 (PHQ-15), general anxiety disorder 7 (GAD-7) and patient health questionnaire 9 (PHQ-9) and after each study period patients completed the Hospital Anxiety and Depression Scale (HADS), Rand 36-Item Health Survey (RAND-36) and Multidimensional Fatigue Inventory (MFI 20).

The study protocol was approved by the University Medical Center Groningen institutional review board. All patients provided written informed consent before participating in the study.

2.3 Outcome Parameters

We defined an adrenal crisis as an acute deterioration of a patient's general health, for which visit to the hospital was deemed necessary and with acute improvement after intravenous administration of glucocorticoids (2, 3). We included retrospectively every AC occurring during the observation period from 2009 to 2019. For every AC, we recorded prior dose adjustments (doubling or tripling the dosage hydrocortisone or cortisone acetate), whether or not hydrocortisone sodium succinate (Solu-Cortef Act-O-Vial®) was injected intramuscularly at home, in-hospital treatment (hydrocortisone dosage, admittance to the general ward or intensive care, length of hospital stay) and likely cause or precipitating factors. Furthermore, plasma concentrations of sodium and potassium were recorded as well as blood pressure and heart rate at the time of admission.

2.4 Laboratory Measurements

A detailed description of the urinary steroid profiling analysis in urine has been described elsewhere (18, 19). In short, urine samples were enzymatically hydrolysed, stable isotope labeled internal standards 11-keto-etiocholanolone -d5, dehydroepiandrosterone -d6, pregnenolone-d4 and tetrahydrocortisone-d5 were added and unconjugated steroids were extracted from urine by using solid phase extraction. The eluate was evaporated and a two-step derivatization was performed before analysis by gas chromatography in combination with tandem-mass spectrometry.

Plasma steroids and serum tryptophan, kynurenine, and 3-hydroxykynurenine concentrations were analyzed by a validated automated online solid-phase extraction–liquid chromatographic–tandem mass spectrometric method with deuterated internal standards, as previously described (20, 21). Plasma equilibrium dialysis for free cortisol was performed using a 10k cellulose membrane, to create CBG free plasma. The procedure was further performed as described by Fiers et al. (22). LC-MS/MS was applied for measurement of free cortisol as well as for measurement of 11-deoxycortisol, corticosterone and 11-deoxycorticosterone (17, 19).

Plasma renin concentration was measured with an immunoradiometric renin assay (Renin III Generation®; Cisbio). Aldosterone in serum was measured by LCMS/MS, essentially as described by Van der Gugten et al, but using additional online solid-phase extraction in combination with LC-MS/MS analysis (19, 23). The method was validated by evaluating imprecision, limit of quantification (LOQ), linearity, carryover, recovery, and ion suppression. Intrassay imprecision (n= 20 at one day) was < 5.6% (at 110, 288, and 1259 pmol/L), whereas interassay imprecision (n = 20 different days) was < 6% (at 110, 292, and 1260 pmol/L). Recovery was evaluated using by spiking three different samples with three increasing concentrations of aldosterone (92, 461 and 925 pmol/L). Recovery was found to be within 92 – 112%. LOQ was 19 pmol/L.

2.5 Pharmacokinetic Parameters

Pharmacokinetic parameters were calculated as described by Werumeus Buning et al. (18). In short, one-compartment and two-compartment population models for plasma total cortisol was calculated using the Kinpop Module of MwPharm version 3.81. The one-compartment model showed the best fit and was used for further analyses. Total body clearance (CL), volume of distribution (V_d), elimination half-life ($T_{½}$) and area under the curve in one hour (AUC_1) and 24 hours (AUC_{24h}) were calculated.

2.6 Glucocorticoid Sensitivity Altering Glucocorticoid Receptor Polymorphisms

We genotyped all patients for GR hypersensitive (1/2 copies BclI and/or N363S) and GR resistant (1/2 copies ER22/23EK and/or 9β) variants (24).

2.7 Statistics

Data were analysed using SPSS version 23.0. Normality of data was assessed by Q-Q plots. Normally distributed data is presented as mean (SD), non-normally distributed data is presented as median (IQR). Analysis of data was primarily performed when using the lower dose of hydrocortisone (i.e. 0.2-0.3 mg/kg/day) as this was considered to better reflect a state of relative hypocortisolism. A two-sided P value <0.05 was considered significant. Because of the exploratory character of this study all $P < 0.1$ were deemed of potential interest. These variables of potential interest were also tested in the higher dose condition (0.4-0.6 mg/kg/day). Differences in baseline characteristics, urinary cortisol, cortisone and steroid profiles, pharmacokinetic parameters and other laboratory measurements in patients with and without an AC were assessed by Mann-Withney U tests or $\chi 2$ tests, where appropriate.

3 RESULTS

3.1 Baseline Characteristics

In total, 52 patients were included in this study. During the timeframe 2009-2019, 9 (17%) of these patients suffered from at

least one AC. This group did not differ from the group without any adrenal crisis in age or sex, nor in educational level, age at diagnosis, duration of substitution therapy, BMI or type and dosage of glucocorticoid treatment (**Table 1**). Out of 52, 13 (25%) patients were treated with medication that interfered with the renin-angiotensin-aldosterone system (**Table 1**).

3.2 Adrenal Crisis

There were 9 (17%) patients with in total 11 (range 1-3) adrenal crisis. This corresponds to 1.7 crisis per 100 patient-years at risk. All the patients were admitted to the regular ward. The average duration of the hospital stay was 2 days with maximum duration of 7 days. All patients had normal electrolytes during admission and were hemodynamically stable (**Table 2**). 5 out of 11 patients had doubled their hydrocortisone dosage during illness. None of the patients had tripled their hydrocortisone. In 4 out of 11 cases, patients had received an intramuscular emergency administration of glucocorticoids at home. The cause of the AC was an infection in 9 out of 11 of the crises.

At the lower hydrocortisone dose (0.2-0.3 mg/kg per day)

3.3 Plasma and Urinary Concentrations of Steroids and Pharmacokinetics

3.3.1 Plasma Cortisol, Cortisone and CBG

Plasma levels of cortisol and cortisone one hour and five hours after ingestion of hydrocortisone did not differ between the AC + group and the AC- group (**Table 3**). Cortisol binding globulin (CBG) levels were also not different between groups.

3.3.2 Pharmacokinetic Parameters

The pharmacokinetic parameters CL, V_d, $T_{½}$, C_{max1}, AUC_1 and AUC_{24h} of both plasma free cortisol and total cortisol did not significantly differ between the two groups (**Table 3**).

3.3.3 Steroid Precursors of Cortisol and Aldosterone

11-deoxycortisol, corticosterone and 11-deoxycorticosterone did not significantly differ among the two groups (data not shown, subset analysis in 17 versus 4 patients). 24-hour urinary excretion of tetrahydro-11-deoxycortisol (THS) was lower in AC+, albeit not significant (0.1 [0.0; 0.1] vs. 0.0 [0.0; 0.1] μmol/24h, P=0.33)

3.3.4 Urinary Steroid Metabolites

The AC+ group had a lower urinary excretion of cortisol and cortisone (0.05 [IQR 0.03; 0.05] vs 0.09[0.05; 0.12] μmol/24h, P =0.01 and 0.13 [0.10; 0.23] vs 0.24 [0.19; 0.38] μmol/24h, P=0.04, **Table 4** and **Figure 1**).

Urinary excretion of metabolites of cortisol and cortisone did not differ significantly among the two groups (**Table 4** and **Figure 1**).

3.4 Glucocorticoid Sensitive Pathways

3.4.1 The Tryptophan-Kynurenine Pathway

Levels of kynurenine in serum were significantly higher in the AC + group (2.64 [2.43; 3.28] vs. 2.23 [1.82; 2.38] μmol/L, P = 0.03). In addition, serum levels of 3-hydroxykynurenine and the ratio kynurenine/tryptophan tended to be higher in the AC+

TABLE 1 | Clinical characteristics of patients with secondary adrenal insufficiency with or without a history of an adrenal crisis.

	Total patient group (n = 52)	Adrenal Crisis - (n = 43)	Adrenal Crisis + (n = 9)	P-value
Age (years), median [IQR]	54 [43; 61]	55 [45; 61]	54 [23; 64]	0.70
Sex (males/females), n	30/22	25/18	5/4	0.91
Educational level (1/2/3/4/5/6/7) ♦	0/2/1/7/24/16/2	0/2/1/6/19/13/2	0/0/0/1/5/3/0	0.85
Age at diagnosis (years), median [IQR]	33 [21; 46]	34 [22;47]	22 [17; 52]	0.67
Duration of adrenal insufficiency (years)	18.2 [11.4; 27.9]	18.4 [11.5; 32.0]	14.6 [8.0; 20.5]	0.16
Childhood onset/Adult onset of SAI, n	7/45	5/38	2/7	0.59
Body weight (kg), median [IQR]	85.7 [72.6; 93.7]	86 [72; 94]	85 [70; 94]	0.63
BMI (kg/m²), median [IQR]	26.8 [24.5; 30.1]	26.7 [24.5; 29.9]	27.1 [24.2; 31.9]	0.65
Systolic blood pressure (mmHg) median [IQR]	135 [126; 147]	136 [127, 147]	126 [117; 156]	0.54
Diastolic blood pressure (mmHg), median[IQR]	78 [70; 86]	78 [70; 85]	79 [67; 93]	0.57
eGFR (CKD-EPI, ml/min/1.73m²), median [IQR]	82 [69; 95]	81 [69; 95]	88 [67; 99]	0.57
No. of hormonal replacement (1/2/3/4/5)	3/10/23/13/3	3/8/20/10/2	0/2/3/3/1	0.73
Thyroid hormone (yes/no)	48/4	39/4	9/0	1.0
Growth hormone (yes/no)	24/28	19/24	5/4	0.72
Sex hormone (yes/no)	29/23	24/19	5/4	1.0
Desmopressin (yes/no)	11/41	8/35	3/6	0.38
Comorbidities				
Diabetes mellitus (type 2/no)	2/50	1/42	1/8	0.32
Asthma/COPD and using inhalation corticosteroids (yes/no)	5/47	4/39	1/8	1.00
Most recent glucocorticoid treatment				
Total daily dose (mg/day), median [IQR]*	25 [20; 30]	25 [20; 30]	30 [20; 37.5]	0.26
Drug (hydrocortisone/cortisone acetate)	35/17	29/14	6/3	0.98
Educated in sick day rules (yes/no)	52/0	43/0	9/0	1.00

♦ *Educational level was classified using a Dutch education system, comparable to the International Standard Classification of Education (ISCED). This scale ranges from 1 (elementary school not finished) to 7 (university level). *Relative potency cortisone acetate: 0.8.*

IQR, interquartile range; SAI, secondary adrenal insufficiency; BMI, body mass index; eGFR, estimated glomerular filtration rate; COPD, chronic obstructive pulmonary disease.

TABLE 2 | Adrenal crisis details.

		Adrenal crisis (n = 11)
Number of crisis per patient, median [min, max]		1 [1;3]
Glucocorticoid dose adjustment at home		
Double dose (yes/no)		5/6
Triple dose (yes/no)		0/11
Emergency glucocorticoid administration i.m. given at home? (yes/no)		4/7
Type of hospital admission (regular ward/intensive care)		11/0
Duration of hospital stay (days), median [min, max]		2 [1; 7]
Precipitating factors		
Infection		9
	Airway/gastrointestinal/other	1/2/4
	Positive culture PCR (yes/no)	4/5
Syncope		1
Medication non-adherence		1
Findings on admission		
Systolic blood pressure (mmHg), median [IQR]		129 [119; 131]
Diastolic blood pressure (mmHg), median [IQR]		76 [70; 81]
Sodium (mmol/L), median [IQR]		142 [140; 143]
Potassium (mmol/L), median [IQR]		4.1 [3.6; 4.3]

BPM, beats per minute; IQR, interquartile range; PCR, polymerase chain reaction.

group (0.04 [0.03; 0.05] vs. 0.05 [0.04; 0.08] µmol/L and 0.03 [0.03; 0.04] vs. 0.04 [0.03; 0.05], both P = 0.06; **Table 5**). None of these analytes were different between the two groups when measured during treatment with the higher hydrocortisone dose (**Table 7**).

3.4.2 Plasma Renin and Aldosterone

There was a trend towards significant for differences in plasma levels of renin in the AC + group (10.75 [6.56; 17.73] pg/mL vs. 16.40 [11.95; 44.60] pg/mL, P=0,09; **Table 5**). Levels of aldosterone did not differ significantly between the AC+ group and the AC- group.

3.4.3 Plasma Metanephrines

Levels of normetanephrine, metanephrine and 3-methoxytyramine did not significantly differ among the two groups (**Table 5**).

3.4.4 Quality of Life Questionnaires

Patients in the AC + group reported significantly more general fatigue (Z-score -0.16 [-0.80; 0.28] vs. 1.02 [-0.11; 1.42], P = 0.04). Levels of physical functioning did not differ among the two groups. There was a trend for more pain and anxiety in the AC + group (Z-score -0.39 [-0.74; 0.05] vs -0.01 [-0.45; 0.91], P = 0.08; Z score -0.58 [-1.14; -0.49] vs. 0.11 [-0.57; 0.74], P = 0.06, **Table 6**).

TABLE 3 | Pharmacokinetic parameters of free cortisol and total cortisol as well as plasma concentrations of cortisol, cortisone and CBG on the lower dose hydrocortisone (0.2-0.3 mg/kg/day) in patients with or without a history of an adrenal crisis.

		Adrenal crisis - (n = 37)	Adrenal crisis + (n = 8)	P value
Total cortisol				
	CL (L/H)	11.7 [8.0; 17.4]	12.6 [8.4; 17.1]	0.69
	V_d (L)	33.7 [26.0; 46.6]	29.8 [22.2; 50.1]	0.76
	$T_{½}$ (h)	1.82 [1.43; 3.05]	1.68 [1.30; 2.47]	0.33
	AUC_{24h} (h*nmol/L)	3956.1 [2791.9; 5364.0]	3539.8 [2969.9; 5066.4]	0.61
Free cortisol				
	CL (L/H)	220.6 [168.2; 334.9]	245.0 [157.1; 364.4]	0.87
	V_d (L)	422.3 [315.9; 627.3]	439.6 [234.7; 746.5]	0.90
	$T_{½}$ (h)	1.20 [1.00; 1.65]	1.42 [0.83; 1.87]	0.78
	AUC_{24h} (h*nmol/L)	217.2 [144.7; 260.2]	188.9 [132.0; 267.9]	0.85
Cortisol level 1h after HC ingestion		498.6 [387.4; 597.8]	516 [341.8; 663.5]	0.73
Cortisone level 1h after HC ingestion		64.8 [52.2; 69.8]	64.4 [55.9; 73.0]	0.90
Cortisol level 5h after HC ingestion		121.7 [76.1; 243.5]	110.9 [68.1; 164.1]	0.46
Cortisone level 5h after HC ingestion		30.6 [21.1; 41.1]	26.9 [19.8; 33.2]	0.51
CBG (ug/mL)		53 [49; 64]	55 [46; 61]	0.68

Data are median [interquartile range].

Cortisol, cortisone levels are nmol/L in plasma.

CL, total body clearance; V_d, volume of distribution; $T_{½}$, elimination half-life; AUC 24h, 24 hour area under the curve; HC, hydrocortisone; CBG, cortisol binding globulin.

Data are on treatment with the lower dose of hydrocortisone (0.2-0.3mg/kg).

*h*nmol/L stand for hours times concentration, the unit of area under the curve. The asterix does not represent anything else.*

TABLE 4 | Urinary steroids and metabolites in patients with and without adrenal crisis on the lower dose hydrocortisone (0.2-0.3 mg/kg/day).

	Adrenal crisis - (n = 43)	Adrenal crisis + (n = 9)	P value
Total glucocorticoids	36.66 [25.84; 51.80]	33.61 [22.85; 53.09]	0.76
Active compounds			
Cortisol	0.09 [0.05; 0.12]	0.05 [0.03; 0.05]	0.01
Cortisone	0.24 [0.19; 0.38]	0.13 [0.10; 0.23]	0.04
Metabolites			
THS	0.05 [0.03; 0.11]	0.03 [0.01; 0.11]	0.33
THE	10.64 [7.10; 14.97]	8.92 [7.52; 13.53]	0.80
THF	9.72 [6.36; 12.33]	5.28 [4.83; 9.44]	0.09
allo-THF	9.86 [6.55; 14.09]	10.21 [5.73; 24.28]	0.76
α-CTLN	3.37 [2.73; 4.35]	2.89 [1.85; 5.38]	0.54
β-CTLN	2.28 [1.54; 3.38]	2.11 [1.80; 3.69]	0.97
Enzymes			
11βHSD type 1	1.90 [1.68; 2.29]	1.60 [1.33; 2.52]	0.85
11βHSD type 2	0.33 [0.24; 0.44]	0.33 [0.16; 0.52]	0.85
5α-reductase	0.89 [0.75; 1.22]	0.87 [0.41; 0.94]	0.44

Data are median [Interquartile range]. Units are µmol/24h.

THS, tetrahydro-11-deoxycortisol; THE, tetrahydrocortisone; THA, tetrahydro-11-dehydrocorticosterone; THB, tetrahydrocorticosterone; 5α-THB, 5α tetrahydrocorticosterone; THF, tetrahydrocortisol; allo-THF, allo-tetrahydrocortisol; α-CTLN, α-cortolone; β-CTLN, β-cortolone.

11βHSD type 1; (THF + allo-THF)/THE, 11βHSD type 2; Cortisol/cortisone; Total glucocorticoid metabolite excretion, THF + allo-THF + THE + α-CTLN + β-CTLN; 5α-reductase: allo-THF/THF.

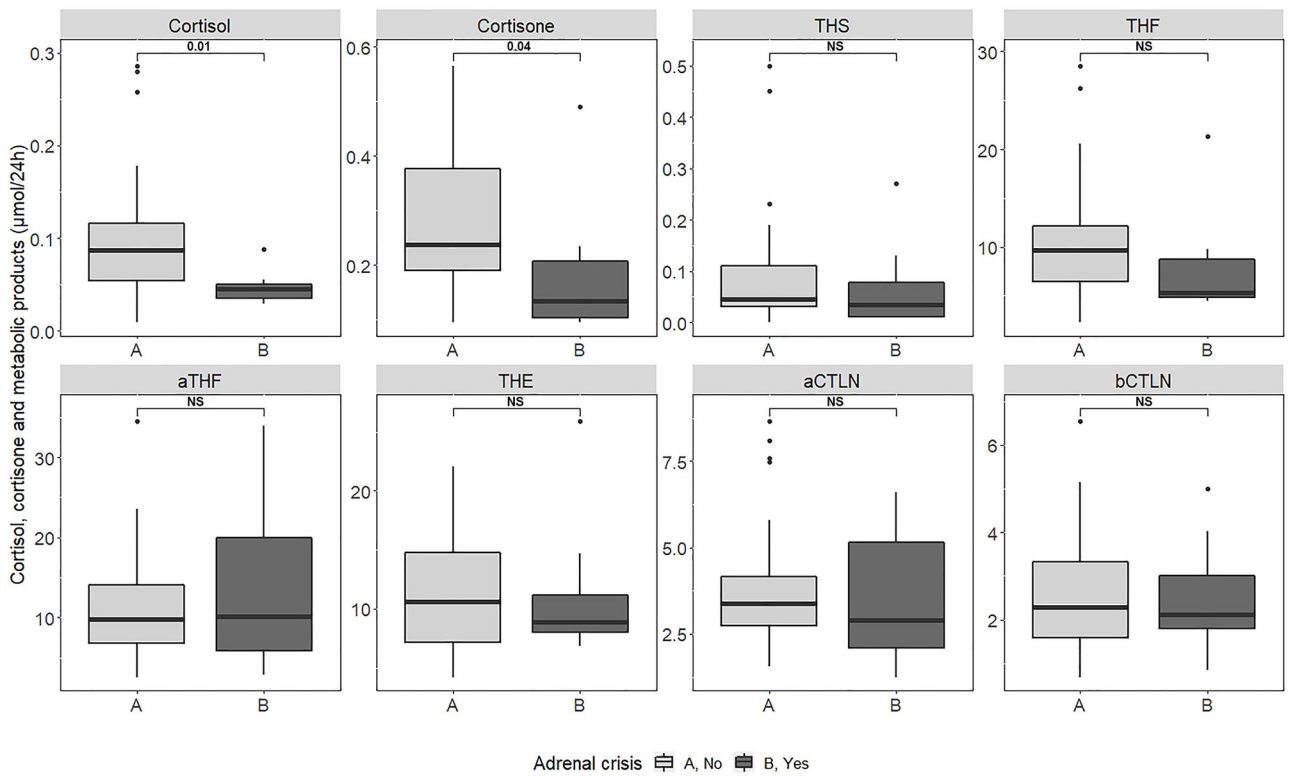

FIGURE 1 | Cortisol and cortisone and metabolic products excretion in 24h urine collections in patients with and without a past history of adrenal crisis. NS, not significant.

TABLE 5 | Total serum levels of tryptophan, kynurenine, and 3-hydroxykynurenine, kynurenine to tryptophan ratio, aldosterone, renin and metanephrines in patients on the lower dose hydrocortisone (0.2-0.3 mg/kg/day) with or without a history of an adrenal crisis.

	Adrenal crisis -	Adrenal crisis +	P value
Tryptophan-kynurenine metabolism	(n=39)	(n=7)	
Tryptophan (μmol/L)	71.07 [58.75; 83.49]	74.82 [58.53; 80.95]	0.72
Kynurenine (μmol/L)	2.23 [1.82; 2.38]	2.64 [2.43; 3.28]	0.03
3-Hydroxykynurenine (μmol/L)	0.04 [0.03; 0.05]	0.05 [0.04; 0.08]	0.06
Kynurenine/tryptophan ratio	0.03 [0.03; 0.04]	0.04 [0.03; 0.05]	0.06
Renin-angiotensin-aldosterone system	(n=38)	(n=6)	
Aldosterone (pmol/L)	135.00 [67.00; 263.00]	200.50 [159.25; 330.25]	0.16
Renin (pg/mL)	10.75 [6.56; 17.73]	16.40 [11.95; 44.60]	0.09
Aldosterone/renin ratio (pmol/ng)	12.20 [3.24; 21.25]	12.26 [7.24; 17.43]	0.98
Use of RAAS interfering medication (yes/no)	13/30	4/5	0.45
Potassium (mmol/L)	3.90 [3.65; 4.00]	3.90 [3.75; 4.15]	0.44
Adrenal medulla activity	(n=39)	(n=6)	
Normetanephrine (nmol/L)	0.61 [0.49; 0.84]	0.59 [0.44; 0.69]	0.46
Metanephrine (nmol/L)	0.13 [0.10; 0.20]	0.16 [0.09; 0.20]	0.79
3-Methoxytyramine (nmol/L)	0.03 [0.02; 0.04]	0.03 [0.02; 0.04]	0.71

Data are median [interquartile range].
Data are on treatment with the lower dose of hydrocortisone (0.2-0.3 mg/kg/day).
RAAS interfering medication: angiotensin-converting enzyme inhibitors, angiotensin receptor blockers, betablockers.
RAAS, renin angiotensin aldosterone system.

3.5 Glucocorticoid Sensitivity-Altering Glucocorticoid Receptor Polymorphisms

There was no association between the GR polymorphisms (typed as GR hypersensitive, resistant or wild-type variants) and the occurrence of an AC (data not shown).

3.6 At the Higher Hydrocortisone Dose (0.4-0.6 mg/kg per Day) for Variables of Interest With P<0.1 at the Lower Hydrocortisone Dose

Serum concentrations of kynurenine did not differ between the two groups at the higher dose of hydrocortisone. Aldosterone was significantly higher in the AC+ group (91.5 [<0.4; 218.5] vs. 249.0 [115.0; 337.0] pmol/L, P = 0.04), but renin was not. Urinary cortisol and cortisone remained lower in the AC+ group (0.31 [0.23; 0.43] vs. 0.18 [0.11; 0.26] μmol/24h, P = 0.01 and 0.48 [0.38; 0.71] vs. 0.32 [0.22; 0.38] μmol/24h, <0.01).

Perceived pain, general fatigue and anxiety did not differ between patients with or without an AC (**Table 7**).

4 DISCUSSION

To the best of our knowledge, this is the first study to investigate a biological predisposition for the risk of an adrenal crisis by means of extensive examination of cortisol pharmacokinetics in combination with several glucocorticoid sensitive pathways in a well-defined cohort of patients with secondary adrenal insufficiency. Patients with a history of an adrenal crisis could be distinguished from those with a negative history by a lower urinary excretion of cortisol and cortisone and alterations in glucocorticoid sensitive pathways like the tryptophan-kynurenine pathway and the level of general fatigue. Collectively, the emerging picture is compatible with a reduced efficacy of hydrocortisone substitution treatment at the usually recommended lower maintenance doses in patients at risk for developing an adrenal crisis.

The AC+ group demonstrated lower urinary cortisol and cortisone excretion compared to the AC-group at both the lower and higher hydrocortisone substitution dose. We did not find differences in pharmacokinetic parameters such as clearance,

TABLE 6 | Perceived pain, fatigue, physical functioning and mood in patient with and without adrenal crisis on the lower dose hydrocortisone (0.2-0.3 mg/kg/day).

	Adrenal crisis - (n = 38)	Adrenal crisis + (n = 8)	P value
PHQ-9			
Pain	-0.39 [-0.74; 0.05]	-0.01 [-0.45; 0,91]	0.08
MFI-20			
General Fatigue	-0.16 [-0.80; 0.28]	1.02 [-0.11; 1.42]	0.04
RAND-36			
Physical functioning	0.42 [-0.12; 0.53]	0.20 [-0.07; 0.31]	0.23
HADS			
Anxiety	-0.58 [-1.14; -0.19]	0.11 [-0.57; 0.74]	0.06
Depression	-0.12 [-0.73; 0.79]	0.18 [-0.66; 1.62]	0.66

Data are represented in Z-scores.
Data are median [interquartile range]. PHQ-9, Physical Health Questionnaire 9; MFI20, multidimensional Fatigue Index-20; RAND-36, 36-Item Short Form Health Survey; HADS, Hospital Anxiety and Depression Scale.

TABLE 7 | Urinary glucocorticoid excretion, tryptophan-kynurenine metabolism, renin-angiotensin-aldosterone system and self-reported aspects of quality in life in patients on the higher dose hydrocortisone (0.4-0.6 mg/kg/day).

	Adrenal crisis -	Adrenal crisis +	P value
Urinary glucocorticoid excretion	(n=38)	(n=7)	
24h Urinary cortisol (µmol/24h)	0.31 [0.23; 0.43]	0.18 [0.11; 0.26]	0.01
24h Urinary cortisone (µmol/24h)	0.48 [0.38; 0.71]	0.32 [0.22; 0.38]	<0.01
THS (µmol/24h)	0.05 [0.03; 0.08]	0.05 [0.02; 0.09]	0.79
THF (µmol/24h)	21.91 [15.70; 25.56]	21.60 [15.37; 27.42]	0.87
Tryptophan-kynurenine metabolism	(n=38)	(n=7)	
Serum kynurenine (umol/L)	1.89 [1.52; 2.45]	2.05 [1.53; 3.18]	0.57
Serum 3-hydroxykynurenine (umol/L)	0.03 [0.02; 0.05]	0.04 [0.04; 0.07]	0.16
Serum kynurenine/tryptophan ratio	0.03 [0.02; 0.03]	0.02 [0.02; 0.03]	0.59
Renin-angiotensin-aldosterone system	(n=38)	(n=7)	
Serum aldosterone (pmol/L)	91.5 [<0.4; 218.5]	249.0 [115.0; 337.0]	0.04
Plasma renin (pg/mL)	8.63 [5.89; 14.78]	22.55 [5.93; 38.72]	0.23
Potassium (mmol/L)	3.70 [3.60; 3.90]	3.90 [3.75; 4.10]	0.03
Reported symptoms	(n=38)	(n=8)	
Pain, z-score	-0.41 [-0.71; 0.12]	-0.24 [-0.55; 0.58]	0.32
General fatigue, z-score	0.01 [-0.75; 0.98]	-0.66 [-1.14; 0.26]	0.17
Anxiety, z-score	-0.53 [-1.14; -0.03]	-0.02 [-0.58; 1.08]	0.18

Data are represented in Z-scores. Data are median [interquartile range].
Variables of interest were selected based on P<0.1 (+ aldosterone) on lower dose hydrocortisone treatment.
THS, tetrahydro-11-deoxycortisol; THF, tetrahydrocortisol.

volume of distribution or elimination half-life of cortisol between these groups. In addition, the urinary excretion profile of metabolites of cortisol and cortisone and their precursors were also comparable in either group. We speculate that the renal excretion of cortisol between the AC+ group and the AC- group possibly explains the differences, but further research should be done to confirm this. The precise mechanism of our findings remains therefore elusive, as we did not find any differences in either the estimated glomerular filtration rate or the enzymatic activity of 11β-hydroxysteroid dehydrogenase type 2.

The pharmacodynamic part of our study showed that the serum kynurenine concentration was significantly higher in the AC+ compared to the AC- group. The first step of the kynurenine pathway is conversion of tryptophan in N′-formylkynurenine, which consecutively becomes hydrolyzed into kynurenine (25). This process involves two enzymes, i.e. tryptophan 2,3-dioxygenase (TDO) and indoleamine 2,3-dioxygenase (IDO). IDO is activated by pro-inflammatory cytokines and reactive oxygen species (ROS). Induction of TDO by activation of the glucocorticoid receptor has been shown in several studies (25–29). These studies were predominantly performed in animals and examined the effect of a single dose of very potent glucocorticoids. However, in prior research we demonstrated an inverse relationship between hydrocortisone dose and serum kynurenine concentration after a treatment period of 10 weeks in study patients of the current RCT (21). Therefore, serum kynurenine could potentially serve as an index of glucocorticoid sensitivity. The elevated level of serum kynurenine in the AC +-group somehow suggests a reduced hydrocortisone efficacy in these patients, which cannot be ascribed to differences in plasma cortisol levels. However, the responsible pharmacodynamic mechanism underlying this lower cortisol sensitivity in the AC+ group remains speculative. On the higher hydrocortisone dose, neither serum kynurenine concentration nor quality of life variables were different between the AC+ and AC- group, suggesting the AC+ group requires a higher substitution dose to compensate for the apparent lower cortisol sensitivity.

Alternative hypotheses to explain differences in the occurrence of adrenal crisis are insufficient education on sick-day rules including self-administration of intramuscular hydrocortisone injections or residual endogenous production of cortisol. Regarding patient education, it should be noted that participants in this study all had received extensive and uniform instructions on sick-day rules during the time of the randomized controlled trial. In addition, during this 20-week study both the AC+ and AC- group did not differ in application of doubling or tripling of the hydrocortisone dose (data not shown), emphasizing that our groups were comparable at this point. Furthermore, with regard to residual cortisol production, we did not find differences in plasma concentration of 11-deoxy-cortisol and urinary THS, although these data must be interpreted with caution as this measurement was not available in all study subjects (30).

We also included aldosterone and renin in our analysis as markers of secondary activation of the RAAS as this was previously shown to be responsive to the hydrocortisone dose and may reflect glucocorticoid mediated mineralocorticoid receptor (MR) activation (19). We found in the AC+ group a trend for higher renin activity at the lower dose, and significantly higher aldosterone at the higher dose. These results suggest increased RAAS activation in AC+ group, secondary to reduced hydrocortisone mediated MR activation at the same dose as the AC- group. However, this should be interpreted with caution as blood pressure between groups was similar and group size was small.

On the higher dose, urinary cortisol and cortisone excretion remained lower in the AC+ group. However, significant differences in the kynurenine pathway and associated quality of life parameters disappeared on the higher dosage of HC. A

possible explanation for this phenomenon could be, that the group who has proved to be vulnerable for an AC is actually undersubstituted. This group might need a higher hydrocortisone dosage, which is not reflected in lower plasma cortisol concentrations in serum or pharmacokinetic parameters but resonates in glucocorticoid sensitive pathways as an expression of shortage of hydrocortisone at the receptor level.

The strength of this study is the high-quality data within the framework of a randomized controlled trial. This trial provides extensive phenotyping of a homogenous group of SAI patients on a standardized dose of hydrocortisone. In addition, we analysed the urine samples using a GC-MS/MS method, which provides higher analytical specificity compared to the previous GC/MS method (31). Furthermore, we used a state-of-the-art LC-MS/MS method to measure analytes in the kynurenine pathway (20).

A few limitations need to be addressed. First, as stated above, this study is hypothesis generating. Further studies should be specifically designed to address our research question. Secondly, as a consequence of retrospective data retrieval on AC, there were some missing data concerning hospital admission. Furthermore, due to the retrospective study design, for some analysis, there was not enough biomaterial available. Therefore not all measurements could be performed in every study participant. In addition, we did not include all cortisol metabolites (e.g. α-cortol) into our analysis. Moreover, our study encompassed only 9 individuals with a past history of one or more AC. This relatively small number not only limited the statistical power but might also have increased the risk of 'false positive' findings, as a result of coincidental outliers. It should be noted, however, that all glucocorticoid variables were assessed under the strictly defined conditions of a randomized controlled trial. In addition, our findings also fit into a plausible pathophysiological model of potentially reduced glucocorticoid bioavailability and sensitivity in patients prone to develop AC.

In conclusion, this study suggests that patients at risk for an adrenal crisis demonstrate differences in urinary excretion of cortisol and cortisone, as well as in glucocorticoid sensitivity, hinting to a biological predisposition for an adrenal crisis in these patients. Prospective studies are needed to elucidate whether these characteristics will identify patients at high risk for an adrenal crisis and if this risk can be reduced successfully by interventions directed at normalization of these alterations.

DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/supplementary material. Further inquiries can be directed to the corresponding author.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by METc Groningen. The patients/participants provided their written informed consent to participate in this study.

AUTHOR CONTRIBUTIONS

Participated in research design: AB and AV. Participated in the writing of the paper: AV, AB, MF, and MK. Participated in the performance of the research: AV, AB, MF, and MK. Contributed new reagents or analytic tools: MF. Participated in data analysis: AV, AB, MF, and MK. All authors contributed to the article and approved the submitted version.

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MINI REVIEW
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Daily Glucocorticoid Replacement Dose in Adrenal Insufficiency, a Mini Review

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The Endocrine Society Guidelines and recent reviews of adrenal insufficiency (AI) recommend a daily glucocorticoid replacement dose of 15 to 25 mg with a midpoint of 20 mg of hydrocortisone (HC) (alternatively 3 to 5 mg prednisolone) in divided doses in otherwise healthy individuals with AI. In contrast, a daily glucocorticoid replacement dose of 4.3 to 26 mg/d HC with a midpoint of 15 mg/d is predicted from current measurements of daily cortisol production rates and oral HC bioavailability. The higher HC doses recommended in the current guidelines may result in glucocorticoid overtreatment of some AI patients and associated long-term adverse outcomes. A titration method for determination of the individual patient's daily glucocorticoid replacement dose and the impact of lower doses are reviewed. Future related research questions are identified.

Keywords: adrenal insufficiency, glucocorticoid, Hydrocortisone, replacement, guidelines, cortisol

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INTRODUCTION

The Endocrine Society guidelines and a recent review in The Lancet both recommend 15 to 25 mg/d of hydrocortisone (HC) or its equivalent in divided doses as the daily glucocorticoid replacement dose in otherwise healthy adults with primary adrenal insufficiency (AI) (1) and with either primary or secondary AI (2). As alternatives, the Endocrine Society suggests either prednisolone (3 to 5 mg) or cortisone acetate (20 to 35 mg) in divided doses. This focused review 1) examines the assertions/assumptions of these guidelines and predicts a broader range and lower midpoint of the daily glucocorticoid replacement dose, 2) summarizes potential complications of overtreatment, 3) presents a pragmatic down titration approach for individualizing the daily glucocorticoid replacement dose, 4) reviews the outcomes of individualized doses, and 5) proposes potential new research questions.

THE DAILY GLUCOCORTICOID REPLACEMENT DOSE

Assertions and Assumptions of the Current Recommendations

Although there are multiple reviews of AI, we have chosen the nearly identical recommendations for the daily glucocorticoid replacement dose from the Endocrine Society Guidelines (1) and those from a recent The Lancet publication by Husebye, et al. (2) as representative of views across Western economies. The Endocrine Society Guidelines address only primary AI (1) while Husebye's treatment recommendations apply to both primary and secondary AI (2). Both recommend a HC dose of 15 to 25 mg/d (1, 2). These recommendations can be derived from the following assumptions/assertions. 1)

The daily glucocorticoid replacement dose equals the daily cortisol production rate (DCPR); 2) the mean DCPR is 7.0 mg/m^2; 3) the individualized daily glucocorticoid replacement dose should reflect the range of mean DCPR from different studies (5 mg/m^2 to 8 mg/m^2); 4) the oral HC bioavailability is about 70 percent; and 5) the DCPR is applied to a male of average height and ideal body weight (175 cm, 73 kg, 1.9 m^2 BSA by the Mosteller formula). The last three of these assumptions/assertions are subject to modification or challenge.

Alternative Predictions of the Daily Glucocorticoid Replacement Dose

Table 1 summarizes the assertions/assumptions used in the current recommendations and compares this to our interpretation of current literature. Without data to the contrary, the unproven assumption that cortisol replacement should equal production is accepted. There is general agreement that the DCPR is accurately measured by a 30-hour stable isotope dilution methodology or by deconvolution analysis, and the mean DCPR in the largest of these studies is 7.0 mg/m^2 (3). Earlier measurements suggested that the DCPR was about 12-15 mg/m^2, and this estimate led to daily HC replacement dose recommendations of about 20 to 30 mg (4). Estaban et al, review the methodologic errors that led to overestimates (5). Some studies used radioactive isotope dilution methodology of brief duration in the morning when cortisol production is at its highest (5, 6). As anticipated, lower DCPR were observed in studies that lasted for ≥ 24 h. Using deconvolutional analysis of serum cortisol measurements at 20 m intervals for 24 h, Kerrigan et al. reported a DCPR of 5.3 ± 0.5 mg/m^2 in 9 Tanner stage IV and V pubertal males and 6.1 ± 0.4mg/m^2 in 9 Tanner stage I and II pubertal males (7). Using the 31-hour stable isotope dilution/mass spectrometry methodology in a group of 12 normal volunteers (mean age = 28 y), Esteban reported a mean DCPR of 5.7 mg/m^2 (5). Using this same technique in a larger group of adult men (n = 24) and women (n = 30) ranging from age 19 y to 70 y, Purnell, et al. observed a some DCPR of 7.0 (range = 2.7-14) mg/m^2. A positive correlation between age and DCPR may account for this latter DCPR estimate exceeding that observed using deconvolutional analysis in pubertal males (7) and the initial measurements by Esteban, et al. (5).

Other Assertions Are Subject to Challenge

First, the guidelines recommend a daily glucocorticoid replacement dose of 15 to 25 mg. The individualized daily glucocorticoid replacement dose should be within the DCPR population range. In their large diverse population Purnell, et al. reported a 5.2-fold DCPR range (2.7 to 14 mg/m^2) (3). This 5.2-fold range is not reflected in the 1.7 fold range of daily glucocorticoid replacement in the current recommendations (1, 2). Second, the bioavailability of oral HC likely exceeds 70 percent. An older study using a cortisol radioimmunoassay reports that the bioavailability of oral HC is about 90 percent (8), and a recent study using more accurate LC-MS/MS cortisol measurement reports that the bioavailability is 100 percent (9). Third, the current recommendations do not account for BSA diversity. By correcting these assertions and accounting for diversity by including a woman of average height and ideal body weight (163 cm, 54.4 kg, 1.6 m^2 BSA by the Mosteller formula), then a combined male and female predicted mean daily HC replacement dose (derived from mean DCPR = 7.0 mg/m^2) is 12 mg; the predicted range (derived from a DCPR range of 2.7 to 14 mg/m^2) is 4.3 to 26 mg. These values vary considerably from those of current recommendations (**Table 1**). There is evidence to support the use of a BSA adjustment. Mah et al, demonstrated that serum cortisol concentrations fall into a narrower range when a BSA adjusted HC dose is compared to a fixed dose (10). Finally, the potential effect of age is not incorporated into the current guidelines (1, 2).

Based on current DCPR measurements some authors have suggested that the daily glucocorticoid replacement dose be less than the standard 15 to 25 mg HC dose or 3 to 5 mg prednisolone (11–13).

POTENTIAL COMPLICATIONS OF GLUCOCORTICOID OVERREPLACEMENT

Accumulating evidence suggests that mild long-term glucocorticoid excess may be harmful. In a variety of AI groups conventional daily glucocorticoid replacement doses are associated with complications of glucocorticoid excess including

TABLE 1 | Comparison of Current Guidelines for treating adrenal insufficiency with our interpretation of the current literature.

	Current Guidelines	Our Interpretation of the Current Literature
Mean daily cortisol prod.	7.0 mg/m2	7.0 mg/m2
Range daily cortisol prod.	5.2 - 8.8 mg/m2 (est)	2.7 - 14 mg/m2
HC bioavailability	70 percent (est)	100 percent
Midpoint HC replacement	20 mg/d	100 percent
Range HC replacement Woman, av height, ibw	15 - 25 mg/d	4.3 - 22.3 mg/d
Midpoint HC replacement Man, av height, ibw	20 mg/d	15.6 mg/d
Range HC replacement Man, av height, ibw	15 - 25 mg/d	5.2 - 26 mg/d
Midpoint HC replacement All patients	20 mg/d	15 mg/d
Range HC replacement All patients	15 - 25 mg/d	4.3 - 26 mg/d

Abbreviations. Average (av), ideal body weight (ibw), our estimate/interpretation by 34 calculating from the current guidelines (est).

death from cardiometabolic disorders and infections (14–16). These and evidence of other potential complications have been carefully reviewed (12). Similarly, mild autonomous cortisol secretion by adrenal adenomas is associated with an increased risk for hypertension and type 2 diabetes mellitus (17). Although associations do not prove cause and effect, these findings are concerning. Unfortunately, for logistical reasons it will be difficult, if not impossible, to perform multi-year, prospective, randomized trials to compare the outcome of conventional daily glucocorticoid replacement doses with lower more physiologic doses. Some valuable information may be derived from two short term trials comparing a lower prednisolone dose to the standard hydrocortisone dose (PRED-AID study, ISRCTN41325341 and HYPER-AID, NCT03608943).

A PRAGMATIC APPROACH TO INDIVIDUALIZING THE DAILY GLUCOCORTICOID REPLACEMENT DOSE

If we accept that the daily glucocorticoid replacement dose range is broad, there is the potential for up to several fold overreplacement, if the current guidelines are applied to an individual of relatively low BSA and a pre-AI DCPR in the lower half of the normal range. It is generally agreed that laboratory measurements of serum plasma ACTH and serum cortisol concentrations and its surrogates are not useful for gauging the correct daily glucocorticoid replacement dose (1, 2). However, these measurements may serve a role in occasional patients that seem to have unusual HC requirements (18).

Consider two opposing approaches to determine the correct daily glucocorticoid replacement dose for an individual. The first approach is to treat with HC doses near the upper normal range (20 to 25 mg/d). Then lower the dose if the patient develops evidence of cortisol excess, such as central obesity, muscle weakness, violaceous abdominal striae, hypertension, diabetes, venous thrombosis, cardiovascular disease, myocardial infarction, stroke or osteoporosis. This approach has obvious disadvantages. These endpoints are delayed and either nonspecific or insensitive markers of mild glucocorticoid excess. Furthermore, irreversible end organ damage may develop before the glucocorticoid dose is decreased. A second approach is to slowly titrate to the lowest tolerated HC dose until there is evidence of mild AI, and then increase the dose slightly. The features of AI are expected to develop relatively rapidly, and be readily reversible. Since patients with AI can survive for many years undiagnosed (2), since mild under-replacement is unlikely to cause adrenal crisis in otherwise healthy individuals, and since many features of AI, such as weight loss and hypotension, are relatively sensitive and specific, then it is anticipated that this down titration approach will identify the correct daily glucocorticoid replacement dose for a given individual in a shorter time frame with potential for fewer adverse outcomes.

One retrospective study employed this down titration approach to empirically determine the daily glucocorticoid replacement dose in 25 otherwise healthy adults with either primary or secondary AI due to a variety of causes (19). Divided doses of HC or prednisone were chosen for the glucocorticoid replacement depending on the patient's preference and drug availability. The prednisone to cortisol equivalence was assumed to be 4 to 1, a generally accepted ratio (20–22), although some suggest that it may be 7:1 (23). In each individual the glucocorticoid replacement was decreased by 5 mg/d HC or 1 mg/d prednisone every 2 to 6 months until one of the following predetermined AI end points precluded further titration: weight loss, symptomatic hypotension, hyponatremia, and otherwise unexplained fatigue. The titration was halted in 3 patients for hyponatremia, 22 patients for fatigue, and 0 patients for weight loss or symptomatic hypotension. For primary AI the mineralocorticoid dose was adjusted to normalize the plasma renin activity.

OUTCOMES USING DAILY GLUCOCORTICOID REPLACEMENT DOSES BELOW THE CONVENTIONAL RECOMMENDED DOSES

The titration method of determining daily glucocorticoid replacement dose was evaluated for safety, and the final doses compared to both the current recommendations and to current estimates of DCPR (19). This empirically determined replacement dose did not carry an increased risk of adrenal crisis as compared to historical controls (19). The mean daily dose expressed in HC equivalents was 13.9 ± 6 mg, a value that is significantly lower than the currently recommended midpoint daily HC dose of 20 mg ($p < 0.001$) (19). The empirically determined daily dose range was approximately 7-fold (4 to 30 mg HC equivalents) in a diverse population and clinically distinguishable from the recommended 1.7-fold range (15 to 25 mg) (19). The mean daily glucocorticoid replacement dose in HC equivalents and corrected for BSA was 7.6 ± 3.4 mg/m^2 (range 1.9-14.4 mg/m^2). This was not significantly different from the reported mean DCPR of 7.0 mg/m^2, and closely approximated the reported DCPR range (2.7-14 mg/m^2) (19). There was no difference in the replacement dose for patients with primary or secondary AI.

In primary AI a 12 week crossover prospective trial reported that a delayed release HC caused small but significant decreases in weight, blood pressure, and glycated hemoglobin as compared to equal doses of three times daily HC. One possible explanation is that the bioavailability of the delayed release HC was about 80% of the standard oral HC dose (24).

In secondary AI, the association of daily glucocorticoid replacement dose and morbidity and mortality has been investigated. In patients with nonfunctional pituitary tumors (NFPT) patients and secondary AI, two studies report that HC equivalent doses ≥ 30 mg/d, as compared to <30 mg/d, were associated with a significantly increased death risk (25, 26) independent of other pituitary deficiencies. In a similar NFPT group, Hammarstrand, et al. reported that a HC equivalent dose > 20 mg/d was associated with significantly increased mortality (HR = 1.88, CI = 1.06 - 1.33), although this study did not account for other hormone deficiencies (27).

Filipsson et al. found that hypopituitary patients with HC equivalent replacement ≥ 20 mg/d had an increased body mass index and lipid abnormalities, but no difference in HbA1C (16). These differences were present at baseline and after 1 year of growth hormone replacement, effectively excluding growth hormone deficiency as a confounding factor. Although associations do not prove cause and effect, these studies raise the concern that conventional glucocorticoid replacement is too high.

In secondary AI mixed results have been reported in prospective short duration studies that compare lower to conventional daily glucocorticoid replacement doses. In a 3-month trial, decreasing HC from 30 mg/d to 15 mg/d in 13 patients was well tolerated, but there was no change in a variety of cardiometabolic end points including blood pressure, weight, plasma and urinary electrolytes, and serum glucose or HbA1C (28). Different results were obtained by Danilowicz, et al, in a trial of 11 patients in whom the glucocorticoid replacement dose was reduced from 20-30 mg/d to 10-15 mg/d for 6 to 12 months. There was a significant reduction in weight, abdominal fat, lipid profile and improved quality of life (29). Petersons, et al. reversed the approach and increased HC dose from about 15 mg/d to 30 mg/d in a 7-day study. There was no change in fasting glucose or glucose tolerance (30). In a prospective crossover trial of 10 men comparing total HC replacement doses of 30 mg/d, 20 mg/d, and 15 mg/d for 6 weeks each, lower HC doses caused a significant improvement in bone turnover markers and arterial stiffness, but no change in blood pressure or insulin sensitivity (31, 32). The effects of excess glucocorticoids manifest slowly. Larger and longer duration studies with individualized lower glucocorticoid doses will be necessary to determine if the conventional glucocorticoid replacement doses are harmful.

POTENTIAL AREAS OF INVESTIGATION

Secondary AI

The appropriate diagnosis and glucocorticoid replacement of secondary AI is not addressed by the current Endocrine Society guidelines (1). In the review by Husebye, the glucocorticoid replacement recommendation is the same for both primary and secondary AI (2). It has been proposed that patients with partial secondary AI may require daily HC doses of 0 to 10 mg (33), and some studies have excluded secondary AI patients who do not require any daily glucocorticoid, so as not to bias results (19, 34). Future studies are necessary to define partial secondary AI more precisely and provide guidelines for daily glucocorticoid replacement.

Individualizing the Daily Glucocorticoid Replacement Dose

The method of individualizing the daily glucocorticoid replacement dose requires further investigation. The current guidelines suggest using the lowest daily glucocorticoid replacement dose within the 15 mg/d to 25 mg/d range, but give no suggestions as to how this should be accomplished (1). In the titration study the individualized daily glucocorticoid replacement dose was determined by slowly decreasing the glucocorticoid by about 4 to 5 mg HC equivalent every 2 to 6 months to avoid confusing glucocorticoid withdrawal symptoms with AI symptoms and to avoid severe AI (19). The rate and timing of the titration method has not been validated by others.

There are special challenges to replacing glucocorticoids in patients with both primary AI and type 1 diabetes and in pregnant patients with AI. These are discussed elsewhere (35–37), and are subject to further investigation at lower replacement doses.

The current recommendations do not suggest adjustments for BSA and age. Patient populations have a large range of BSA, and the total daily cortisol production is BSA dependent. A significant positive association of DCPR with age has been reported (3), but the mechanism for this alteration and its physiologic importance are unknown. Investigations into adjustments for BSA and age are indicated.

Glucocorticoid Replacement Timing and Alternate Glucocorticoids

As reviewed by others, methodologies such as continuous infusion, delayed absorption formulations, multiple small daily doses, and intermediate activity glucocorticoids have been studied at conventional replacement doses (11, 38). Using these methods to examine the outcome of a daily glucocorticoid replacement dose that is lower and more physiologic than current recommendations would be of interest.

The biologic equivalence and clinical utility of synthetic glucocorticoids require further investigation. This is particularly important for prednisolone and its hepatically activated precursor prednisone, that have widespread availability at a relatively low cost. A broad prednisolone/HC equivalence of 4 to 7 has been suggested (1, 22, 23). An observational study suggests a 6:1 equivalence, since AI subjects on an average of 3.7 mg/d prednisolone or 20.5 mg/d HC had equal cardiometabolic risk factors (39). A specific prednisolone assay may assist in determining equivalence and dosing schedules (39–41).

The Normal DCPR May Be Too High in the Current Western Environment

We hypothesize that for some individuals their normal DCPR may be too high and harmful in the modern Western environment. Possibly the normal DCPR and its diurnal variation evolved as an orexigenic modifier to [1] drive appetite and food/salt seeking during the daylight hours, [2] to be low enough at night to allow for adequate sleep and recovery, and [3] modulate energy stores and help sustain blood pressure for 24 h. In the current Western environment, little activity is required to find, prepare and ingest adequate calories and salt to meet current modest energy requirements and maintain blood pressure. Within this environment the normal DCPR for some individuals may contribute to such morbidities as obesity, diabetes and hypertension. This may explain why some AI patients tolerate very low daily glucocorticoid replacement doses and why some patients with partial AI require no daily glucocorticoid replacement. Some extreme experiments of nature are also consistent with this hypothesis. Patients with hypothalamic

obesity due to genetic disorders affecting hypothalamic MSH and ACTH production and patients with craniopharyngioma surgery affecting the hypothalamic satiety center have excessive appetites and weight gain even with AI (42, 43). Therefore, in the current environment, multiple orexigenic pathways may overcome the anorexigenic effects of glucocorticoid deficiency. The hypothesis could be tested using tools such as cortisol synthesis inhibitors and glucocorticoid receptor antagonists that are recently available.

SUMMARY AND CONCLUSIONS

Our interpretation of the literature suggests that the current daily glucocorticoid replacement dose recommendations for otherwise healthy AI patients are based on assertions that do not account for the DCPR range, oral HC bioavailability of 100 percent, BSA diversity or age. The current midpoint recommended daily glucocorticoid replacement dose is about 30 percent greater than that predicted from the DCPR. Long-term therapy with conventional glucocorticoid replacement doses is associated with adverse outcomes, but cause and effect has not been established. A titration approach is safe and yields a daily glucocorticoid replacement dose that approximates the mean and range of the known DCPR.

AUTHOR CONTRIBUTIONS

The authors CC and CM contributed to the data extraction, data interpretation, and writing the manuscript.

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High Mortality Rate in Oral Glucocorticoid Users: A Population-Based Matched Cohort Study

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Objective: The aim of the study was to investigate all-cause and disease-specific mortality in a large population-based cohort of oral glucocorticoid (GC) users.

Methods: This was a retrospective, matched cohort study. Information on dispensed prescriptions was obtained from the Swedish Prescribed Drug Register. The cause of death was obtained from the Swedish Cause-of-Death Registry. Patients receiving prednisolone ≥5 mg/day (or equivalent dose of other GC) for ≥21 days between 2007-2014 were included. For each patient, one control subject matched for age and sex was included. The study period was divided into 3-month periods and patients were divided into groups according to a defined daily dose (DDD) of GC used per day. The groups were: Non-users (0 DDD per day), low-dose users (>0 but <0.5 DDD per day), medium-dose users (0.5-1.5 DDD per day) and high-dose users (>1.5 DDD per day). Hazard ratios (HRs), unadjusted and adjusted for age, sex and comorbidities, were calculated using a time-dependent Cox proportional hazard model.

Results: Cases (n=223 211) had significantly higher all-cause mortality compared to controls (HR adjusted for age, sex and comorbidities 2.08, 95% confidence interval 2.04 to 2.13). After dividing the cases into subgroups, adjusted HR was 1.31 (1.28 to 1.34) in non-users, 3.64 (3.51 to 3.77) in low-dose users, 5.43 (5.27 to 5.60) in medium-dose users and, 5.12 (4.84 to 5.42) in high-dose users. The highest adjusted hazard ratio was observed in high-dose users for deaths from sepsis 6.71 (5.12 to 8.81) and pulmonary embolism 7.83 (5.71 to 10.74).

Conclusion: Oral GC users have an increased mortality rate compared to the background population, even after adjustment for comorbidities. High-dose users have an increased risk of dying from sepsis, and pulmonary embolism compared to controls. Whether the relationship between GC exposure and the excess mortality is causal remains to be elucidated.

Keywords: glucocorticoids, mortality, adrenal insufficiency, cohort study, corticosteroids

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INTRODUCTION

Glucocorticoids (GCs) are commonly used worldwide for the treatment of various diseases (1, 2). GC treatment is associated with several adverse effects such as osteoporosis, hypertension, insulin resistance, infections, mood disturbances, cataract formation, and increased risk of cardiovascular disease (3–8). Previous studies have shown that GC use is associated with increased all-cause mortality and cardiovascular mortality in patients with rheumatoid arthritis, where both longer duration of treatment and higher doses predict worse outcome (9–11). Similarly, oral GC use is associated with increased mortality in patients with asthma and chronic inflammatory diseases (12–15). Moreover chronic GC users have a 1.4-fold higher 5-year all-cause mortality (16).

Most previous studies on mortality in GC users are limited to patients with specific underlying disease and none has investigated pulmonary embolism as a specific cause of death.

The aim of this study was to investigate all-cause and disease-specific mortality in a large population-based cohort of oral GC users. Our main hypothesis was that mortality in oral GC users is higher than in the background population.

METHODS

This was a retrospective, matched cohort study based on data from five Swedish healthcare registries. Data on GC prescriptions were collected from the Prescribed Drug Register by using the Anatomical Therapeutic Chemical codes for prednisolone, hydrocortisone, betamethasone, and dexamethasone. The Prescribed Drug Register has information on all prescriptions that are dispensed at Swedish pharmacies since July 2005 (17). We included patients living in Västra Götaland County, Sweden, with dispensed equivalent daily oral GCs doses of prednisolone ≥5 mg, hydrocortisone ≥20mg, betamethasone ≥0.5 mg, or dexamethasone ≥0.5 mg, for more than 21 days, from 1 January 2007 to 31 December 2014. For every prescription the number of Defined daily dose (DDD) is registered. In the registry, DDD is defined according to the World Health Organization (WHO) definition (18). For GC the definition was 1 DDD=10mg Prednisolone=1.5 mg Betamethasone=1.5mg Dexamethasone=30mg hydrocortisone. The patients, defined as cases, were divided into four groups according to GC use: 1. Non-users (0 DDD per day). 2. Low-dose users (more than 0 DDD per day but lower than 0.5 DDD per day). 3. Medium-dose users (0.5-1.5 DDD per day) 4. High-dose users (more than 1.5 DDD per day). A case may at any time point in the study move between those DDD groups based on the previous 3-month period. For example, one case could be classified as a high user in the beginning of the study and at a later time point as a non-user, depending on the GC dose dispensed during the previous 3 months. A non-user is a case that meets the inclusion criteria and has dispensed at least one prescription for GC but has not dispensed GC in the previous 3 months.

For each case, one control subject from Västra Götaland's population register (Västfolket), matched for age (same year of birth) and sex, was included. Controls with any dispensed systemic GC (i.e., tablets or injections) during 2005-2014 were excluded. In cases, the date of inclusion was defined as the date of the first dispensed GC prescription. Follow-up time was calculated from study inclusion to death or end of study (December 31, 2014). For controls, the same inclusion date was used as for their matched GC user.

By using a personal identification number, cases and controls were cross-linked with the Swedish Cause-of-Death Registry. Information on date of death, the primary cause of death, and contributing causes of death were collected and used for the mortality analysis. All-cause mortality and mortality due to ten pre-specified diseases (i.e., ischemic heart disease, myocardial infarction, heart failure, pulmonary embolism, stroke, stroke unspecified, cerebral infarction, intracerebral hemorrhage, sepsis, and pneumonia) were analyzed. A list of the pre-specified diseases, and the corresponding International Classification of Disease 10th edition (ICD-10) codes, is provided in **Table 1**.

The Swedish National Patient Register (NPR) and the Västra Götaland Regional Healthcare Database (VEGA) were used to collect information on the indication for GC treatment, and other comorbidities, by gathering ICD-10 codes during a 24-month period prior to the date of inclusion. The NPR contains all diagnoses for inpatients and hospital-based outpatient care in Sweden (19). VEGA comprises information on diagnoses from primary healthcare and private care in Västra Götaland County. Information on cancer diagnoses was collected from the Swedish Cancer Registry that covers all cancer diagnoses in Sweden (20).

TABLE 1 | Cause of death and ICD-10 codes.

Cause of death	ICD-10 code
Ischemic heart disease	I20 to I25
Myocardial infarction	I21
Heart failure	I50
Pulmonary embolism	I26
Cerebral infarction	I63
Intracerebral hemorrhage	I61 and I62
Stroke (total)	I61 to I64
Stroke UNS	I64
Sepsis	A40 and A41
Pneumonia	J12 to J18

ICD, International Classification of Diseases; UNS, unspecified.

Statistical Analysis

Categorical variables are presented as number and percent and age as mean, standard deviation, median, and range. Years of follow-up are presented as mean, median, and sum. Mortality rate was calculated as number of deaths per follow-up days and then converted to number of deaths per 1000 observation years. Hazard ratio with 95% confidence interval (CI) for mortality in GC users, relative to controls, was evaluated by using Cox proportional hazard models. Difference in survival was determined by the log-rank test. When evaluating mortality by DDD groups time-dependent Cox proportional hazard models presenting hazard ratios with 95% CI and p-values. Both unadjusted hazard ratio, and hazard ratio adjusted for age, sex and comorbidities (diabetes ICD-10 code E10-E14, deep vein

thrombosis I80-I82, pulmonary embolism I26, hypertension (more than 2 dispensed prescription of antihypertensive drug), stroke I64, ischemic heart disease I20-I25, heart failure I50, pneumonia J12-J18, malignant neoplasm C00-C97), diagnosed from two years prior to inclusion, were calculated. For the adjusted models the p-value from the Cox model is presented. All significance tests were two-sided and conducted at a 5% significance level. All analyses were performed using SAS® version 9.4 (Cary, NC).

Ethical Approval

The study was approved by the Regional Research Ethics Committee in Gothenburg, Sweden (reference number 773-14; approved 9 March 2015) and by the National Board of Health and Welfare, Sweden.

RESULTS

Of 1 585 335 inhabitants in Västra Götaland, 223 211 cases (55.6% women) and an equal number of matched controls were included in the study (**Table 2**). Mean age was 48.4 years (standard deviation 24.2). Chronic obstructive pulmonary disease and asthma were the most common indications for GC treatment (17.2%), followed by allergy (12.5%) and malignant neoplasms (11.5%).

All-Cause Mortality

The mortality rate was 26 550 deaths per 3.6 observation years in cases, compared to 12 384 deaths per 3.9 observation years in controls. The mortality rate per 1000 patient years was 14.05 in controls and 31.98 in cases (**Table 3**). Unadjusted hazard ratio for all-cause mortality in GC users was 2.26 (95% CI 2.21 to 2.30) and after adjustment for age, sex and comorbidities 2.08 (95% CI 2.04 to 2.13) (**Figure 1**). The hazard ratio, adjusted for age, sex and comorbidities, for all-cause mortality in non-users was 1.31 (95% CI 1.28 to 1.34), 3.64 (95% CI 3.51 to 3.77) in low-dose users, 5.43 (95% CI 5.27 to 5.60) in medium-dose users and, 5.12 (95% CI 4.84-5.42) in high-dose users (**Table 4**).

Disease-Specific Mortality

Cox regression was used to analyze mortality due to ten pre-specified diseases (**Table 5**). After adjustment for sex, age and comorbidities, the hazard ratio for death from pulmonary embolism was 1.51 (95% CI, 1.28 to 1.78), 5.16 (95% CI 4.18 to 6.37), 6.77 (95% CI 5.59 to 8.19), and 7.83 (95% CI 5.71 to 10.74) in non-users, low-dose users, medium-dose users and high-dose users, respectively. Mortality from stroke (cerebral infarction, intracerebral hemorrhage or stroke not otherwise specified) was increased in low-dose users, medium-dose users and high-dose users, adjusted hazard ratio 1.74 (95% CI 1.53 to 1.99), 1.68 (95% CI 1.45 to 1.93) and 2.03 (95% CI 1.52 to 2.72), respectively (**Figure 2**). Adjusted hazard ratio for death from sepsis was 1.46 (95% CI 1.28 to 1.65) in non-users, 3.00 (95% CI 2.48 to 3.62) in low users, 4.89 (95% CI 4.16 to 5.75) in medium-dose users and 6.71 (95% CI 5.12 to 8.81) in high-dose users. Increased mortality from pneumonia was found in all GC users´ groups, hazard ratio being 3.34 (95% CI 3.01 to 3.71) in low-dose users, 3.01 (95% CI 2.69 to 3.36) in medium-dose users, and 3.82 (95% CI 3.10 to 4.70) in high-dose users. Adjusted hazard ratio was also increased for death from heart failure, with the highest hazard ratio in low-dose users 2.73 (95% CI 2.54 to 2.93).

DISCUSSION

In this population-based matched cohort study of 223 211 oral GC users, we found an increased all-cause mortality compared to controls. The study illustrates that patients receiving oral GC treatment have a two-fold overall risk of dying during follow-up than matched controls, mainly due to deaths from pulmonary embolism, pneumonia, and sepsis.

Previous studies have shown that oral GC use is associated with an increased mortality rate in patients with chronic

TABLE 2 | Baseline characteristics.

	GC users (n = 223 211)	Controls (n = 223 211)
Age (years)		
Mean (standard deviation)	48.4 (24.2),	48.4 (24.2)
Median (range)	50.8 (0.1 to 107)	50.8 (0.0 to 107)
Gender		
Men	99 172 (44.4%)	99 172 (44.4%)
Women	124 039 (55.6%)	124 039 (55.6%)
Comorbidities prior to inclusion*		
Diabetes mellitus	14 249 (6.4%)	12 449 (5.6%)
Heart failure	6898 (3.1%)	3260 (1.5%)
Ischemic heart disease	11 629 (5.2%)	7978 (3.6%)
Hypertension	56 874 (25.5%)	45 896 (20.6%)
Stroke	3418 (1.5%)	2759 (1.2%)
Deep vein thrombosis	2795 (1.3%)	1486 (0.7%)
Pulmonary embolism	1024 (0.5%)	385 (0.2%)
Sepsis	924 (0.4%)	323 (0.1%)
Malignant neoplasm	15927 (7.1%)	3270 (1.5%)

Comorbidities during a 24-month period prior to the date of inclusion. GC, glucocorticoid.

TABLE 3 | All-cause mortality and disease-specific mortality in GC users compared to age- and sex-matched controls.

		No of deaths* (%)	Follow-up (years) mean; median; sum	No of deaths per 1000 patient years	Unadjusted		Adjusted for age, sex and comorbidities**	
					Hazard ratio (95% CI)	p-value	Hazard ratio (95% CI)	p-value
Death	Cases	26550 (11.9)	3.72; 3.60; 830294	31.98				
	Controls	12384 (5.5)	3.95; 3.87; 881731	14.05	2.26 (2.21-2.30)	<.0001	2.08 (2.04-2.13)	<.0001
Ischemic heart disease	Cases	4243 (1.9)	3.72; 3.60; 830294	5.11				
	Controls	3205 (1.4)	3.95; 3.87; 881731	3.63	1.40 (1.34-1.47)	<.0001	1.33 (1.27-1.39)	<.0001
Myocardial infarction	Cases	1547 (0.7)	3.72; 3.60; 830294	1.86				
	Controls	1368 (0.6)	3.95; 3.87; 881731	1.55	1.20 (1.11:1.29)	<.0001	1.20 (1.11:1.29)	<.0001
Heart failure	Cases	5030 (2.3)	3.72; 3.60; 830294	6.06				
	Controls	3139 (1.4)	3.95; 3.87; 881731	3.56	1.69 (1.62:1.77)	<.0001	1.55 (1.48:1.62)	<.0001
Pulmonary embolism	Cases	762 (0.3)	3.72; 3.60; 830294	0.92				
	Controls	265 (0.1)	3.95; 3.87; 881731	0.30	3.02 (2.63:3.48)	<.0001	2.54 (2.20:2.93)	<.0001
Stroke total †	Cases	1462 (0.7)	3.72; 3.60; 830294	1.76				
	Controls	1477 (0.7)	3.95; 3.87; 881731	1.68	1.05 (0.97:1.13)	0.2087	1.09 (1.01:1.17)	0.0258
Cerebral infarction	Cases	510 (0.2)	3.72; 3.60; 830294	0.61				
	Controls	509 (0.2)	3.95; 3.87; 881731	0.58	1.06 (0.94:1.20)	0.3459	1.08 (0.95:1.23)	0.2268
Intracerebral	Cases	314 (0.1)	3.72; 3.60; 830294	0.38				
hemorrhage	Controls	268 (0.1)	3.95; 3.87; 881731	0.30	1.24 (1.05:1.46)	0.0098	1.24 (1.05:1.47)	0.0106
Stroke UNS	Cases	687 (0.3)	3.72; 3.60; 830294	0.83				
	Controls	746 (0.3)	3.95; 3.87; 881731	0.85	0.97 (0.88:1.08)	0.6239	1.04 (0.93:1.15)	0.5021
Sepsis	Cases	1006 (0.5)	3.72; 3.60; 830294	1.21				
	Controls	482 (0.2)	3.95; 3.87; 881731	0.55	2.20 (1.98:2.46)	<.0001	2.07 (1.85:2.31)	<.0001
Pneumonia	Cases	2280 (1.0)	3.72; 3.60; 830294	2.75				
	Control	1481 (0.7)	3.95; 3.87; 881731	1.68	1.63 (1.52:1.74)	<.0001	1.63 (1.53:1.75)	<.0001

**The cause of death can be both the primary cause of death and contributing causes of death, so the total number of deaths is therefore higher than the total deaths due to disease-specific deaths.*
***Hazard ratio adjusted for comorbidities (diabetes ICD-10 code E10-E14, deep vein thrombosis I80-I82, pulmonary embolism I26, hypertension (more than 2 dispensed prescription of antihypertensive drug), stroke I64, ischemic heart disease I20-I25, heart failure I50, pneumonia J12-J18, malignant neoplasm C00-C97).*
†Cerebral infarction, intracerebral hemorrhage, and stroke not specified as hemorrhage or infarction.
CI, confidence interval; GC, glucocorticoid; UNS, unspecified; ICD, International Classification of Disease.

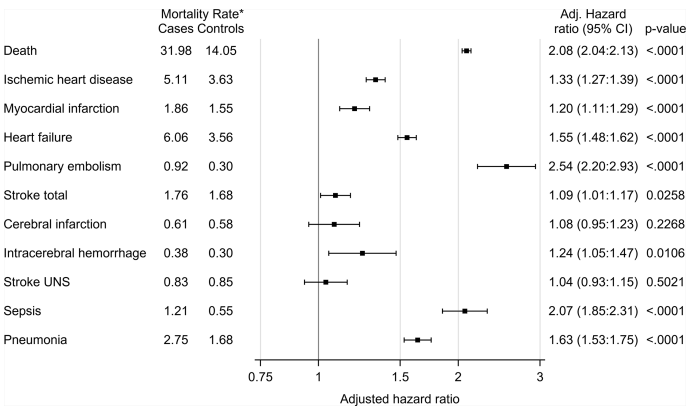

FIGURE 1 | All-cause mortality and disease-specific mortality in glucocorticoid users (cases) compared to age- and sex-matched controls. The hazard ratio was calculated using Cox proportional hazard model. The hazard ratio was adjusted for age, sex and comorbidities (diabetes, deep vein thrombosis, pulmonary embolism, hypertension, stroke, ischemic heart disease, heart failure, pneumonia, malignant neoplasm). *Number of deaths per 1000 patient years.

inflammatory diseases (12–14, 21). These studies showed a hazard ratio for all-cause mortality of 2.17 (95% CI 2.04 to 2.31) in patients with asthma (14), 2.48 (95% CI 1.85 to 3.31) in patients with Crohn's disease, 2.81 (95% CI 2.26 to 3.50) in patients with ulcerative colitis (13), and 1.97 (95% CI 1.81 to 2.15) in patients with rheumatoid arthritis (21). The almost doubled risk of death is in agreement with our findings, although our results derive from a population-based cohort and cannot be directly compared to previous studies. A recent population-based cohort study showed that chronic (≥30 days) GC users had a 1.4-fold higher 5-year all-cause mortality compared with controls (16). For chronic high-dose GC users (>5 mg/day of

TABLE 4 | All-cause mortality in oral glucocorticoid (GC) users compared to age- and sex-matched controls.

		No of deaths (%)	Follow-up (years)	No of deaths per 1000 patient years	Unadjusted		Adjusted for age, sex and comorbidities*	
					Hazard ratio (95% CI)	p-value	Hazard ratio (95% CI)	p-value
All-cause mortality	Controls	12384 (5.5)	881731	14.0				
	Non-users (0 DDD per day)	12191 (5.9)	712482	17.1	1.22 (1.19-1.25)	<.0001	1.31 (1.28-1.34)	<.0001
	Low-dose users (lower than 0.5 DDD per day)	5318 (3.0)	70807	75.1	5.30 (5.11-5.49)	<.0001	3.64 (3.51-3.77)	<.0001
	Medium-dose users (0.5-1.5 DDD per day)	7685 (11.4)	39376	195.2	13.10 (12.72-13.50)	<.0001	5.43 (5.27-5.60)	<.0001
	High-dose users (more than 1.5 DDD per day)	1356 (7.7)	8000	169.5	10.81 (10.22-11.44)	<.0001	5.12 (4.84-5.42)	<.0001

**Hazard ratio adjusted for comorbidities (diabetes ICD-10 code E10-E14, deep vein thrombosis I80-I82, pulmonary embolism I26, hypertension (more than 2 dispensed prescription of antihypertensive drug), stroke I64, ischemic heart disease I20-I25, heart failure I50, pneumonia J12-J18, malignant neoplasm C00-C97).*
CI, confidence interval; GC, glucocorticoid; ICD, International Classification of Diseases.

TABLE 5 | Disease-specific mortality in GC users compared to age- and sex-matched controls.

		No of deaths* (%)	Follow-up (years)	No of deaths per 1000 patient years	Unadjusted		Adjusted for age, sex and comorbidities**	
					Hazard ratio (95% CI)	p-value	Hazard ratio (95% CI)	p-value
Ischemic heart disease	Controls	3205 (1.4)	881731	3.6				
	Non-users (0 DDD per day)	2548 (1.2)	712482	3.6	0.98 (0.93-1.03)	0.35	1.07 (1.01-1.13)	0.014
	Low-dose users (lower than 0.5 DDD per day)	914 (0.5)	70807	12.9	3.81 (3.50-4.14)	<.0001	2.18 (2.01-2.36)	<.0001
	Medium-dose users (0.5-1.5 DDD per day)	678 (1.0)	39376	17.2	4.76 (4.37-5.18)	<.0001	2.09 (1.91-2.27)	<.0001
	High-dose users (more than 1.5 DDD)	103 (0.6)	8000	12.9	3.48 (2.86-4.24)	<.0001	1.88 (1.55-2.30)	<.0001
Myocardial infarction	Controls	1368 (0.6)	881731	1.6				
	Non-users (0 DDD per day)	946 (0.5)	712482	1.3	0.85 (0.78-0.92)	<.0001	0.97 (0.89-1.05)	0.45
	Low-dose users (lower than 0.5 DDD per day)	333 (0.2)	70807	4.7	3.32 (2.90-3.81)	<.0001	2.09 (1.84-2.38)	<.0001
	Medium-dose users (0.5-1.5 DDD per day)	224 (0.3)	39376	5.7	3.72 (3.22-4.30)	<.0001	1.73 (1.50-2.00)	<.0001
	High-dose users (more than 1.5 DDD per day)	44 (0.3)	8000	5.5	3.53 (2.61-4.78)	<.0001	1.97 (1.46-2.67)	<.0001
Heart failure	Controls	3139 (1.4)	881731	3.6				
	Non-users (0 DDD per day)	2931 (1.4)	712482	4.1	1.13 (1.08-1.19)	<.0001	1.22 (1.15-1.28)	<.0001
	Low-dose users (lower than 0.5 DDD per day)	1211 (0.7)	70807	17.1	5.71 (5.30-6.16)	<.0001	2.73 (2.54-2.93)	<.0001
	Medium-dose users (0.5-1.5 DDD per day)	767 (1.1)	39376	19.5	5.73 (5.29-6.21)	<.0001	2.40 (2.21-2.61)	<.0001
	High-dose users (more than 1.5 DDD per day)	121 (0.7)	8000	15.1	4.35 (3.62-5.22)	<.0001	2.43 (2.02-2.92)	<.0001
Pulmonary embolism	Controls	265 (0.1)	881731	0.3				
	Non-users (0 DDD per day)	332 (0.2)	712482	0.5	1.54 (1.31-1.81)	<.0001	1.51 (1.28-1.78)	<.0001
	Low-dose users (lower than 0.5 DDD per day)	163 (0.1)	70807	2.3	8.02 (6.46-9.96)	<.0001	5.16 (4.18-6.37)	<.0001
	Medium-dose users (0.5-1.5 DDD per day)	219 (0.3)	39376	5.6	17.62 (14.65-21.18)	<.0001	6.77 (5.59-8.19)	<.0001
	High-dose users (more than 1.5 DDD per day)	48 (0.3)	8000	6.0	17.78 (13.01-24.28)	<.0001	7.83 (5.71-10.74)	<.0001
Stroke total †	Controls	1477 (0.7)	881731	1.7				
	Non-users (0 DDD per day)	878 (0.4)	712482	1.2	0.73 (0.67-0.79)	<.0001	0.87 (0.80-0.95)	0.0021
	Low-dose users (lower than 0.5 DDD per day)	303 (0.2)	70807	4.3	2.76 (2.40-3.17)	<.0001	1.74 (1.53-1.99)	<.0001
	Medium-dose users (0.5-1.5 DDD per day)	233 (0.3)	39376	5.9	3.59 (3.12-4.14)	<.0001	1.68 (1.45-1.93)	<.0001

(Continued)

TABLE 5 | Continued

		No of deaths* (%)	Follow-up (years)	No of deaths per 1000 patient years	Unadjusted		Adjusted for age, sex and comorbidities**	
					Hazard ratio (95% CI)	p-value	Hazard ratio (95% CI)	p-value
	High-dose users (more than 1.5 DDD per day)	48 (0.3)	8000	6.0	3.58 (2.68-4.79)	<.0001	2.03 (1.52-2.72)	<.0001
Cerebral infarction	Controls	509 (0.2)	881731	0.6				
	Non-users (0 DDD per day)	321 (0.2)	712482	0.5	0.78 (0.67-0.89)	0.0004	0.91 (0.79-1.05)	0.21
	Low-dose users (lower than 0.5 DDD per day)	107 (0.1)	70807	1.5	2.73 (2.15-3.46)	<.0001	1.72 (1.37-2.15)	<.0001
	Medium-dose users (0.5-1.5 DDD per day)	65 (0.1)	39376	1.7	2.88 (2.21-3.75)	<.0001	1.33 (1.02-1.74)	0.033
	High-dose users (more than 1.5 DDD per day)	17 (0.1)	8000	2.1	3.67 (2.25-5.97)	<.0001	2.07 (1.27-3.38)	0.0035
Intracerebral hemorrhage	Controls	268 (0.1)	881731	0.3				
	Non-users (0 DDD per day)	205 (0.1)	712482	0.3	0.93 (0.78-1.12)	0.46	1.05 (0.87-1.26)	0.61
	Low-dose users (lower than 0.5 DDD per day)	32 (0.0)	70807	0.5	1.66 (1.12-2.48)	0.012	1.21 (0.83-1.78)	0.33
	Medium-dose users (0.5-1.5 DDD per day)	64 (0.1)	39376	1.6	5.42 (4.09-7.18)	<.0001	2.53 (1.90-3.37)	<.0001
	High-dose users (more than 1.5 DDD per day)	13 (0.1)	8000	1.6	5.24 (2.99-9.20)	<.0001	2.80 (1.59-4.93)	0.0004
Stroke UNS	Controls	746 (0.3)	881731	0.8				
	Non-users (0 DDD per day)	387 (0.2)	712482	0.5	0.63 (0.56-0.72)	<.0001	0.79 (0.70-0.90)	0.0002
	Low-dose users (lower than 0.5 DDD per day)	174 (0.1)	70807	2.5	3.19 (2.65-3.85)	<.0001	1.90 (1.60-2.27)	<.0001
	Medium-dose users (0.5-1.5 DDD per day)	108 (0.2)	39376	2.7	3.34 (2.71-4.10)	<.0001	1.59 (1.29-1.95)	<.0001
	High-dose users (more than 1.5 DDD per day)	18 (0.1)	8000	2.3	2.71 (1.70-4.34)	<.0001	1.61 (1.01-2.59)	0.047
Sepsis	Controls	482 (0.2)	881731	0.5				
	Non-users (0 DDD per day)	530 (0.3)	712482	0.7	1.36 (1.20-1.54)	<.0001	1.46 (1.28-1.65)	<.0001
	Low-dose users (lower than 0.5 DDD per day)	168 (0.1)	70807	2.4	4.39 (3.61-5.34)	<.0001	3.00 (2.48-3.62)	<.0001
	Medium-dose users (0.5-1.5 DDD per day)	246 (0.4)	39376	6.2	11.25 (9.59-13.19)	<.0001	4.89 (4.16-5.75)	<.0001
	High-dose users (more than 1.5 DDD per day)	62 (0.4)	8000	7.8	13.64 (10.42-17.84)	<.0001	6.71 (5.12-8.81)	<.0001
Pneumonia	Controls	1481 (0.7)	881731	1.7				
	Non-users (0 DDD per day)	1181 (0.6)	712482	1.7	0.97 (0.90-1.05)	0.46	1.13 (1.05-1.23)	0.0015
	Low-dose users (lower than 0.5 DDD per day)	568 (0.3)	70807	8.0	5.46 (4.90-6.09)	<.0001	3.34 (3.01-3.71)	<.0001
	Medium-dose users (0.5-1.5 DDD per day)	433 (0.6)	39376	11.0	6.74 (6.04-7.52)	<.0001	3.01 (2.69-3.36)	<.0001
	High-dose users (more than 1.5 DDD per day)	98 (0.6)	8000	12.3	7.32 (5.95-8.99)	<.0001	3.82 (3.10-4.70)	<.0001

**The cause of death can be both the primary cause of death and contributing causes of death, so the total number of deaths is therefore higher than the total deaths due to disease-specific deaths.*
***Hazard ratio adjusted for comorbidities (diabetes ICD-10 code E10-E14, deep vein thrombosis I80-I82, pulmonary embolism I26, hypertension (more than 2 dispensed prescription of antihypertensive drug), stroke I64, ischemic heart disease I20-I25, heart failure I50, pneumonia J12-J18, malignant neoplasm C00-C97).*
†Cerebral infarction, intracerebral hemorrhage, and stroke not specified as hemorrhage or infarction.
CI, confidence interval; GC, glucocorticoid; UNS, unspecified; ICD, International Classification of Diseases.
Mortality analysis was performed for controls and for non-users (0 DDD per day), low-dose users (more than 0 DDD per day but lower than 0.5 DDD per day), medium-dose users (0.5-1.5 DDD per day) and high-dose users (more than 1.5 DDD per day).

prednisolone) the hazard ratio was 1.5 (95% CI 1.3 to 1.8; P < 0.001) and for low-dose GC users 1.3 (1.2 to 1.5; P < 0·001) (16).

Previous studies have not focused on pulmonary embolism as a specific cause of death in patients receiving GC treatment (9, 14, 16, 21). Malignant neoplasm and previous history of thromboembolic disease may increase the risk of pulmonary embolism. Therefore, it is important to emphasis that we adjusted for comorbidities such as cancer, as well as deep vein thrombosis and pulmonary embolism at baseline (before prescription of GCs) in the mortality analysis. The increased mortality rate from deaths due to pulmonary embolism in the current report is in line with the increased incidence of thromboembolism in patients with endogenous hypercortisolism (22, 23). Patients with endogenous

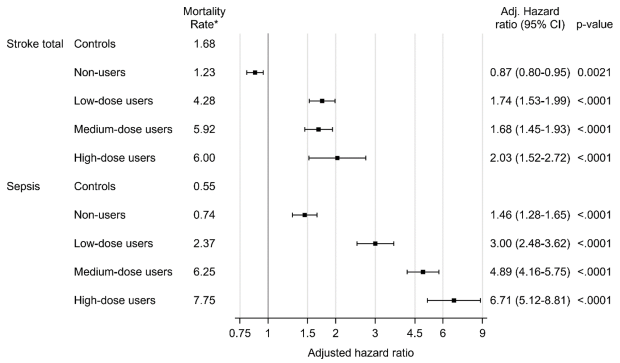

FIGURE 2 | Mortality due to stroke and sepsis in glucocorticoid users compared to age- and sex-matched controls. Mortality analysis was performed for controls and for non-users (0 DDD per day), low-dose users (more than 0 DDD per day but lower than 0.5 DDD per day), medium-dose users (0.5-1.5 DDD per day) and high-dose users (more than 1.5 DDD per day). Time-dependent Cox proportional hazard models was used. The hazard ratio was adjusted for age, sex and comorbidities (diabetes, deep vein thrombosis, pulmonary embolism, hypertension, stroke, ischemic heart disease, heart failure, pneumonia, malignant neoplasm). *Number of deaths per 1000 patient years.

hypercortisolism have increased levels of procoagulant factors and impaired fibrinolytic capacity, that leads to hypercoagulability with an up to ten-fold increased risk of venous thromboembolism (22, 24, 25). Data on the association between GC use and hypercoagulability are, however, sparse (25, 26). A population-based case-control study from Denmark showed that current systemic GC use was associated with an approximately two-fold increased incidence of both pulmonary embolism and deep vein thrombosis (26). Thus, our results are in line with these previous results and suggest that GC treatment at supraphysiological doses is associated with increased morbidity and mortality due to thromboembolic diseases.

GCs have immunosuppressive and anti-inflammatory effects that, consequently, increase the susceptibility to infections (27, 28). Our study showed a six-fold risk of death from sepsis and three-fold risk of death from pneumonia in high GC-dose users, and that the risk of death from sepsis is dose dependent. Patients with endogenous hypercortisolism also have an increased risk of dying from infections (29, 30). In a recent nationwide study on patients with Cushing disease, 11% of all deaths were due to infections and half of them due to pneumonia (30). Furthermore, according to a recent study from the European Register on Cushing's syndrome (ERCUSYN), one-third of all deaths were due to infections (29). These, and our data, strongly indicate that the immunosuppressive effects of GCs may have deleterious consequences for patients with endogenous hypercortisolism as well as GC users.

Our study showed increased mortality from ischemic heart disease and heart failure, although not in dose-dependent pattern. GC use has in fact previously been associated with increased morbidity and mortality from cardiovascular disease (8, 9). GC use in patients with rheumatoid arthritis has been associated with a dose-dependent increase in cardiovascular mortality rates, with a daily threshold dose of 8 mg of Prednisolone (9). Another study showed a dose-dependent relation between current users of GC and risk of heart failure (adjusted odds ratio 2.66, 95% CI 2.46 to 2.87), and ischemic heart disease (adjusted odds ratio 1.20, 95% CI 1.11 to 1.29) (8). In our study, the hazard ratio was highest in low-dose users for deaths both from heart failure and ischemic heart disease. Due to this, and the retrospective design, a causal role between GC treatment and the increased mortality from cardiovascular diseases can however not be confirmed.

Previous studies have shown that prednisolone doses lower than 5 mg/day do not increase mortality and do not suppress the hypothalamic-pituitary-adrenal axis (21, 31). Similarly, GC treatment for less than 2-3 weeks does not seem to suppress the hypothalamic-pituitary-adrenal axis (32–34). Patients receiving prednisolone equivalent doses of <5 mg/day for <21 days were therefore not included in our study. On the contrary, higher doses and/or longer treatment duration frequently causes transient GC-induced cortisol deficiency, also called GC-induced adrenal insufficiency (6). In such cases, GC cessation can be hazardous and lead to acute adrenal crisis (35). A recent study including 70,638 oral GC users showed increased mortality during the first 2 months after cessation of oral GC treatment and then decreased mortality over time after the first 3 months of cessation (15). This may have been caused by adrenal crisis due to undiagnosed GC-induced adrenal insufficiency. An increased mortality in GC users due to sepsis and pneumonia could be related to adrenal crisis. However, this is only speculative since our data does not contain information on whether the GC treatment was tapered slowly or not. More studies are needed to investigate if GC-induced adrenal insufficiency is underdiagnosed in GC users and whether it is associated with premature and avoidable death.

The main strength of our study is the access to large healthcare databases with information about dispensed prescriptions at all Swedish pharmacies, causes of death, and comorbidities. The Swedish Prescribed Drug Register has information on all dispensed prescriptions in Sweden offering the opportunity to evaluate mortality in oral GC users, both adults and children, in a large population-based cohort, in contrast to previous studies with focus on mortality in GC

users with specific diseases (rheumatoid arthritis, inflammatory bowel disease, asthma, and chronic inflammatory disease) (12–14, 21). However, the true causal relationship between oral GC use and mortality is challenging to uncover due to a large number of confounders, including the underlying disease itself and its severity (9, 10). In fact, high-dose GC users are more likely to have more severe underlying diseases than low-dose users, that consequently may explain the increased mortality rate. Previous studies that have investigated mortality in GC treated patients with one specific disease have the same limitations and a true causal relationship between GC use and mortality can therefore not be proven (10, 12, 14). Further research on this topic is therefore needed.

This large matched cohort study showed that oral GC users have a high all-cause mortality compared to the background population, mainly due to deaths from sepsis, pulmonary embolism, and heart failure.

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by The Regional Research Ethics Committee in Gothenburg, Sweden (reference number 773-14; approved 9 March 2015) and by the National Board of Health and Welfare, Sweden. Written informed consent for participation was not required for this study in accordance with the national legislation and the institutional requirements.

AUTHOR CONTRIBUTIONS

ME, OR, GJ, DO, and PT designed the study. OR supervised the study. PE and MM had full access to data in the study and performed the statistical analysis. GJ and ME obtained funding. ME and OR drafted the manuscript and all authors revised it. All authors approved the final manuscript. OR and ME are guarantors. All authors contributed to the article and approved the submitted version.

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Impaired quality of life, but not cognition, is linked to a history of chronic hypercortisolism in patients with Cushing's disease in remission

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Context: Impaired cognition and altered quality of life (QoL) may persist despite long-term remission of Cushing's disease (CD). Persistent comorbidities and treatment modalities may account for cognitive impairments. Therefore, the role of hypercortisolism *per se* on cognitive sequelae remains debatable.

Objective: To investigate whether memory and QoL are impaired after long-term remission of CD in patients with no confounding comorbidity.

Design and Setting: Cross-sectional case-control study in two tertiary referral centers

Patients: 25 patients (44.5 ± 2.4 years) in remission from CD for 102.7 ± 19.3 Mo and 25 well-matched controls, without comorbidity or treatment liable to impair cognition.

Main Outcome Measure(s): Hippocampus- and prefrontal cortex-dependent memory, including memory flexibility and working memory, were investigated using multiple tests including sensitive locally-developed computerized tasks. Depression and anxiety were evaluated with the MADRS and HADS questionnaires. QoL was evaluated with the SF-36 and CushingQoL questionnaires. The intensity of CD was assessed using mean urinary free cortisol and a score for clinical symptoms.

Results: CD patients displayed similar performance to controls in all cognitive tests. In contrast, despite the absence of depression and a minimal residual clinical Cushing score, patients had worse QoL. Most of the SF36 subscales and

the CushingQoL score were negatively associated only with the duration of exposure to hypercortisolism ($p \leq 0.01$ to 0.001).

Conclusions: Persistent comorbidities can be a primary cause of long-lasting cognitive impairment and should be actively treated. Persistently altered QoL may reflect irreversible effects of hypercortisolism, highlighting the need to reduce its duration.

Clinical Trial Registration number: https://clinicaltrials.gov, identifier NCT02603653

KEYWORDS

Cushing's disease, hypercortisolism, cognition, memory, quality of life

Introduction

Chronic hypercortisolism significantly impacts brain function and morphology (1–5). Cognition is frequently negatively affected during Cushing's disease (CD) including deficits in memory, verbal and visual learning (4, 6, 7). Imaging has revealed cerebral atrophy and functional changes in areas rich in glucocorticoid receptors, involved in processing of cognitive functions, such as the hippocampus, amygdala, and prefrontal cortex (1, 8). Remission of CD is usually associated with cognitive improvements (4, 6, 7, 9), but controversies persist as to the degree of recovery (4, 6, 7). Numerous factors may account for the heterogeneity between the results of studies. These include variations in recruitment, duration of hypercortisolism and time elapsed between remission of CS and cognitive evaluation, assuming that cognitive recovery may be delayed (2, 10, 11), discrepancies between cross-sectional and longitudinal studies, and differences in neuropsychological tests used (6, 7, 11, 12). Besides these caveats, variable memory recovery between individuals questions the specific role of hypercortisolism and suggests that different causes, directly or indirectly linked to hypercortisolism, may be involved in persistent alterations (12, 13). Indeed, patients may have confounding factors impairing cognition and, more specifically, memory. These include advanced age (14), obesity, poorly-controlled diabetes (15, 16), cerebral vascular disease and poorly controlled hypertension (17), psychopathology and depression including psychotropic treatments (18), excessive hydrocortisone replacement, imperfect hormonal replacement for hypothyroidism and untreated GH deficiency (19–21). Among the treatment modalities, pituitary radiotherapy may be associated to cognitive deficits (22, 23). A number of these putative interfering factors were not controlled or specified in prior publications. Therefore, the specific long-term consequences of hypercortisolism after its cessation on cognition remain debatable. In addition, identifying the association of confounding factors with persistent cognitive deficits is clinically relevant, because they may lead, along with somatic sequelae, to a lasting deterioration in quality of life (QoL) (2–4, 6, 7, 24, 25). To address this, we investigated memory function with multiple tests, including sensitive computerized tests for declarative and working memory, and QoL in patients with long-term remission of CD, with no confounding comorbidity.

Patients and methods

Patients

Clinical charts of 192 patients with cured CD and followed for > 1 year were reviewed in two expert centers (Endocrinology departments of Haut Leveque Hospital, Bordeaux, France and Hospital Sant Pau, Barcelona, Spain). CD was confirmed on histopathological analysis, remission of CD following transsphenoidal surgery, and the results of bilateral inferior petrosal sinus sampling (BIPSS). Patients were selected using two main criteria: 1) being in definitive remission following surgery for at least one year and 2) the absence of confounding factors or comorbidity that may alter cognitive functions.

Remission was ascertained during the 3 months preceding cognitive evaluation and was defined by persistent adrenal insufficiency or normal 24h urinary free cortisol excretion (UFC) associated with serum cortisol < 50 nmol/L following 1 mg overnight dexamethasone suppression test. Patients with adrenal insufficiency had to receive hydrocortisone supplementation at doses ≤ 15 mg/m^2. Conditions known to interfere with cognitive function (considered exclusion criteria) included: age > 60 years, remission of CD induced by drugs, previous pituitary radiotherapy, BMI > 30 kg/m^2, drugs- and

alcohol-abuse (past and present), untreated GH-deficiency or hypothyroidism, uncontrolled diabetes (HbA1c > 7.5% or FBG > 1.40 g/L) or diabetes-induced organ damage, past-history of neurological/vascular disease including uncontrolled hypertension, intake of psychotropic drugs including sleeping tablets and intake of exogenous steroids. GH secretion was assessed using dynamic testing and was considered normal in presence of a GH peak > 3 ng/mL during insulin tolerance test and glucagon stimulation test or a GH peak > 8 ng/mL during the GHRH -arginine stimulation test.

Control group

Each patient with CD recruited a control subject matched for age, gender, living area, socioeconomic status and educational level. Five educational levels were defined: 1: no school, 2: primary school, 3: 5-9 years at school, short secondary school, 4: more than 9 years at school, long secondary school, 5: more than 12 years, university level. Exclusion criteria for controls were identical to those of CD patients; thyroid function was evaluated, but GH secretion was not.

Informed consent was obtained from all participants and the study was approved by the ethical committees of both centers. The study was registered under Clinical Trials (ClinicalTrials.gov Identifier: NCT02603653).

Methods

All evaluations for the patients and the controls were performed at the same time during one day.

Cognitive evaluation

Cognitive and psychological evaluation was performed by a dedicated psychologist to explore memory (hippocampal- and frontal- dependent forms), fluency, mood (including depression and anxiety); and QoL. The neuropsychological outcomes were explored using: 1) The Rey Auditory Verbal Learning Test (RAVLT) to evaluate verbal episodic memory (26), 2) the Isaac' Set tests assessing phonemic and semantic verbal fluency abilities and speed of verbal production (27), 3) two subtests of the Wechsler Memory Scale, 4th Edition that evaluates visual and visuo-spatial working memory: the spatial addition task and the symbol span task (28), 4) the Rey-Osterrieth Complex Figure (ROCF) test dedicated to the evaluation visuospatial constructional ability and visual memory (29). Only the delayed recall was performed in this test.

In addition, patients underwent two locally developed computerized maze memory tests assessing cardinal features of declarative memory and working memory such as flexibility of declarative memory; and organization of information in memory and sensibility to interference (30–32) (see Supplemental Data).

Evaluation of depression and anxiety

Depression and anxiety were assessed using the Depression Rating Scale (MADRS) questionnaire and the Hospital Anxiety and Depression Scale (HADS) (33, 34).

Evaluation of QoL

The generic Short Form 36 (SF-36) questionnaire (35) was used in both patients and controls. The specific CushingQoL questionnaire was also used in patients (36).

Evaluation of Cushing's disease

We used an arbitrarily defined score for symptoms of hypercortisolism to evaluate clinical intensity of CD. The score was calculated following clinical examination at the time of cognitive evaluation and retrospectively calculated from medical reports at diagnosis. A score of 1 was recorded for the presence of any symptom like hirsutism, menstrual irregularities, buffalo neck, facial plethora and central obesity, diabetes, dyslipidemia, hypertension and cognitive or psychopathological complaints. A score of 2 was recorded for the presence of more specific symptoms such as large purple striae, proximal muscle weakness, spontaneous ecchymosis and glucocorticoid-induced osteoporosis. The maximum score was 20. The biological intensity of CD at diagnosis was calculated using the mean results of 2-3measurements of 24h UFC expressed relative to the upper normal range of the assay (x ULN).

The apparent duration of hypercortisolism was calculated retrospectively from the time between onset of symptoms according to the patient interview, and the date of remission of hypercortisolism.

Statistical analysis

A power analysis was performed: according to a hypothesis that the percentage of correct answers to the Bordeaux maze test would be 80% in controls, with a standard deviation of 13% and 60% with a standard deviation of 23% in CD patients, 25 patients per group were necessary to have a power of 80% associated with a type 1 risk of 5%. Cognitive performance parameters recorded in each task and scores recorded in the different scales assessing QoL, anxiety and depression were analyzed with ANOVAs to assess potential effect of group factor. Comparison of data

between patients with recovery of the HPA axis and those with persistent corticotropic insufficiency were analyzed using the t-test or Mann-Whitney's test according to the distribution of values. All results are expressed as mean ± SEM. Multiple correlation analysis were performed with Spearman test after Log transformation of data using GraphPad software (San Diego, CA). We also compared Z-scores (computed using log data for each participant taking into account the mean and SD of each measure) of SF36 items in controls versus patients. A negative z-score indicates that patients has lower quality of life than the corresponding normative population. The Bonferroni method was used to reduce the likelihood of type I error. Since we performed 4 different evaluations (cognitive, depression, anxiety, and quality of life), a p value ≤0.01 was considered as significant.

Results

Characteristics of patients and controls

Clinical files of 192 patients with CD were analyzed. Twenty-five (19 women and 6 men, aged 44.5 ± 2.4 y) in long-term remission of CD (102.7 ± 19.3 months; range =17-364) satisfied the inclusion criteria and accepted to participate (Table 1 and Figure 1). Ten were recruited in Barcelona and 15 in Bordeaux. Twenty-two were cured following a single pituitary surgery. Among these, 13 patients had a normal biological evaluation of the HPA axis and 9 had persistent corticotropic insufficiency. Three underwent bilateral adrenalectomy following 1 (n=1) or 2 (n=2) unsuccessful pituitary surgical attempts. Hydrocortisone was given at 11.3 ± 1.9mg/m^2 dose divided in 2-3 daily intakes in 12 patients (e.g. 10 mg/day in 3, 11 to 20 mg/day in 5 and 21 to 30 mg/day in 4.

Thirteen patients had a BMI ≥ 25 and < 30 kg/m^2. Five were supplemented with levothyroxine and 2 with recombinant GH. Blood pressure was controlled pharmacologically in 5 hypertensive patients (≤135/85 mmHg); only one patient was treated with antidiabetic hypoglycemic oral drugs (HbA1C = 6.8%).

Mean UFC at diagnosis was 4.4 ± 0.6 x ULN (range 1.5 – 12.4). An improvement in the clinical score was observed between diagnosis and the time of evaluation (12.6 ± 0.7 vs 2.2 ± 0.5, respectively; p < 0.0001). Clinical scores at diagnosis and at the time of evaluation were similar between patients with and without adrenal insufficiency (13.5 ± 1.0 and 11.7 ± 0.9; p=0.2 and 2.3 ± 0.8 vs 2.2 ± 0.5, p = 0.91, respectively).

Twenty-five subjects (19 women and 6 men, aged 44.3 ± 2.3 y), were recruited as controls. CD patients and controls had similar characteristics (Table 1). Apart from one individual receiving adequate levothyroxine supplementation for hypothyroidism, no control subject had a history of endocrine disease.no control subject had a history of endocrine disease.

CD patients in long-term remission have preserved cognitive performance

CD patients in long-term remission displayed similar performance to controls in all cognitive tests. Regarding declarative memory, total words recalled in the RAVLT test was not different between control and CD groups [group effect: F (1,48)=1.81; p=0.18] and CD patients in remission performed as controls in all RAVLT subtests [i.e. delayed recall: F (1,48)=1.7;

TABLE 1 Characteristics of participants in the Memocush study.

	Patients (n=25)	Controls (n=25)
Age (years)	44.5 +/- 2.4	44.3 +/- 2.3
Women (%)	76	76
Level of education (from 1 to 5)	Level 4 (n=8) & Level 5 (n=17)	Level 4 (n=7) & Level 5 (n=18)
BMI (kg/m^2)	24.2 +/- 0.6	23.1 +/- 0.6
Treated hypertension (N)	5	0
Systolic blood pressure (mm Hg)	123.2 +/- 3.2	116.6 +/- 2.6
Diastolic blood pressure (mm Hg)	74.4 +/- 2.0	71.3 +/- 2.3
Diabetes mellitus (N)	1	0
Fasting glycemia (mmol/l)	4.9 +/- 0.1	5.1 +/- 0.1
HbA1c (%)	5.4 +/- 0.1	5.4 +/- 0.1
Hydrocortisone treatment (Number of patients)	12	0
Hydrocortisone total dose (mg/m^2 per day)	11.3 +/- 1.9	NA
Treated Hypothyroidism (Number of patients)	5	1
Treated GH deficiency (Number of patients)	2	0
Clinical score at diagnosis (/20)	12.6 +/- 0.7	NA
Clinical score at evaluation (/20)	2.3 +/- 0.5	NA

No significant difference was found in the non-specific parameters between patients with a history of Cushing's disease and their matched controls.

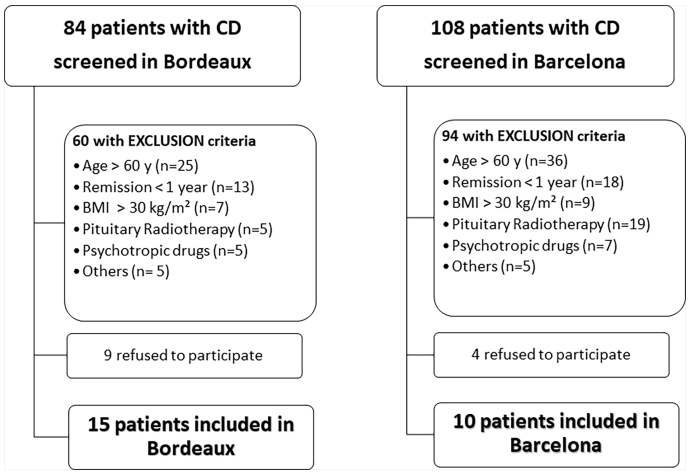

FIGURE 1
Flow chart of the recruitment of patients with Cushing's disease (CD) in the two investigating centers.

p=0.19, for all the subtests, mean and p value in Table 2]. In the Bordeaux Maze declarative memory test, patients learned accurately the initial 6 pairs, needing similar number of training sessions as controls [group effect: F (1,48)=1.7; p=0.19]. When considering the evolution of performance during the last 6 trials preceding achievement of criterion performance, CD patients progressively reached a mean percent of correct choices (96%) close to that of controls (98%), with no difference between both groups (group effect: F (1,48)=1.8; p=0.31; interaction trials X group: F (5,240)=1.2; p=0.19). When submitted to flexibility probes, CD patients accurately expressed their memories of previously acquired information similarly to controls [group effect: F (1,48)=0.7; p=0.39]. 6/25 CD patients displayed performance in the flexibility probes below or equal to 62.5% of correct responses, similar to that observed in the controls.

Regarding working memory, symbol span score was not different between CD patients and controls [group effect: F (1,48) =0.2; p=0.63]. In the spatial addition subtest, CD patients in

TABLE 2 Mean responses during various cognitive tests.

	Controls		CD		p value
List A-1	7.1 ± 0.5	(1/25)	6.4 ± 0.5	(5/25)	p=0.24
List A-2	10.6 ± 0.6	(1/25)	9.7 ± 0.5	(1/25)	p=0.26
List A-3	12.0 ± 0.6	(1/25)	11.0 ± 0.4	(1/25)	p=0.16
List A-4	12.7 ± 0.4	(1/25)	12.2 ± 0.4	(1/25)	p=0.4
List A-5	13.2 ± 0.3	(1/25)	12.7 ± 0.4	(1/25)	p=0.31
List A-6-Retention	12.0 ± 0.5	(1/25)	10.7 ± 0.6	(1/25)	p=0.09
Delayed A	12.2 ± 0.5	(1/25)	11.2 ± 0.5	(1/25)	p=0.19
P	26.0 ± 1.3	(0/25)	26.6 ± 1.3	(0/25)	p=0.76
R	22.5 ± 1.3	(2/25)	20.5 ± 1.3	(1/25)	p=0.28
V	16.5 ± 0.9	(1/25)	17.2 ± 1.0	(0/25)	p=0.59
Animal	33.5 ± 1.5	(2/25)	33.6 ± 1.6	(0/25)	p=0.94
Fruit	17.0 ± 1.0	(9/25)	16.5 ± 1.0	(7/25)	p=0.74
Furniture	21.7 ± 1.0	(0/25)	21.4 ± 0.7	(0/25)	p=0.27
Spatial Addition	14.1 ± 0.8	(4/25)	13.7 ± 0.7	(4/25)	p=0.69
Symbol	25.2 ± 1.3	(3/25)	26.2 ± 1.6	(4/25)	p=0.63
ROCF Copy	55.6 ± 3.6	(0/25)	54.6 ± 3.6	(0/25)	p=0.87
ROCF delayed	33.1 ± 2.6	(1/25)	31.4 ± 2.6	(1/25)	p=0.1

Correct responses in the successive free recall subtests of the RAVLT (26); words produced for each letter or category subtest of the Isaac Set Test (27), performance in the spatial addition test, the symbol test and the ROCF test (29). For each parameter, the number of deficient participants according to the published normative value is specified and the p value indicates the effect of group. CD: patients with a history of Cushing's disease.

remission performed similarly to controls [group effect: F (1,48) =0.2; p=0.69]. In the Bordeaux Working memory test, the total mean correct performance of the CD group was above chance level and not different from controls [group effect: F (1,48)=1.9; p=0.16].

Regarding verbal fluency, CD patients in long-term remission performed similarly to controls both in the semantic subtest [group effect: F (1,48)=0.2; p=0.67] and in the phonetic subtest [group effect: F (1,48)=0.03; p=0.85].

The ROCF scores were all found to be within acceptable limits. Performance on the ROCF copy or the ROCF recall test were not different between both groups [copy: F (1,48)=0.03, p=0.87; recall: F (1,48)=0.09, p=0.76]

According to published normative values for RAVLT-, Isaacs-, spatial addition- and symbol tests, the proportion of patients with abnormal responses was similar in CD and control groups.

Patients with normal HPA axis function performed better than patients requiring hydrocortisone supplementation only during the Isaac lexical test (24.0 ± 1.2 vs 18.6 ± 1.1; p < 0.005).

CD patients in long-term remission have substantially impaired QoL

CD patients scored worse than controls in most subscales of the SF-36 questionnaire taken independently with differences reaching statistical significance for physical functioning, role-physical, general health and vitally:[physical functioning: F (1,48) =15.6; p<0.005; role-physical: F (1,48)=9.2; p<0.005; general health: F (1,48)=10.2 p<0.005; vitality: F (1,48)=6.3; p<0.01; social functioning: F (1,48)=5.1 p<0.05; role-emotional: F (1,48)=3.5; p=0.06; bodily pain: F (1,48)=1.7; p=0.19; mental health: F (1,48) =3.04; p=0.08] (Table 3). Z scores of SF36 (especially physical functioning, general Health and vitality) were also significantly lower in CD patients than in controls (Supplementary Table 1).

CD patients scored worse than controls in the depression rating scale [MADRS: F (1,48)=3.8; p = 0.01]. 10/25 CD patients displayed mild (8/25) or moderate (2/25) depression as opposed to 12% 3/25 of the controls (p=0.04). These mild alterations were not apparent in the depression subscale of the HADS questionnaire [F (1,48)=0.51; p=0.47]. Similarly, no difference was found between patients and controls in the anxiety subscale of the HADS questionnaire [F (1,48)=2.46; p=0.12] (Table 4). The CushingQoL global score was 62.6 ± 3.9.

There was no significant difference in the results of tests between patients requiring hydrocortisone supplementation and patients with a normal HPA axis. Only a nominal association was found for the general health perception of the SF-36 questionnaire (47.3 ± 7.8 vs 66.1 ± 4.9 for patients taking hydrocortisone and patients with normal HPA axis function respectively; p = 0.05).

Worse QoL is correlated to the duration of exposure to cortisol excess

Biological intensity of hypercortisolism was not correlated to the clinical score at diagnosis, nor to the clinical score at the time of evaluation (r=0.39; p=0.10 and r=0.32; p=0.19, respectively).

TABLE 3 Mean score in each subscale of the SF-36 for participants.

	SF36-PF	SF36-RP	SF36-BP	SF36-GH	SF36-VIT	SF36-SF	SF36-RE	SF36-MH
Controls	94.8 ± 1.6 2/25	87.7 ± 5.1 2/25	86.1 ± 3.8 1/25	74.8 ± 2.7 1/25	70.0 ± 3.7 3/25	92.4 ± 3.8 3/25	90.7 ± 4.4 1/25	81.2 ± 2.9 1/25
CD	78.9 ± 3.7 10/25	58.9 ± 7.1 8/25	78.5 ± 4.4 1/25	57.0 ± 4.8 7/25	54.2 ± 5.1 12/25	77.8 ± 5.5 6/25	74.9 ± 7.2 5/25	72.7 ± 3.8 3/25
p value	*p*=0.0003	*p*=0.0039	*p*=0.19	*p*=0.0024	*p*=0.015	*p*=0.03	*p*=0.068	*p*=0.087

CD, patients with a history of Cushing's disease. For each parameter; the number of deficient participants according to the published normative value is specified and the p value indicates the effect of group. PF, physical functioning; RP, Role Physical; BP, Bodily Pain; GH, General Health; VIT, Vitality; SF, Social Function; RE, Role Emotional; MH, Mental Health.

TABLE 4 Mean score in each subscale of the Hospital Anxiety and Depression Scale (HADS) and the Depression Rating Scale (MADRS) for each group of participants.

	HADS Depression	HADS Anxiety	MADRS
Controls	3.1 ± 0.7 Doubtful cases : 1/25 Certain cases : 1/25	6.6 ± 0.7 Doubtful cases : 1/25 Certain cases : 4/25	5.0 ± 1.4 Mild depression : 2/25 Moderate depression : 1/25
CD	3.8 ± 0.6 Doubtful cases : 3/25 Certain cases : 0/25	7.4 ± 0.7 Doubtful cases : 3/25 Certain cases : 7/25	9.8 ± 1.1 Mild depression : 8/35 Moderate depression : 2/25
p value	*p*=0.47	*p*=0.12	*p*=0.04

CD, patients with a history of Cushing's disease. For each test, the number of participants with abnormal score is specified according to normative values and the p value indicates the effect of group.

None of the QoL, cognition and anxiety/depression evaluations including all SF-36 subscales correlated with the biological intensity of hypercortisolism and clinical score at diagnosis (p = 0.16 to 0.99). In contrast, the general health, vitality, role physical and physical functioning subscales of the SF-36, as well as CushingQoL were significantly and nominally negatively associated with the duration of exposure to hypercortisolism: r=-0.54, p= 0.004; r=-0.61, p=0.001; r=-0.53, p=0.01; r=-0.49, p=0.02; r=-0.43, p=0.04; respectively. No parameter was associated with the time elapsed between remission and time of testing (p=0.19 to 0.85).

Discussion

The main finding of our study is that, in the absence of obvious confounding comorbidity, adult patients younger than 60 years in surgical remission of CD displayed no long-term memory impairment. These findings were obtained after multiple tests for memory evaluation, including the Bordeaux Maze relational memory test that has been shown to be exquisitely sensitive to mild changes in declarative memory, as occurs with ageing (30, 31, 37, 38). In contrast, and in the absence of obvious psychopathology and major physical sequelae, patients displayed impaired QoL, the intensity of which was proportional to the duration of active hypercortisolism.

Studies evaluating patients in remission of CD show variable improvement in cognitive domains and a recent publication pinpointed that the scarcity of data did not allow metanalysis of studies for memory (6). We hypothesized that cortisol-related comorbidities and treatments of CD may account for persistent memory deficits and impaired QoL. This study was therefore carried out in an attempt to discriminate the respective impact of a direct effect of hypercortisolism on the brain versus that of other causes, directly or indirectly linked to hypercortisolism in persistent memory impairment and altered QoL.

Thus, we strictly excluded putative confounding factors which may explain the discrepancy with more pessimistic studies on the recovery of cognitive functions (9–13, 39–43). For example, while most investigators used an age limit of 70, we decided, based on studies devoted to the decline in memory performance with age (31, 37, 44), to use an age limit of 60 years. Several studies included patients treated for depression. However, both depression and long-term intake of antidepressant drugs can induce cognitive impairments (18, 45). Similarly, the use of any psychotropic drug including sleeping pills, described in almost 1/3 of patients with a history of CD (46), was an exclusion criterion. No major psychological disturbance was found in our patients. A trends towards increased prevalence of depression was found using the MADRS, but depression was rated as mild and patients did not differ from controls during the HADS screening questionnaire for depression and anxiety. Accordingly, the mental health dimension of the SF-36 questionnaire was similar to that of controls. Given the debate on the consequences of brain irradiation, patients after pituitary radiotherapy were excluded (9, 22). Hydrocortisone replacement dosage and GH deficiency were also controlled to avoid interference between imperfect hormonal replacement and cognitive performance (19–21). A delay between remission of CD and recovery of cognitive impairments has also been suggested (2, 10). The longer duration of remission (mean = 8.6 years) before cognitive evaluation in our study as compared to several published studies may partly account for our findings. Interestingly, one of the largest published studies with the longest duration of remission, that also considered a number of confounding comorbidities for selecting patients, only found subtle long-term residual alterations in cognitive function and memory (9). The mild differences with the results of our study, in which several similar cognitive tests were used may also be related to some residual confounders. For instance, our patients were younger (44 ± 2 years vs 52 ± 1 years; P = 0.008) and had a higher educational level, two parameters associated with the outcome of most cognitive tests (9, 13, 43). In addition, 27% of the Dutch patients underwent pituitary radiotherapy, a treatment modality negatively associated with memory and executive functioning (9).

In accordance with previous studies using generic and disease-specific questionnaires (2, 6, 7, 24, 25, 47), CD patients in remission demonstrated persistent worse QoL, with a similar CushingQoL score to those previously published (48). Depression and physical sequelae are major determinants of altered QoL (48). Therefore, an intriguing finding of our study is the contrast between persistently altered QoL on the one hand, and lack of memory impairment, evident psychopathology and major residual somatic comorbidity as illustrated by the minimal residual clinical score on the other hand. These differences between perception and objective findings are reminiscent of the description of an increased negative illness perception in CD (49). Subtle psychopathological alterations and unconscious fears related to the mental experience of hypercortisolism that cannot be detected by questionnaires cannot be excluded. Close links between glucocorticoids and addiction have been described in animal models including mediation of addictive properties of drugs by glucocorticoids (50, 51). The positive reinforcing effects of sustained hypercortisolism may determine reward-related psychopathologies (51) and, due to their psychostimulant properties, corticosteroids have been labelled as drugs of addiction (52). The negative emotional state and symptoms of drug abuse withdrawal (53) are also observed after remission of hypercortisolism, at least in the short-term (54). One could hypothesize that prolonged impaired QoL may share common mechanisms with those observed following withdrawal from drug abuse. In this conceptual framework, it is worth mentioning that improvements in QoL in this last situation can be delayed for decades, with lower indices of well-being over

time in women with a history of psychostimulant abuse as compared to alcohol and cannabis abuse (55). Improvement in psychological care of patients with a history of CD may also benefit from neuroendocrine research in the field of addiction.

Importantly, most of the SF-36 subscales and the CushingQoL score were negatively associated with the calculated duration of exposure to hypercortisolism, but not with the duration of remission. This has also been described in a retrospective survey (47) and is reminiscent of studies showing that the persistence of somatic and psychological cortisol-related comorbidities correlates with the delay to diagnosis (56).

Our study has several limitations. Being cross-sectional, it does not allow comparison with alterations observed during the active phase of CD or concluding that cognitive dysfunction in CD is reversible. However, a cross-sectional study avoids learning the cognitive tests that occurs with their repetition and may result in an apparent improvement of performances. In addition, our study probably escapes the selection bias of longitudinal studies with high losses at follow-up, that may select patients who perform worse (6, 7). Dissociations in the recovery between various cognitive domains have been reported (10, 11) and we cannot exclude the persistence of other less frequently observed cognitive deficits, not explored within our protocol.

The number of patients is relatively small, a problem practically unavoidable in rare diseases, especially if followed up over years, but compares well to most series related to memory performance published to date (6, 10, 12, 39, 41–43). As potent confounders are very common in patients with a history of CD, an important proportion of patients were excluded. Although we agree that this selection excludes the most common situation in which patients have persistent comorbidities, it was mandatory to delineate the specific role of past hypercortisolism and that of persistent cortisol-dependent or non-cortisol-dependent comorbidities on cognition and QoL. However, the comparison of parameters between patients with adrenal insufficiency and those with normal HPA axis function may lack statistical power given the small number of patients.

Among the methodological merits of our study, the careful matching of patients with controls is worth mentioning, a condition that likely resulted in a higher accuracy than studies involving population-based cohorts. In addition, the selection of controls by patients enabled a perfect match for socioeconomic status, which may be a determinant of the outcomes of the questionnaires, along with age, gender, and education level.

In conclusion, our study challenges the concept of irreversible memory impairment due to a specific and direct effect of hypercortisolism in patients below the age of 60. Although an increased vulnerability of the brain to cortisol excess in aged people cannot be excluded, our results suggest that various persisting co-morbidities of CD may be responsible for long-lasting impaired memory and should therefore be actively sought and adequately treated by expert specialists (46). The contrast between lasting impairment of QoL on the one hand and absence of major physical, psychological and cognitive sequelae on the other hand, may reflect irreversible consequences of cortisol excess. The association of negative health perception and impaired QoL with the duration of cortisol excess reinforces the importance of shortening this exposure, especially following diagnosis, to reduce the long-term burden of CD.

Data availability statement

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

Ethics statement

The studies involving human participants were reviewed and approved by CPP Sud-Ouest et Outre-Mer III and ethical committee of hospital de la Santa creu I Sant-Pau. The patients/participants provided their written informed consent to participate in this study.

Author contributions

AS, NE, AM, DC, and AT conceptualized the study. NE and AM developed the Bordeaux maze investigations. EP, AL, and AS performed the investigations and collected the data. NE, EP, and AT analyzed the data. AS, ER, DC, SW, and AT wrote the manuscript. AT takes responsibility for the integrity of the data analysis AF performed the investigations and analyzed the data. All authors contributed to the article and approved the submitted version.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fendo.2022.934347/full#supplementary-material

SUPPLEMENTARY DATA SHEET 1
Computerized maze memory tests assessing cardinal features of declarative memory and working memory.

SUPPLEMENTAL TABLE 1
Z-scores of each SF36 subscale in controls and patients with Cushing's disease in remission Z-scores of log data were computed for each participant taking into account the mean and SD of log SF36 measures. A negative z-score indicates that the population has lower quality of life than the corresponding normative population.

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Trends in hospital admissions for adrenal insufficiency in adolescents and young adults in the 21st century

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Background: Very little is known about the epidemiology of adrenal crises (AC) and adrenal insufficiency (AI) in adolescents and young adults.

Methods: Data on all admissions to Australian hospitals between 2000/1 to 2019/20 for a principal diagnosis of AI (including AC) in 10-24 year olds were extracted from a national repository. Age and sex-specific rates and age-adjusted rates were compared.

Findings: Over the study, there were 3386 admissions for a principal diagnosis of AI; 24.0% (n=812) were for an AC and 50·7% (n=1718) were for secondary AI. Age-adjusted AI admissions increased from 31·70/million in 2000/1 to 54·68/million in 2019/20 (p<0·0001). Age-adjusted AC admissions also increased, most notably in the second decade (from 5·80/million in 2010/11 to 15·75/million in 2019/20) (p<0·00001). Average AI and AC admission rates were comparable between the sexes, but rates increased significantly in females, especially in those aged 20 to 24 years, whose AC rate in 2019/20 (39·65/million) was significantly higher than the corresponding rate in 2000/1 (3·15/million) (p<0·00001). Average age-adjusted SAI admission rates were higher in males (23·92/million) than females (15·47/million) (p<0·00001). However, SAI admission rates increased only among females (from 11·81/million to 22·12/million in 2019/20), with an increase in 20-24 year old females in the second decade from 5·07/million in 2010 to 20·42/million (p<0·00001). Age adjusted admissions for congenital adrenal hyperplasia, primary AI (PAI) and drug-induced AI did not change significantly over the study.

Interpretation: AC/AI admissions increased over the first two decades of this century in the emerging adult population, particularly among females who also experienced a marked increase in AC admission rates, most evident in the

second decade. Although uncertain, possible explanations include: dose of glucocorticoid replacement; non-adherence to therapy; psychosocial factors; and difficulty in transition to adult services. Admissions for SAI also increased, while rates of PAI and CAH remained constant.

KEYWORDS

adrenal, adrenal crisis, adrenal insufficiency, adolescence, emerging adults

Introduction

Adrenal insufficiency (AI) is a rare cause of morbidity and occasional mortality among adolescents and young adults (1–4). The estimated prevalence of AI in this age group is approximately 120/million, with aetiological factors that include congenital and autoimmune disorders, tumours, and traumatic brain injury (1–4). Congenital adrenal hyperplasia (CAH) is the commonest cause of primary AI (PAI) in children (incidence between 1/14000 and 1/18000) (4). Secondary AI (SAI) is less common in this age group, having an estimated prevalence of 25/million, with causes that include congenital anomalies, cerebral tumours and their treatment, and traumatic brain injuries (5). Glucocorticoid induced AI, on the other hand, is thought to be common, may require ongoing glucocorticoid replacement, and is often unrecognised.

All patients with AI are at risk of an adrenal crisis (AC), which is an acute episode of AI characterised by hypotension, electrolyte abnormalities (hyponatraemia and hyperkalaemia), alterations in consciousness, acute abdominal symptoms and hypoglycaemia (in children) (6, 7). Education of patients and their carers, especially with regard to domiciliary emergent glucocorticoid stress dosing (oral, intramuscular or subcutaneous, when necessary), is a key component in AC prevention and can be life-saving (6, 7). Other preventive measures include use of medical jewellery and carriage of a steroid dependency card (6 7). Despite efforts directed at education and prevention, ACs continue to occur at an estimated rate of 6 to 8 ACs/100 patient years in treated AI (8, 9).

AC episodes have been studied in infancy and early childhood, particularly in relation to AC incidence in CAH, and among adults (2, 8–11). However, the occurrence of ACs, and the epidemiology of AI more generally in adolescents and young adults, has not been a focus of research, despite the important personal and social challenges to the self-management of AI that feature during this period of transition into adulthood (12–14). The present study aims to address the paucity of information on AI/AC epidemiology among emerging adults by examining AC/AI hospitalisations in this age group in Australia between 2000/1 and 2019/2020.

Methods

Admission data

Information on all admissions to all Australian hospitals (public and private) is collected by each State or Territory health department. The Australian Institute of Health and Welfare (AIHW) stores these data, which are available for each Australian financial year (July 1 to June 30) in its Principal Diagnosis Datacubes (https://www.aihw.gov.au/reports/hospitals/principal-diagnosis-data-cubes/contents/data-cubes) according to year and principal diagnosis, coded using the International Statistical Classification of Diseases and Related Health Problems, Tenth Revision, Australian Modification Australian modification (ICD CM 11-AM) (15). Variables available in the datacubes and included in the dataset comprise: the principal diagnosis (main reason for the admission); age in five-year categories; sex; and financial year of admission (July 1 to June 30).

For this study, data were extracted from the AIHW for the years 2000/1 to 2019/20 for all admissions of adolescents and young adults aged 10 to 24 years for the following principal diagnoses: hypopituitarism (E23.0 and E23·1); post-procedural hypopituitarism (E89·3); congenital adrenal hyperplasia (E25); primary adrenal insufficiency (PAI) (E27·1); Addisonian (adrenal) crisis (E27·2); drug-induced adrenal insufficiency (E27·3) (excluding information on the agent causing AI); and "Other and unspecified adrenal insufficiency" (E27·4) (including secondary AI, tertiary AI and where the specific form of AI is not given).

For the years 2015/16 and 2016/17, data on the age and sex breakdown of CAH admissions were not published, so mean values of the remaining admissions for CAH for each age and sex group were substituted.

Population data

Information on the age and sex structure of the Australian population is available from the Australian Bureau of Statistics

(https://www.abs.gov.au/statistics/people/population/national-state-and-territory-population/latest-release#data-downloads-data-cubes). Data on the sex and age group (10-14, 15-19 and 20-24 years) of the population between 2001 and 2020 were downloaded to correspond with each year of the study.

Statistical analysis

Downloaded data were entered into Microsoft Excel. Age and sex-specific admission rates were calculated for AC and AI subtypes (according to the ICD-10 CM AM code) for each year. Age-adjusted admission rates were calculated by using the population structure of the year 2000 as the base year.

Z-scores were calculated to assess the difference between admission rates in the first and last years of the study. Poisson regression models were constructed using R version 4·0·2 to assess the effect of secular trends in admission rates (presented as 'trend' with an accompanying p value) overall and for each age and sex group. ANOVA was used to assess the differences in mean admission rates between groups. Where results were only significant for certain sex and age groups, these are presented in isolation.

As the sample sizes for each year were large and multiple comparisons were conducted, a conservative p-value of $p<0·001$ was regarded as significant.

Ethics

As all data used in these analyses were from publicly available datasets, no ethics clearance was required to conduct the study.

Results

Admissions for adrenal insufficiency

There were 3386 admissions for a principal diagnosis of AI or hypopituitarism over the study period, half (50·7%, n=1718) of which were for SAI; 24·0% (n=812) were for an AC; 16·6%, (n=561) were for primary AI; and 8·7% (n=295) were for CAH. Males comprised 52·4% (n=1774) of the sample; 39·5% (n=1339) were aged 10-14 years and fewer (27·4%, n=929) were aged 20-24 years.

Annual AI admissions increased from 126 in 2000/1 to 263 in 2019/20, corresponding to a 72.5% increase in the age-adjusted total AI admission rate (from 31·70/million in 2000/1 to 54·68/million in 2019/20, $p<0·00001$) (Figure 1).

Average age-adjusted admission rates were comparable between the sexes (males: 39·86/million and females: 36·98/million). Within the sexes, however, average age-specific admission rates did not vary significantly by age in females but differed according to age among males ($p<0·00001$) in whom the rate was highest in those aged 10-14 years and lowest among young adults (Figure 2 and Table 1).

Admission rates did not change significantly over the study period in any age category among males or in females aged 10 to 14 years. However, in females aged 15-19 years, AI admission rates increased by 33·4% (from 28·97/million in 2000/1 to 38·64/million in 2019/20, trend, $p<0·001$) (Table 2). There was a more marked (426·1%), increase in the AI admission rate among 20 to 24 year old females (from 14·16/million in 2000/1 to 74·49/million in 2019/20, trend, $p<0·00001$), with the increase being most prominent in the period 2010-2020 (Figure 3).

Admissions for adrenal crises

There were 812 AC admissions over the study period; 43·6% (n=354) of which were in males and nearly half (47·3%, n=384) were in patients aged 20-24 years. Admissions increased from 27 in 2000/1 to 77 in 2019/20, corresponding to a 132·0% rise in the age-adjusted AC admission rate from 6·79/million to 15·75/million ($p<0·0001$), with the increase being most marked in the second decade (Table 2 and Figure 1).

Average age-adjusted AC admission rates were comparable between the sexes (male: 7·79/million and female: 10·06/million). There was significant variation in the average age-specific AC admission rates in females ($p<0·0001$), but not in males (Table 1 and Figure 4). Average AC admission rates among females were lowest in those aged 10-14 years (5·0/million) and highest in those aged 20-24 years (16·1/million) (Figure 4).

AC admission rates in males were steady (9.87/million in 2000/1 and 11.14/million in 2019/20, $p=0.4$) over the study period. In contrast, the female rate increased by 474·7%, from 3·60/million in 2000/1 to 20·69/million in 2019/20 ($p<0·00001$). Within the age-sex specific categories, a significant secular trend in AC admissions was identified only among females aged 20-24 years, in whom the AC admission rate increased substantially. In this group, admission rates rose from 3·15/million in 2000/1 to 39·65 in 2019/20, which included a 152·9% increase in the last 5 years of the study (from 15·68/million in 2015/16 to 39·65 in 2019/20) (trend $p<0·00001$) (Figure 5).

Admissions for secondary adrenal insufficiency

There were 1718 admissions for the combined SAI group (hypopituitarism, "other AI" and drug-induced AI). Admissions rose from 60 in 2000/1 to 137 in 2019/20, corresponding to a 90·6% increase in the age-adjusted admission rate from 15·10/

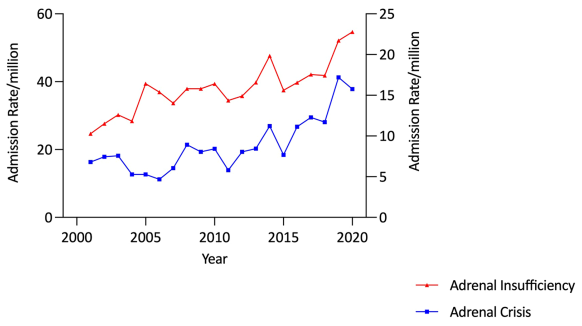

FIGURE 1
Adjusted adrenal insufficiency and adrenal crisis admission rates, 10-24 years, Australia, 2000/1 to 2019/20.

million in 2000/1 to 28·78/million in 2019/20 (trend, p<0·00001). Males comprised 61·5% (n=1056) of these admissions, corresponding to an average adjusted SAI admission rate of 23·92/million which was higher than that in females (15·47/million) (p<0·00001). There was significant variation in the average SAI admission rate in both sexes according to age group (both p<0·00001), with the average rate decreasing with age (Table 1 and Figure 6).

Significant secular trend increases over the study period were observed in females, whose SAI admission rates increased by 87·3% (from 11·81/million to 22·12/million, trend p<0·00001) but similar changes were not evident among males. Within age-sex-specific categories, significant secular trends (p<0·00001) were only identified in young adult females, whose admission rates increased by 302·8% over the second decade (from 5·07/million in 2010 to 20·42/million in 2019/20) after being stable in the first decade (Table 2 and Figure 7).

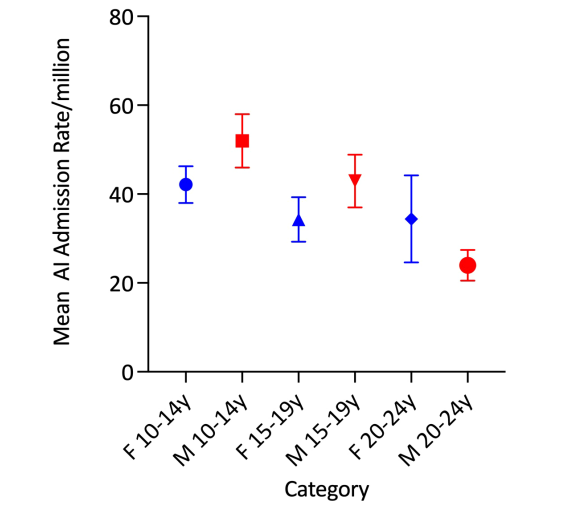

FIGURE 2
Mean total adrenal insufficiency admission rates by age group and sex.

Admissions for "other" adrenal insufficiency

Of the 502 admissions for a principal diagnosis of "Other AI", 51·8% (n=260) were in males. Overall, the age-adjusted "Other AI" admission rate rose by 493·2%, from 1·76 in 2000/1 to 10·44/million in 2019/20 (trend p<0·00001) (Figure 8). The average age-adjusted male admission rate (5·81/million) was comparable to the female rate of (5·48/million). Among males, average age-specific admission rates decreased with age (p<0·00001), with the highest rate being among males aged 10-14 years (10·33/million) but there was no difference in mean rates according to age among females.

Age-adjusted admission rates increased in both males (from 0·99/million to 9·85/million in 2000/1 to 2019/20) and females (2·57/million in 2000/1 to 10·37/million in 2019/20) (both p<0·00001). Within the age-sex specific categories, admission rates for "Other AI" increased in the younger two age groups in males (both p<0·00001) and in the 15 to 19 year and 20 to 24 year age group in females (both p<0·00001) (Table 2).

Admissions for hypopituitarism

There were 1160 admissions for hypopituitarism over the study period, two thirds (66·3%, n=769) of which were in males; 52·1% (n=604) were in aged 10-14 years, and 13·8% (n=160) were in the oldest age group. Age-adjusted admissions for hypopituitarism increased from 13·34/million in 2000/1 to 22·56/million in 2004/5 and then decreased to 17·49/million in 2019/20 (Figure 8).

Average age-adjusted rates were higher in males than females (17·60/million vs 9·32/million, p<0·0001). In both sexes, average admission rates decreased with age (both p<0·00001) (Table 1). No significant secular changes in hypopituitarism admission rates were identified on regression modelling.

Admissions for drug-induced adrenal insufficiency

Of the few (n=56) admissions with a principal diagnosis of drug-induced AI, 48·2% (n=27) were in males and few (n=18) were recorded in the 15 to 19 year or the 20 to 24 year (n=15) groups. Age-adjusted admission rates increased from 0·0/million in 2000/1 to 0·85/million in 2019/20 (p=0.3). There were no significant secular trends identified.

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TABLE 1 Average admission rates by sex and age group and adrenal Insufficiency Subtype*.

	Male			Female			Total		
Age (years)	10-14	15-19	20-24	10-14	15-19	20-24	10-14	15-19	20-24
Primary AI									
PAI	4.1 (3.2, 5.1)	7.3 (5.5, 9.1)	6.6 (5.2, 8.0)	4.3 (3.1, 5.6)	7.9 (6.1, 9.7)	7.5 (5.4, 9.5)	4.2 (3.2, 5.3)	7.6 (5.8, 9.3)	7.0 (5.3, 8.7)
CAH	3.3 (2.0, 4.7)	2.3 (1.1, 3.6)	0.5 (-0.02, 1.0)	9.4 (7.5, 11.3)	3.8 (2.9, 4.6)	1.4 (0.8, 2.0)	6.3 (4.2, 8.5)	3.1 (2.0, 4.1)	0.9 (0.4, 1.5)
Secondary AI									
Hypopituitarism	25.9 (21.9, 29.9)	19.8 (15.1, 24.5)	6.6 (3.8, 9.4)	16.7 (14.3, 19.1)	7.5 (6.1, 8.9)	3.6 (1.8, 5.3)	21.3 (17.4, 25.1)	13.7 (9.3, 18.1)	5.1 (2.7, 7.4)
Other AI	10.3 (7.0, 13.7)	4.9 (2.9, 6.9)	2.0 (1.1, 2.9)	6.0 (4.2, 7.8)	5.1 (3.2, 7.1)	5.3 (3.1, 7.5)	8.2 (5.4, 10.9)	5.0 (3.1, 6.9)	3.6 (1.9, 5.4)
All SAI	37.1 (32.2, 42.0)	25.3 (20.2, 30.3)	8.9 (6.1, 11.8)	23.4 (20.1, 26.7)	13.4 (11.1, 15.6)	9.4 (6.1, 12.8)	30.3 (25.1, 35.4)	19.3 (14, 6, 24.0)	9.2 (6.2, 12.2)
AC	7.4 (5.5, 9.2)	8.0 (6.2, 9.8)	8.0 (6.2, 9.7)	5.0 (3.8, 6.2)	9.3 (6.6, 12.0)	16.1 (10.4, 21.7)	6.2 (4.6, 7.8)	8.7 (6.4, 10.9)	12.0 (7.6, 16.5)
All AI	51.9 (46.0, 57.8)	42.9 (37.0, 48.9)	24.0 (20.5, 27.4)	42.2 (38.0, 46.3)	34.3 (29.3, 39.3)	34.4 (24.6, 44.2)	47.0 (41.6, 52.5)	38.6 (33.0, 44.3)	29.2 (21.7, 36.7)

*Average admission rate/million (95% confidence intervals).

Admissions for primary adrenal insufficiency

Of the 561 admissions for a principal diagnosis of PAI, 51·3% (n=288) were in females; 39·0% (n=219) were in patients aged 15-19 years and 39·6% (n=222) were for patients aged 20-24 years. Age-adjusted PAI admission rates were constant over the study period; 6·29/million in 2000/1 and 7·62/million in 2019/20.

Average age-adjusted admission rates were 5·99/million for males and 6·55/million in females, and there were no significant differences in the age-specific admission rates in either sex (Table 1). No significant secular trends were identified in age-sex specific PAI admission rates over the time period.

TABLE 2 Admission rates for adrenal insufficiency by sex and age group in 2000/1 & 2019/20*.

		Male			Female			Total		
		10-14	**15-19**	**20-24**	**10-14**	**15-19**	**20-24**	**10-14**	**15-19**	**20-24**
2019/2020	***Primary AI***									
	PAI	6.10	9.12	6.82	7.72	4.14	12.01	6.89	6.70	9.34
	CAH	0.00	1.30	2.27	3.86	5.52	2.40	1.88	3.35	2.34
	Secondary AI									
	Hypopituitarism	21.98	26.06	21.59	16.74	5.52	12.01	19.43	16.09	16.94
	Other AI	18.31	14.33	1.14	15.45	5.52	7.21	16.92	10.05	4.09
	All SAI	40.29	41.70	22.73	33.47	12.42	20.42	36.97	27.48	21.61
	AC	10.99	14.33	7.95	6.44	16.56	39.65	8.77	15.42	23.36
	All AI	57.38	66.46	39.77	51.50	38.64	74.49	54.52	52.95	56.65
2000/1			**Male**			**Female**			**Total**	
		10-14	**15-19**	**20-24**	**10-14**	**15-19**	**20-24**	**10-14**	**15-19**	**20-24**
	Primary AI									
	PAI	5.81	8.77	9.17	1.53	9.15	3.15	3.72	8.96	6.20
	CAH	8.72	1.46	0.00	6.10	3.05	1.57	7.44	2.24	0.78
	Secondary AI									
	Hypopituitarism	29.05	19.00	3.06	15.25	7.62	4.72	22.32	13.43	3.88
	Other AI	2.91	0.00	0.00	3.05	3.05	1.57	2.98	1.49	0.78
	All SAI	31.96	19.00	3.06	18.30	10.67	6.29	25.30	14.93	4.65
	AC	**2.91**	**10.23**	**16.81**	**1.53**	**6.10**	**3.15**	**2.23**	**8.21**	**10.08**
	All AI	49.39	39.46	29.03	27.45	28.97	14.16	38.69	34.33	21.70

*Rate/million.

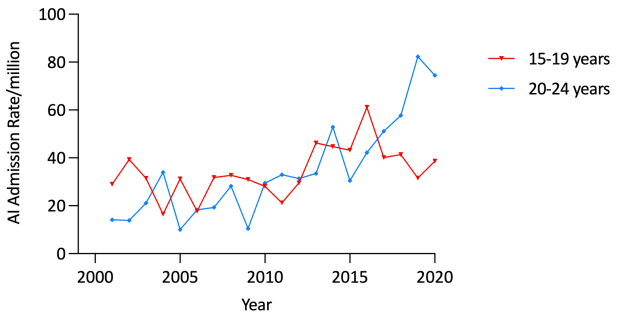

FIGURE 3
Age-specific admission rates for adrenal insufficiency in females aged 15 to 24 years, Australia, 2000/1 to 2019/20.

Admissions for congenital adrenal hyperplasia

Of the 295 admissions for a principal diagnosis of CAH, 69·2% (n=204) were in females. Age-adjusted admission rates for CAH remained stable over the study period (3·52/million in 2000/1 and 2·52/million in 2019/20). Average age-adjusted admission rates were 4·89/million in females and 2·07/million in males. Among females, average age-specific admission rates diminished with increasing age ($p<0·00001$), but rates did not change with age among males (Table 1). No significant secular trends in the age-sex specific categories were identified.

Discussion

This study is the first to analyse trends in hospital admissions for AI/AC specifically in adolescents and young adults, a population about which little is known regarding the epidemiology of AI. It showed that, between 2000/1 and 2019/20, admissions for both AI and AC increased but that there was a greater increase in AC relative to AI admissions. The secular changes in AI/AC admissions were most evident in the second decade, especially among women aged 20-24 years, in whom AC admission rates increased from 3·15/million in 2000/1 to 39·65/million in 2019/2020. Although unknown, possible reasons for this increase include: psychosocial factors; difficulties with

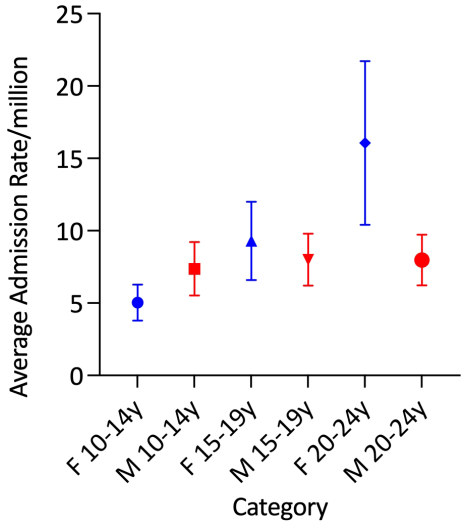

FIGURE 4
Mean adrenal crisis admission rates by age group and sex.

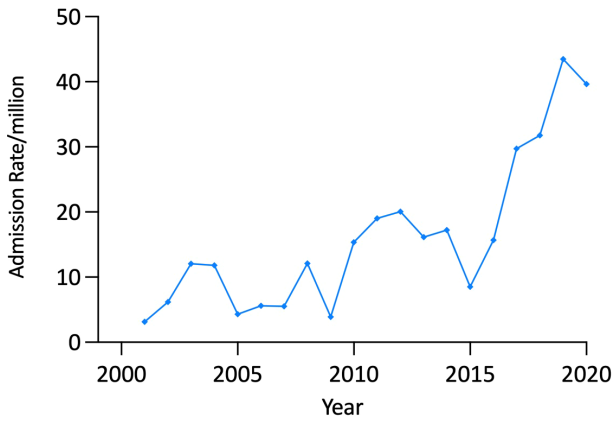

FIGURE 5
Adrenal crisis admission rate, females aged 20-24 years by year.

transition to adult health services; non-adherence to therapy or lower dosing of glucocorticoid replacement. Admission rates for all forms of SAI also increased, particularly in females. Over the same period, there was a substantial rise in "Other AI" admissions, which was partially offset by a relative decline in admissions for hypopituitarism which occurred in both sexes and across the age-spectrum. This suggests a change in diagnostic classification of patients, possibly to reflect more accurately the reason for hospitalisation in patients with SAI and at least one other pituitary hormone deficit. In contrast, admissions for PAI, CAH and drug-induced AI remained constant, with the latter two diagnoses being rare in this population.

The reason for the increase in AI and AC admissions is uncertain, although the comparatively greater rise in AC relative to AI admissions in the second decade suggests an increase in severity of presentations. This may be attributable, at least in part, to the uptake of recommendations advocating use of lower doses of short acting glucocorticoid replacement therapy (hydrocortisone or cortisone acetate) which may expose patients to periods of hypocortisolaemia, potentially predisposing them to an AC (9, 16, 17). Alternatively, diagnostic classifications of AI/AC may have evolved, leading to an increased use of "AC" when previously the more general "AI" diagnosis may have been used (18). However, the variations in AC rate increases identified between the age and sex groups in

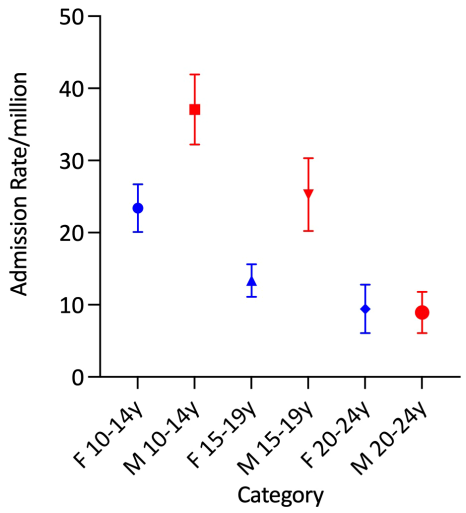

FIGURE 6
Mean secondary adrenal insufficiency admission rates by age group and sex.

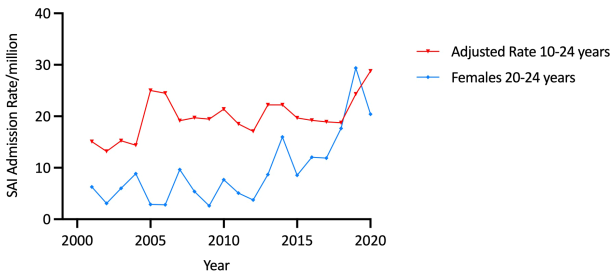

FIGURE 7
Age-adjusted 10-24 years and female 20-24 year admission rates for secondary adrenal insufficiency by year, Australia, 2000/1 to 2019/20.

this study suggest that a generalised trend to diagnose patients who may be symptomatic but not necessarily severely ill with an AC rather than "AI" did not occur. Increased AC rates have also been observed among older adults in the same population, suggesting that factors underpinning the observed change affect a wide range of patients and are persistent (16).

Although average AC admission rates were comparable between the sexes, and admissions increased in both males and females, the increase among females was considerably larger than that in males, especially in the 20-24 year age group, despite relative parity in the underlying epidemiology of AI and its causes between the sexes. The reasons for this gender disparity are unknown. While physiological changes during puberty often require recalibration of glucocorticoid replacement doses, this would not be a prominent issue in young adults. One possible contributory factor may be the modern approach to glucocorticoid replacement, mentioned above, which is recommended for all patients but, possibly due to greater concern about steroid-related weight gain, may be used by some females at a lower dose/BSA than males. Eating disorders also tend to arise in this age group, are more common in females, and have been shown to increase the incidence of diabetic ketoacidosis (DKA) in young women with type 1 diabetes mellitus (T1DM) (19). By extension, eating disorders among young adult females with AI may be associated with patient-initiated dose omission or reduction in the prescribed daily dose of glucocorticoid replacement therapy, which would increase AC risk. Patients in this age group may also present with an AC in the context of previously undiagnosed AI, often after several attendances for health care with typical symptoms but without appropriate treatment (20).

Variation in AC predisposition has been reported in cohort studies in which some patients had no AC events while others had more than one episode (8, 9). Recurrent admissions for individuals could not be identified in this study but it is likely that some patients contributed more than one AI/AC admission. In the more common endocrine emergency, DKA, clinical experience, together with evidence from a recent meta-analysis, suggests that psychosocial factors appear to account for more frequent hospital presentations in some patients (21–23). In Australia, DKA incidence is highest among 15-19 year old females and, in the young adult age group, there is a female predominance at a ratio of 1·4:1 to males (22). DKA is also more common in lower socioeconomic groups (21, 22). Overall, about 40% of cases of DKA are thought to be due to non-adherence with the treatment regimen (22). Given that AI and T1DM both require daily commitment to self-management and changes to protocols during intercurrent illness, it is likely that similar

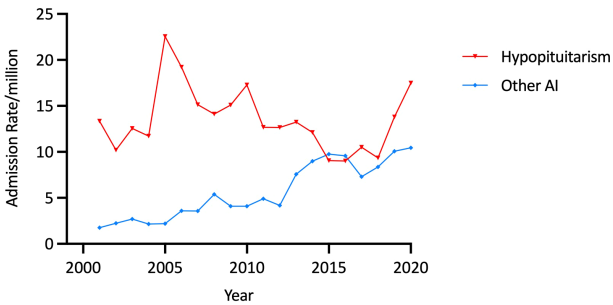

FIGURE 8
Age-adjusted admission rates for "Other AI" and hypopituitarism, 10-24 years, Australia, 2000/1 to 2019/20.

psychosocial factors to those identified in T1DM patients would influence self-management in emerging adults with AI, and that this may be reflected in AC incidence, including the high rate of AC events in women aged 20-24 years (2). This phenomenon needs further exploration, particularly in light of the secular trends observed in this study.

Admissions for SAI were more common in males, which is consistent with evidence from previous studies, but females experienced a greater increase in SAI admission rates, to reach near equivalence in the last year of the study (24). Males in this age group are considered to have a higher prevalence of SAI due to increased rates of traumatic brain injury and cerebral tumours (and their treatment) (6, 24, 25). In this study, the proportionate increase in SAI among females exceeded the 14-fold increase in AC rates in young adult women, a pattern of occurrence that requires further investigation, both with regard to the aetiology of SAI and to the identification of the underlying AC precipitants. While pituitary adenomas increase in prominence as a cause of SAI in adulthood, and are typically identified earlier in females due to menstrual disturbances, this does not explain the increase in SAI admissions or the disparity in rates of change between the sexes found in this analysis. Contributory factors may be increased cerebral imaging uncovering asymptomatic pituitary tumours in young women resulting in treatment-related SAI, and an unrelated but possible increase in autoimmune hypophysitis (26, 27).

Studies have shown that adolescents and young adults with chronic health conditions, such as AI, face a range of challenges and have unique vulnerabilities as they progress from reliance on specialist paediatric care and parental oversight to effective self-management (14). In Australia, this typically occurs after 18 years of age, when young people are required to move from the paediatric environment to the less well-defined setting of adult healthcare services. Accessing developmentally appropriate support for these patients may be difficult, particularly in non-urban areas, and financial burdens may lead to lower levels of engagement with healthcare services and lower levels of use of appropriate management and AC preventative strategies in this age group (28). It is likely that these vulnerabilities vary between individuals, and that other factors, such as use of recreational drugs and alcohol, would also affect treatment adherence and increase AC risk (18).

This analysis found that admissions for a principal diagnosis of CAH were rare, despite this being the most common underlying cause of AI in young people. This may be indicative of the phenomenon of "missing" CAH patients that has been reported following discharge of individuals with CAH from specialist paediatric care (29). It is possible that teenage and young adult patients with CAH were admitted with an AC or were included in the "Other AI" diagnostic category when admitted for treatment of symptomatic AI. Alternatively, their AI diagnosis may be seen as a secondary problem relative to other reasons for admission, such as management of fertility and genitourinary issues. Admissions for a principal diagnosis of "drug-induced AI" were also very uncommon, despite drug-related iatrogenic AI being considered a common, often undiagnosed, and potentially increasing cause of AI in populations (30). It is possible that, in this dataset of hospital admissions, some patients coded as having an AC or another subtype of AI may have had drug-induced AI, thereby leading to an underestimate of the true rate of admissions for this category of AI. In addition, patients with comorbid drug-induced AI who were admitted for another related principal diagnosis, such as asthma, would not have been identified in this analysis.

The data used in this study are from a large, nationwide, population-based database of all admissions for a principal diagnosis of AI/AC spanning 20 years. As the data were for the principal diagnosis of each admission only, the rates of admission found in this study would be an underestimate of the true AI-related admission rate in this population. Whether a sick patient was diagnosed as having an AC or the less severe, AI (or its subtypes), was dependant on the diagnostic criteria used by the treating clinician. As there was only one diagnosis per patient, when an AC was nominated as the principal diagnosis, neither the underlying subtype of AI nor the precipitant of the AC could be determined. Further, these data were not matched for individual patients and, therefore, the influence of a small group of patients having repeat admissions could not be addressed. Age-adjustment was used to control for the effect of changing population demographics and a conservative level of statistical significance was chosen to address the effects of large sample sizes and multiple comparisons.

In conclusion, this is the first study to comprehensively examine national rates of hospital admissions for AI/AC in adolescents and young adults over the most recent twenty years. Increases in AI/AC admissions in these patients, particularly among females aged 20-24 years, are of concern, have not been reported previously, and are unexplained. Possible reasons include psychosocial factors, problems with transition to adult services; non-adherence or patient-initiated changes to medication; or type and dose of glucocorticoid replacement therapy. Given the patterns observed and knowledge of behavioural and social influences that increase the vulnerabilities of patients in this age group, the causes of the increase are likely to be multifactorial and complex and require further investigation.

Data availability statement

Publicly available datasets were analyzed in this study. This data can be found here: https://www.aihw.gov.au/reports/hospitals/principal-diagnosis-data-cubes/contents/data-cubes.

Ethics statement

Ethical review and approval was not required for the study on human participants in accordance with the local legislation and institutional requirements. Written informed consent from the participants' legal guardian/next of kin was not required to participate in this study in accordance with the national legislation and the institutional requirements.

Author contributions

GC: downloading data, age standardisation and analysis and preparation of results and manuscript. MQ: interpretation of analysis and results and manuscript preparation. DT: planning, interpretation of results and manuscript preparation. HF: interpretation of results and manuscript preparation. RR: analysis and preparation of results and manuscript. All authors contributed to the article and approved the submitted version.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

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Trabecular bone score and sclerostin concentrations in patients with primary adrenal insufficiency

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Background: Patients with primary adrenal insufficiency need lifelong replacement therapy with glucocorticoids and mineralocorticoids, which may influence their bone quality.

Aim: The aim of the study was to evaluate densitometry parameters, trabecular bone score and sclerostin concentrations in patients with primary adrenal insufficiency in comparison to control group.

Materials and methods: We included 29 patients (62% females) with diagnose of autoimmune primary adrenal insufficiency (mean age 49.7 ± 11.7 years, mean duration of the disease 13.2± 13.6 years) and 33 healthy subjects (adjusted with age, sex and body mass index). Bone mineral density at the femoral neck, lumbar spine, total body and trabecular bone score were evaluated. Serum sclerostin concentrations were measured.

Results: There were no significant differences in densitometry parameters (T-score, Z-score, bone mineral density in all locations) as well as in trabecular bone score in patients with adrenal insufficiency in comparison to control group. Mean serum sclerostin concentration was significantly higher in patients with adrenal insufficiency than in control group (44.7 ± 23.5 vs 30.7 ± 10.4 pmol/l, p=0.006). There was a negative correlation between trabecular bone score and the duration of adrenal insufficiency and age, also a negative correlation between femoral neck and total densitometry parameters and 24-hour urine cortisol as a marker of hydrocortisone daily dose in patients with adrenal insufficiency.

Conclusions: The bone status in patients with primary adrenal insufficiency was not impaired in comparison to control group, while sclerostin concentration was higher. The duration of the disease and higher hydrocortisone doses may affect negatively bone status.

KEYWORDS

primary adrenal insufficiency, sclerostin, trabecular bone score, bone mineral density, Addison's disease

Introduction

Primary adrenal insufficiency (Addison's disease) is a rare disorder, characterized by an inability of the adrenal cortex to produce sufficient amounts of glucocorticoids and mineralocorticoids. The prevalence in European countries is about 100-140 cases per million (1, 2) and it is increasing (3). Despite receiving hormonal replacement therapy, patients suffer from impaired quality of life (4–6) and a higher mortality rate - especially if diagnosed at a young age (7–9). It may be a consequence of coexisting autoimmune disorders in about 60% of patients (10), but also adrenal crises (8), malignancies, infections, and cardiovascular complications (7, 11). Another important problem is the risk of osteoporosis, but data concerning patients with primary adrenal insufficiency are inconsistent or even contradictory. The cross-sectional study comprising 292 patients from Norway, Britain, and New Zealand showed a reduced bone mineral density (BMD) at the femoral neck and lumbar spine (12). Another population-based cohort study from Sweden showed an increased risk of hip fracture in patients in comparison to control group, especially females diagnosed with autoimmune primary adrenal insufficiency < 50 years of age (13). There are also studies reporting no significant impairment of BMD (14, 15).

It has been shown, that standard glucocorticoid replacement therapy provides doses higher than produced in healthy adrenal glands, which may affect bone status (16). Hypercortisolemia leads to increased fracture risk due to inhibition of calcium absorption, impaired bone formation, and increased resorption, especially in bone with high trabecular content (17). One of the mediators of the adverse effect of glucocorticoids on bone may be the influence on Wnt/βcatenin signaling, a pathway known to promote differentiation of mesenchymal stem cells into osteoblasts. Canonical Wnt signaling also stimulates osteoblast maturation and survival of osteoblasts and osteocytes, while decreasing osteoclast generation (18). Glucocorticoids inhibited intracellular Wnt signaling, resulting in the suppression of osteoblast differentiation *in vitro* (19, 20). Sclerostin, encoded by the Sost gene, is a protein secreted mainly by osteocytes, that inhibits Wnt/βcatenin signaling mainly through interaction with the lipoprotein receptor-related protein (LRP) family (18). In mice, glucocorticoids increased the expression of Sost gene, while knockout mice lacking Sost were protected from bone loss in conditions of glucocorticoid excess (21). Additionally, treatment with antibodies against sclerostin in mice prevented glucocorticoid-induced osteoporosis (22). In line with animal results, in a study comprising patients treated with more than 7.5 mg of prednisolone per day for a year, sclerostin concentration increased significantly and correlated with glucocorticoid dose (23). On the other hand, in 21 patients with chronic endogenous Cushing syndrome, sclerostin concentration in serum was significantly reduced in comparison to control group, and increased after treatment (24). In a recent randomized intervention study, 64 healthy males were given a placebo or prednisolone 7.5 mg daily, or prednisolone 30 mg daily for 2 weeks. Compared with placebo, prednisolone high-dose decreased serum sclerostin concentrations, while low-dose did not alter sclerostin concentrations (25). Another study did not show any significant influence of short-term hypercortisolemia after adrenocorticotropin (ACTH) infusion on sclerostin concentrations in 17 healthy subjects (26). In summary, studies *in vitro* and in mice suggest that inhibiting the canonical Wnt/β-catenin signaling pathway, especially through increased expression of its antagonists like sclerostin, is an important factor contributing to glucocorticoid-induced osteoporosis. In humans results are inconsistent. The duration and type of used glucocorticoid may be crucial. Some authors also suggest, that chronic hypercortisolemia affects the number and function of osteocytes, cells that are the main source of sclerostin, rather than affects directly sclerostin concentration (24).

The trabecular bone score (TBS) is a new diagnostic method, providing indirect information on trabecular bone microarchitecture (27). It is obtained from the computed evaluation of pixel grey-level variations in lumbar spine dual-energy X-ray absorptiometry (DXA) images. TBS is correlated with trabecular bone volume, the number of trabeculae and their connectivity (28). It has been shown, that in patients with endogenous hypercortisolemia TBS was significantly lower than in control group and correlated with disease duration (29).

To our best knowledge, there was no study evaluating TBS and sclerostin concentrations in patients with primary adrenal insufficiency so far. It may be important because of

glucocorticoid deficiency – a mainstay of the disease, but also common overtreatment with hydrocortisone in the replacement therapy. There are other factors contributing to osteoporosis in patients with primary adrenal insufficiency, like deficiency of adrenal androgens (30) or other autoimmune diseases that need to be taken into consideration.

In this context, the aim of our study was to evaluate TBS and sclerostin serum concentrations in patients with autoimmune primary adrenal insufficiency.

Materials and methods

Subjects

We included 29 patients with diagnose of autoimmune primary adrenal insufficiency (18 women, 11 men; mean age 49.7 ± 11.7 years) and sex-, age-, body mass index (BMI) matched 33 controls (20 women, 13 men, mean age 54.8 ± 9.5 years), who were patients of Department of Endocrinology, Diabetes and Isotope Therapy in Wrocław, Poland, between February 2020 and February 2022. All subsequent patients followed up in our department were included in the study. Only one patient denied to take part in the study. Three patients were newly diagnosed with Addison`s disease, so we waited 6 months of stable therapy to perform examinations. The patients with longer duration of the disease had stable doses of replacement therapy for at least a year. In case of infections doses were increased for a short period of time, however 3 months before our examinations doses were not modified.

According to the Endocrine Society Clinical Guidelines, the primary adrenal insufficiency diagnosis was established on the basis of the following criteria: peak cortisol concentrations below 18 μg/dl at 30 or 60 minutes after corticotropin stimulation test (250 μg) as a gold standard, alternatively morning cortisol concentration below 5 μg/dl in combination with ACTH concentration increased twofold the upper reference range as a preliminary test (31). The inclusion criteria was the diagnosis of autoimmune primary adrenal insufficiency treated with a hormonal replacement therapy for at least 3 months. The autoimmune cause of the adrenal insufficiency was made on the basis of positive testing for autoantibodies against adrenal 21 hydroxylase or coexistence of other autoimmune diseases, without any other known cause of adrenal insufficiency.

A thorough medical history was taken, including detailed information about hormonal replacement therapy (current doses and mean doses for the last 3 months) and osteoporotic risk factors. The physical examination included measurement of height in cm, weight in kg, on this basis BMI was calculated. The patients did not report changes in body height. The waist circumference was measured with tape, halfway between the lowest rib and the top of the hipbone. The hip circumference was measured with tape, at the widest part of the buttocks.

We collected concentrations of following parameters in blood: morning ACTH, direct renin concentration, 25(OH) vitamin D, dehydroepiandrosterone sulfate (DHEA-S), estradiol (E2), follicle-stimulating hormone (FSH), luteinizing hormone (LH), total testosterone (T), sex hormone binding globulin (SHBG), thyroid-stimulating hormone (TSH), free thyroxine (fT4), insulin-like growth factor (IGF-1), parathormone (PTH), calcium, and alkaline phosphatase (ALP). The free androgen index (FAI) was calculated as the ratio of T divided by SHBG and multiplied by 100. We also measured urinary free cortisol (UFC). An oral glucose tolerance test (OGTT) with 75 g glucose in patients without known diabetes was conducted.

In the group with adrenal insufficiency 5 patients had type 1 diabetes treated with insulin, one patient had type 2 diabetes, 21 patients had primary autoimmune hypothyroidism treated with L-thyroxine. In the control group, 4 patients had type 2 diabetes, 4 patients had autoimmune hypothyroidism treated with L-thyroxine. The doses of L-thyroxine and insulin were stable for at least 3 months, there were no exacerbations of concomitant diseases for at least 3 months.

In patient`s group there were 10 postmenopausal women (56%), four of them received hormonal replacement therapy, 8 women were premenopausal (44%). In the control group there were 12 postmenopausal women (60%), none of them received hormonal replacement therapy, 8 women were premenopausal (40%).

On the basis of the WHO densitometric criteria (32), in the adrenal insufficiency group at the lumbar spine 13 patients had osteopenia, 3 had osteoporosis. At the femoral neck 14 patients had osteopenia, 2 had osteoporosis. In the control group at the lumbar spine 8 subjects had osteopenia, 3 had osteoporosis. At the femoral neck 14 controls had osteopenia, no one had osteoporosis.

No patient from the adrenal insufficiency group or control group had pituitary insufficiency, hyperprolactinemia, hyperparathyroidism (based on current hormonal measurements) or osteoporotic fracture (based on medical history and clinical examination). All patients had normal kidney and liver function.

Laboratory examinations

The ACTH, LH, FSH, E2, T, DHEA-S, PTH,fT4 concentrations were measured by chemiluminescence immunoassay method using Immulite 2000 (Siemens Healthcare Diagnostics, USA). Following reference ranges were used: ACTH <46 pg/ml (analytical sensitivity: 5.0 pg/ml); LH premenopausal women: follicular phase 1.1-11.6 mIU/ml, ovulation phase 17.0-77.0 mIU/ml, luteal phase 0.7-14.7 mIU/ml; postmenopausal women: 11.3-39.8 mIU/ml; men: 0.8-7.6 mIU/ml (analytical sensitivity: 0.05 mIU/ml); FSH premenopausal women follicular phase 2.8-11.3 mIU/ml,

ovulation phase 5.8-21.0 mIU/ml, luteal phase 1.2-9.0 mIU/ml; postmenopausal women 21.3-153.0 mIU/ml; men 0.7-11.1 mIU/ml (analytical sensitivity: 0.1 mIU/ml); E2 premenopausal women: follicular phase <160.0 pg/ml, ovulation phase 34–400.0 pg/ml, luteal phase 27.0–246.0 pg/ml; postmenopausal women <30.0 pg/ml; men <56.0 pg/ml (analytical sensitivity: 15.0 pg/ml); T premenopausal women 0.2–0.72 ng/ml, postmenopausal women 0.2–0.43 ng/ml, men 0.72–8.53 ng/ml, >50 years 1.29–7.67 ng/ml (analytical sensitivity: 0.15 ng/ml); DHEA-S: women 35.0–430.0 µg/dl; men 80.0–560.0 µg/dl (analytical sensitivity: 3.0 µg/dl), PTH 11.0-76.0 pg/ml (analytical sensitivity: 3.0 pg/ml), fT4 10-22 pmol/l (analytical sensitivity: 0.5 pmol/l).

UFC was measured using a radioimmunoassay method (Immunotech, Beckman Coulter Inc., Prague, Czech Republic), reference range in control group 14.0–120.0 µg/24 h. There is no available reference range in patients receiving hydrocortisone replacement therapy.

25(OH)D concentrations were measured by chemiluminescent immunoassay using Architect i1000 (Abbott Laboratories, USA), reference ranges: vitamin D deficiency <20 ng/ml, suboptimal status 20–30 ng/ml, optimal status 30–70 ng/ml. Limit of detection (LOD) was 2.2 ng/ml, limit of quantitation (LOQ) was 2.4 ng/ml.

Serum calcium and alkaline phosphatase were measured using colorimetric assays on an Architect c4000 (Abbott Laboratories, USA). Reference ranges were as follows: calcium 8.4–10.5 mg/dL (LOD: 0.5 mg/dl; LOQ: 1.0 mg/dl); alkaline phosphatase 40–150 IU/l (LOD: 5.0 IU/l; LOQ: 5.0 IU/l).

Sclerostin in the serum was measured using a sandwich ELISA kit from Biomedica (Biomedica Wien, Austria). The analysis was performed in accordance with the attached protocol in sample duplicate during a single session.

BMD and TBS assessment

BMD at the femoral neck, lumbar spine (L1-L4), and total body were evaluated using the DXA technique (Hologic Horizon A densitometer). The coefficients of variation (CV) were as follows: females - femoral neck 1.69%, lumbar spine 1,6%, total body 1%; males - femoral neck 1.8%, lumbar spine 1.3%, total body 1%. Results were presented as BMD (g/cm^2), T-score, and Z-score. According to WHO criteria in postmenopausal women and men aged >50 years, we used the following categories: T-score ≥-1 standard deviations (SD) – normal; T-score between -1 and -2.5 SD – osteopenia; T-score ≤-2.5 SD – osteoporosis. In premenopausal women or men aged < 50 years we used Z-score: values of -2.0 SD or lower are stated "below the expected range for age" and those above -2.0 SD "within the expected range for age (32).

TBS values were obtained from lumbar spine DXA images using TBS iNsight software, version 3.0.3.0 (Med-Imaps, Pessac, France). In line with other studies, the following criteria were used: TBS ≥1.31 normal, 1.31–1.23 partially degraded microarchitecture, ≤1.23 degraded microarchitecture (33).

Statistical analysis

Statistical analysis was performed using Statistica software for Windows, version 13.3 (StatSoft),. The mean, median, standard deviation (SD), and interquartile ranges (IQR) were determined for all variables. The Shapiro–Wilk test was used to check the normality of the data distribution. Student's t-test or Mann-Whitney test was applied to compare quantitative variables, whereas categorical variables were compared by the chi-square test or Fisher's exact test. Correlations between parameters were evaluated using Pearson's test or Spearman's rank correlation test as appropriate. Moreover, a multiple regression analysis was used to identify the predictors of TBS. P-value <0.05 was considered statistically significant

Ethics

The Bioethics Committee of Wroclaw Medical University approved the protocol of the study. All subjects signed informed consent forms in accordance with the Declaration of Helsinki. The participants provided their written informed consent to participate in this study.

Results

The general characteristics of the group with primary adrenal insufficiency and controls are presented in Table 1. The adrenal insufficiency group comprised 29 patients (62% females, mean age 49.7 ± 11.7 years) and the control group comprised 33 subjects (61% women, mean age 54.8 ± 9.5 years). There were no significant differences in age, sex, body mass, and BMI between the studied groups. The patient`s group and controls did not differ significantly in case of the frequency of postmenopausal women.

Mean plasma ACTH concentration and UFC were significantly higher in group with the adrenal insufficiency (p<0.000 and p=0.013, respectively), while controls had higher DHEA-S concentration (p<0.000).

The characteristic of disease duration and hormonal replacement therapy in the adrenal insufficiency group is presented in Table 2. Mean disease duration was 13.2 ± 13.6 years, mean age at diagnosis was 36.6 ± 10.9 years. All patients were treated with hydrocortisone (mean daily dose 25.8 ± 6.2 mg), one patient received also 0.125 mg of dexamethasone per day. 19 patients received ≥ 25 mg of hydrocortisone daily. 26 patients received fludrocortisone (mean dose 0.07 ± 0.06 mg).

TABLE 1 Characteristics of the patients with primary adrenal insufficiency and controls.

	Adrenal insufficiency (n = 29)			Control group (n = 33)			p-value
	Mean ± SD	Median	IQR	Mean ± SD	Median	IQR	
Age (years)	49.7 ± 11.7	48	39-58	54.8 ± 9.5	55	46-64	0.052
Body mass (kg)	72.8 ± 16.3	70	59-82	78.7 ± 11.4	80	70-85	0.098
BMI (kg/m^2)	25.6 ± 4.4	26.4	21.9-28.7	27.4 ± 2.7	27.5	25-29.2	0.064
ACTH (pg/ml)	584.5 ± 477	421	152-1184	19.4 ± 19.2	14.7	10.6-20.1	**<0.000**
UFC (µg/day)	88.5 ± 61.6	72.8	39.9-123.9	49.7 ± 23.3	43.8	34.3-59.6	**0.013**
DHEA-S (µmol/l)	24.1 ± 17.7	15	15-28.6	124.04 ± 67.5	112	77.2-158.0	**<0.000**
25(OH) vitamin D (ng/ml)	31.3 ± 14.0	28.8	22.2-40.5	25.9 ± 6.5	25.6	22.1-29.5	**0.049**
PTH (pg/ml)	28.7 ± 12.8	26.2	19.0-37.3	41.8 ± 21.7	34.9	25-57	**0.019**
ALP (U/l)	55.1 ± 15.1	52.5	43.4 ± 65.0	63.2 ± 17.7	61	50-72	0.059
SCL (pmol/l)	44.7 ± 23.5	35.4	30.5-48.9	30.7 ± 10.4	28.7	24.3-35.5	**0.006**
LS T-score	-0.83 ± 1.3	-0.9	-1.9-0.4	-0.90 ± 1.0	-0.5	-1.5-(-)0.1	0.817
LS Z-score	-0.18 ± 1.3	-0.2	-1.1-0.8	0.06 ± 0.9	0.1	-0.6-0.6	0.371
LS BMD (g/cm^2)	0.97 ± 0.2	1.0	0.9-1.1	0.96 ± 0.1	1.0	0.9-1.0	0.744
FN T-score	-0.90 ± 0.9	-1.1	-1.5-(-)0.5	-0.78 ± 0.9	-0.8	-1.5-(-)0.2	0.617
FN Z-score	-0.17 ± 0.9	-0.2	-0.8-0.3	0.13 ± 0.9	0.2	-0.5-0.5	0.179
FN BMD (g/cm^2)	-0.77 ± 0.1	0.8	0.7-0.9	0.80 ± 0.1	0.8	0.7-0.9	0.380
TBS	1.32 ± 0.1	1.3	1.25-1.37	1.30 ± 0.1	1.29	1.22-1.39	0.630

SD, standard deviations; IQR, interquartile range; BMI, body mass index; ALP, alkaline phosphatase; SCL, sclerostin; BMD, bone mineral density; LS, lumbar spine; FN, femoral neck; TBS, trabecular bone score.
Bold values are considered statistically significant ($p < 0.05$).

There were no significant differences in BMD, T-score or Z-score values at the lumbar spine and femoral neck between the studied groups. TBS also did not vary between groups (p=0.63), as presented in Figure 1. Mean TBS values in both groups indicated partially degraded bone microarchitecture (between 1.31 and 1.23).

The sclerostin concentration was significantly higher in the group with adrenal insufficiency in comparison to the control group (mean values 44.7 ± 23.5 pmol/l vs 30.7 ± 10.4 pmol/l, p=0.006), as presented in Figure 2. There were no significant differences in sclerostin concentration, TBS, BMD at the LS and FN between patients receiving higher vs lower doses of hydrocortisone (we divided groups according to mean daily dose 25.8 mg and median of the daily dose – 25 mg, p> 0.05).

We performed further analyses to assess correlations between sclerostin and other clinical parameters. We only found correlation with serum fT4 concentration (R=0.486, p=0.009; no significant correlation with serum TSH). Other

TABLE 2 Characteristics of the patients with autoimmune primary adrenal insufficiency (n = 29).

	Mean ± SD	Median	IQR
Duration of the disease (years)	13.2 ± 13.6	6	3-26
Age at diagnosis	36.6 ± 10.9	36	29-41
Daily dose of hydrocortisone (mg)	25.8 ± 6.2	25	20-30
Mean daily dose of hydrocortisone from last 6 months (mg)	26.2 ± 6.1	28	22.5-30
Daily dose of fludrocortisone (mg)	0.07 ± 0.06	0.05	0.025-0.1
Mean daily dose of fludrocortisone from last 6 months (mg)	0.07 ± 0.06	0.05	0.034-0.1
Sodium (mmol/l)	139.4 ± 3.2	139	137-141
Potassium (mmol/l)	4.2 ± 0.3	4.2	4.0-4.5
Fasting glucose (mg/dl)	93.3 ± 23	88	81-93
Systolic blood pressure (mmHg)	128 ± 14.4	125	120-140
Diastolic blood pressure (mmHg)	79 ± 10.4	80	75-85

SD, standard deviations; IQR, interquartile range.

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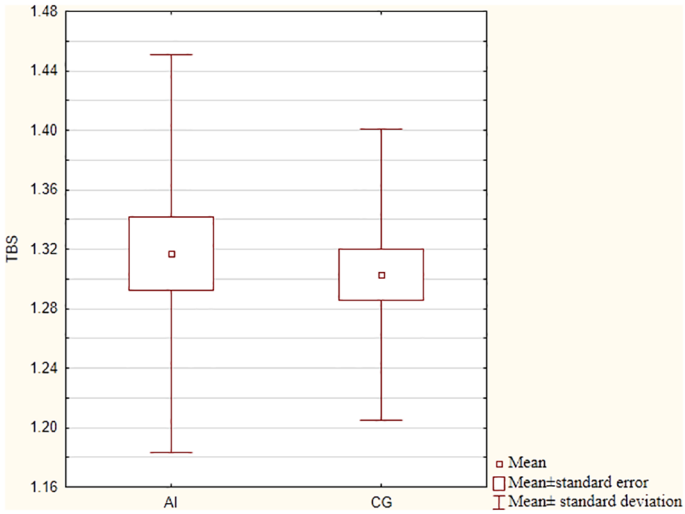

FIGURE 1
TBS values in patients with primary adrenal insufficiency and control group. TBS, trabecular bone score; AI, adrenal insufficiency group; CG, control group.

clinical factors (hydrocortisone dose, duration of adrenal insufficiency, duration of autoimmune hypothyroidism) and hormones were not associated significantly with sclerostin serum concentration.

There were no significant differences in calcium or alkaline phosphatase concentrations between patients and controls, but vitamin D concentration was higher in the adrenal insufficiency group (p=0.049), while controls had higher PTH concentrations (p=0.019).

In patients with primary adrenal insufficiency further analyses were conducted to assess correlations between TBS/densitometry parameters with other clinical characteristics (Table 3). TBS correlated negatively with age (p=0.004), the duration of the disease (p=0.009) and gonadotropin

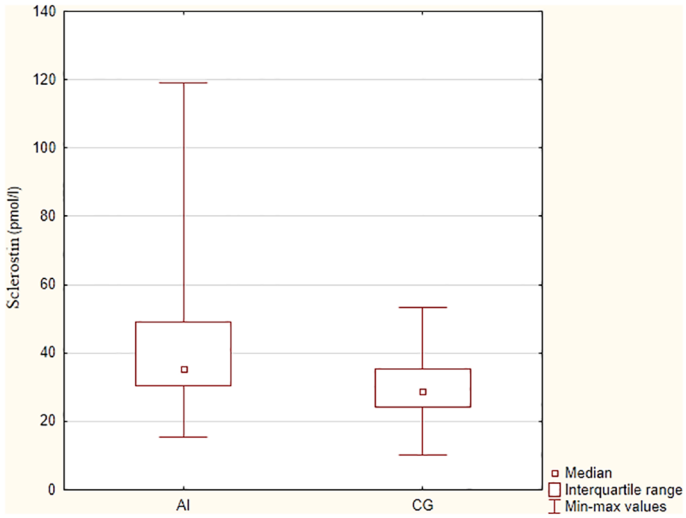

FIGURE 2
Sclerostin concentrations in patients with primary adrenal insufficiency and control group. AI – adrenal insufficiency group, CG – control group.

TABLE 3 Correlations between TBS/densitometry parameters/sclerostin and other clinical characteristics in 29 patients with autoimmune primary adrenal insufficiency.

		R	p
TBS			
	age	-0.523	0.004
	disease duration	-0.479	0.009
	LS BMD	0.615	0.000
	total body BMD	0.475	0.011
	FSH	-0.572	0.001
	LH	-0.387	0.038
LS BMD			
	DHEA-S	0.372	0.046
	FSH	-0.628	0.0003
FN T score			
	UFC	-0.654	0.0003
	FSH	-0.519	0.004
	estradiol	0.381	0.045
	FAI	0.482	0.013
FN Z score			
	UFC	-0.448	0.022
	FAI	0.504	0.009
FN BMD			
	UFC	-0.664	0.0002
	DHEA-S	0.622	0.0003
	FSH	-0.595	0.001
	testosterone	0.515	0.004
	FAI	0.620	0.001
total body T score			
	UFC	-0.476	0.016
	estradiol	0.384	0.048
total body BMD			
	UFC	-0.462	0.020
	DHEA-S	0.603	0.0007
	FSH	-0.733	0.000009
	testosterone	0.529	0.004
sclerostin			
	fT4	0.486	0.009

fT4, free thyroxine concentration; BMD, bone mineral density; DHEA-S, dehydroepiandrosterone-sulfate; FAI, free androgen index; FSH, foliculotropin; LH, lutropin; TBS, trabecular bone score; TBS, trabecular bone score; UFC, urinary free cortisol.

concentrations (p=0.001 for FSH, p=0.038 for LH). There was a positive correlation between TBS and BMD at the lumbar spine (p=0.000) and total body (p=0.011). Further, we conducted a multiple regression analysis to determine the influence of clinical factors and hormones on TBS value. At the beginning, we included factors such as age, disease duration, DHEA-S, FSH, LH, TSH, PTH, IGF-1, BMI. The final model comprised age and LH concentration. The multiple regression analysis revealed that age (ß - (–)0.0042, standard error (SE)- 0.00197, p=0.042) and LH concentration (ß - (-)0.0035, SE - 0.00172, p=0.049) are independent predictors of TBS.

BMD in all examined locations (LS, FN, total body) correlated positively with serum DHEA-S concentrations and negatively with gonadotropin concentrations (Table 3, data not shown for LH). Gonadotropin concentrations were also negatively associated with T-score and Z-score in total body densitometry (T-score and FSH: p=0.00003, R=(-)0.702). Estradiol concentrations were positively correlated with T-score value at the femoral neck (p=0.045) and total body (p=0.048).

DHEA-S was also positively associated with T-score and Z-score at the femoral neck (p=0.016 and p=0.02, respectively) and

total body T-score (p=0.016). Testosterone correlated positively with BMD in FN and total body. FAI was positively associated with FN T-score, Z-score, and BMD.

UFC was significantly negatively associated with densitometry parameters in FN (T-score, Z-score, BMD) and total body (BMD, T-score) (Table 3).

Additionally, we analyzed the influence of most prevalent concomitant autoimmune diseases (autoimmune hypothyroidism in 21 patients and autoimmune diabetes in 5 patients with primary adrenal insufficiency). There were no significant differences in sclerostin concentration, TBS and BMD between patients with and without diabetes in subject with primary adrenal insufficiency (p>0.05). Similarly, we did not observe significant differences in sclerostin, TBS, BMD between patients with and without the diagnose of autoimmune hypothyroidism. There was also no correlation between the duration of autoimmune hypothyroidism and sclerostin (R=0.186, p= 0.34), TBS (R=-0.046, p=0.81), BMD, T-score and Z-score.

Discussion

We found no significant difference between TBS or BMD in patients with autoimmune primary adrenal insufficiency in comparison to controls. To our best knowledge, this is the first report of TBS in patients with Addison's disease. Previous studies examined BMD or osteoporotic fractures in this group of patients with varying results. The biggest cross-sectional study from Norway, UK and New Zealand showed reduced BMD at the FN and LS in comparison to the control group (12). Some authors reported no significant difference in BMD between patients with Addison's disease and healthy subjects, however, numbers of patients were mostly small (14, 15). Some studies showed also an increased prevalence of vertebral and hip fractures in patients with primary adrenal insufficiency (13, 34). Maybe our study group was too small to demonstrate differences in TBS/BMD values between patients and controls because even in larger cohorts those differences were subtle (12). Another interesting aspect is that in our study vitamin D concentration was higher in the adrenal insufficiency group, while controls had higher PTH concentrations. It may suggest, that patients with Addison`s disease used more effective vitamin D supplementation than controls.

The evaluation of osteoporosis risk in patients with primary adrenal insufficiency is difficult because there are many contributing factors. The first of them is overtreatment with glucocorticoids. Studies with the use of thermospray liquid chromatography-mass spectrometry showed that daily steroid production by healthy adrenal glands is about 9.9 ± 2.7 mg/day, (5.7 mg/m^2/day) (16) and the suggested dose in replacement therapy is 15-20 mg of hydrocortisone per day by many authors (35). According to European Adrenal Insufficiency Registry (EU-AIR) data, 42% of patients with adrenal insufficiency received daily 20 to 25 mg of hydrocortisone, while 12.6% over 30 mg per day (36). In our group mean daily dose of hydrocortisone was 26.2 ± 6.1 mg, while 19 patients received 25 mg or more. We found that UFC was significantly higher in patients receiving hydrocortisone than in controls. Espiard et al. have shown, that cortisol and all of its metabolites correlated positively with daily hydrocortisone dose in patients with Addison`s disease and UFC was 3-fold higher than in controls (37) UFC in patients with Addison`s disease may be used as a marker of glucocorticoid dose. We found a negative correlation between UFC and densitometry parameters at the FN and total body. Similarly, in the Norwegian Registry of Addison's disease the Z-scores at the FN, total hip, and total body, but not those at the lumbar spine, were significantly associated with weight-adjusted glucocorticoid dose (12) We found no association between TBS and glucocorticoid dose or UFC. It is known, that hypercortisolemia has a negative impact on bone by many mechanisms, not only reduction in mineralization (17). That is why sole BMD evaluation is not sufficient in estimating glucocorticoid-related fracture risk. TBS has strong positive correlations with the trabecular bone volume to tissue volume ratio, number of trabeculae, and their connectivity and stiffness (38). In this context, TBS appears to be a valuable tool in assessing the bone status and estimating fracture risk in patients with glucocorticoid-induced osteoporosis. It has been shown that in patients with endogenous hypercortisolemia TBS values were significantly lower than in controls, moreover, subjects with Cushing`s syndrome with fractures had low TBS values (29). However, the effect of exogenous glucocorticoids on bone may be different, because it is modulated by various mechanisms at a tissue level, like variations in the expression and sensitivity of the glucocorticoid receptors (GRs), the action of transmembrane transporters, and enzymatic metabolism of glucocorticoids to more or less active forms by 11β-hydroxysteroid dehydrogenases (11β-HSDs) (17). It has been shown, that a common polymorphism in the efflux transporter P-glycoprotein was associated with reduced BMD and increased susceptibility to glucocorticoid-induced osteoporosis in patients with Addison`s disease (12). It suggests that other factors contributing to glucocorticoid action on tissue level may play an important role in glucocorticoid induced osteoporosis development. In our group more than half of the patients received over 25 mg hydrocortisone daily, suggesting overtreatment, while TBS/BMD values did not differ from the control group. We also did not find differences in bone parameters between groups on higher vs lower doses of hydrocortisone. Maybe factors influencing steroids metabolism played important role in this case. This aspect needs further studies.

In our group, TBS correlated negatively with the duration of the disease. It stays in accordance with the previous studies showing that patients with a longer history of primary adrenal insufficiency had a higher prevalence of vertebral fractures (34).

Also, the age of patients and postmenopausal status (higher gonadotropin levels) were associated with lower TBS values. In multiple regression analysis, age and gonadotropin concentration were independent factors influencing TBS values in patients with primary adrenal insufficiency. Previously it has been shown, that a linear decline of 14.5% in TBS was seen between 45 and 85 years of age (6% before 65 years and 8.5% after age 65 years) (39).

The deficiency of adrenal androgens has also been suggested as an osteoporotic risk factor in patients with primary adrenal insufficiency. Gurnell et al. showed, that 12 months of DHEA-S substitution therapy slightly but significantly increased FN BMD, but not at other skeletal sites (30). In our group, DHEA-S concentration correlated significantly with BMD in all examined locations (LS, FN, total body). In accordance with the work of Gurnell et al., the associations between androgens and densitometry parameters were more visible at the FN than in other locations. Apart from DHEA-S, we found associations between FAI and FN T score, Z score and BMD, and also between testosterone and FN BMD.

To our best knowledge, this is the first study to examine sclerostin concentrations in patients with autoimmune primary adrenal insufficiency. We have found that in patients with Addison`s disease the serum sclerostin concentration was significantly higher than in the control group (p=0.006). Studies in mice and *in vitro* are rather consistent, showing increased expression of sclerostin after glucocorticoid therapy and a protective role of anti-sclerostin antibodies against glucocorticoid-induced osteoporosis (19, 20, 22, 40), but in humans, results are so far contradictory. Gifre et al. showed an increase in sclerostin concentrations in patients treated for a year with prednisone (mostly due to hematological diseases). Moreover, sclerostin was correlated with glucocorticoid dose (23). Other studies comprising healthy patients treated with prednisone for a shorter period (25) or patients with endogenous hypercortisolemia (24) reported a glucocorticoid-related decrease in sclerostin concentrations. In this context, our result is very interesting. Our study group was homogenous, treated with hydrocortisone for a long time, and presented no decrease in BMD or TBS in comparison to controls, so the difference in sclerostin concentrations was probably not an effect of the changes in osteocyte number, which are the main source of sclerostin. There was a significant correlation between serum sclerostin and fT4 concentration in patients with primary adrenal insufficiency. There was no correlation with any other parameters (including TSH, daily dose of hydrocortisone or thyroxine, UFC), so the relevance of this correlation remains unclear. Previously it has been shown that after the successful treatment of thyrotoxicosis, the level of serum sclerostin decreases (41). Also, in the group of patients with different thyroid status, serum sclerostin correlated positively with fT4 and negatively with TSH (42). In the current study, fT4 concentration did not differ between patients with Addison`s disease and controls, so we cannot draw conclusions, that different thyroid status explains higher serum sclerostin in patients with primary adrenal insufficiency. However, thyroid status is known to affect bone metabolism and most probably affects sclerostin level, this fact needs to be taken into consideration in further studies on this topic.

Since this is the first study concerning sclerostin in Addison's disease, it is difficult to draw conclusions why sclerostin was higher in patients than in controls. We present few hypotheses of this result. Firstly, treatment with steroids may increase sclerostin concentration like in animals and some human studies. This association may occur before the impairment of bone status and may be differently pronounced depending on steroid type and duration of treatment. Maybe our study was too small to detect influence of mild hydrocortisone overtreatment on sclerostin. Also factors influencing steroid metabolism may be important, as discussed above. Secondly, the autoimmune process may promote other sources of sclerostin production. It has been suggested that in rheumatoid arthritis fibroblast-like synoviocytes were a major source of sclerostin (43). Interestingly, the autoimmune disease was present in all our patients and most of patients from the study of Gifre et al., both groups were treated with steroids for longer period, resulting in significant sclerostin increase. Also, thyroid status may affect sclerostin concentration, as discussed above. In our study group more patients than controls were treated with L-thyroxine and sclerostin correlated with fT4 concentration. However, fT4 did not differ significantly between patients and controls, so this aspect needs further studies.

In this context, we believe that our study should be considered as preliminary one, we are planning follow up. The strength of our work is the homogeneity of our study group (autoimmune etiology of the disease, treatment with hydrocortisone) and the use of novel methods (first report of TBS and sclerostin in Addison`s disease). The limitations of our study are low number of patients and lack of radiological imaging focused on osteoporotic fractures. This topic needs further studies, especially multicenter, with greater number of patients, with follow up and radiological assessment of fractures. To further explore the reasons of sclerostin alterations, studies could assess also patients without autoimmune disease (for example after bilateral adrenalectomy).

In conclusion, we performed the first published study comparing TBS values and sclerostin concentrations between patients with autoimmune primary adrenal insufficiency and healthy controls. TBS results were not impaired in patients with Addison`s disease while sclerostin concentrations were significantly increased in comparison to healthy subjects.

Data availability statement

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

Ethics statement

The studies involving human participants were reviewed and approved by The Bioethics Committee of Wroclaw Medical University. The patients/participants provided their written informed consent to participate in this study.

Author contributions

All authors contributed to the study conception and design. Material preparation, data collection, and analysis were performed by AZ-W, JS, and JH-Z. The first draft of the manuscript was written by AZ-W, and all authors commented on previous versions of the manuscript. AZ-W conceptualized the study. JH-Z contributed to the methodology. JS performed TBS examination and analysis. NS performed the sclerostin examination and analysis. AZ-W, JH-Z, ŁG, and MB performed the Formal analysis and investigation. AZ-W and JH-Z wrote and prepared the original draft. ŁG, JS, and MB wrote, reviewed, and edited the manuscript. All authors contributed to the article and approved the submitted version.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Significant risk of COVID-19 and related-hospitalization among patients with adrenal insufficiency: A large multinational survey

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Objective: To determine self-reported incidence and potential risk factors for COVID-19 in patients with adrenal insufficiency (AI).

Methods: A 27-item AI survey was developed for AI and COVID-19 status, vetted by specialists and patients, and distributed *via* social media, websites, and advocacy groups. Participation was voluntary and anonymous. Data were collected from September 20th, 2020 until December 31st, 2020.

Results: Respondents (n=1291) with self-reported glucocorticoid treatment for AI, completed the survey, with 456 who reported having symptoms and were screened for COVID-19 during 2020; 40 tested positive (+ve), representing an 8.8% incidence. Of the COVID-19^{+ve}, 31 were female (78%), with mean age of 39.9 years. COVID-19 among AI patients occurred most commonly in those aged 40–59 years (n=17; 42.5%); mean time since AI diagnosis was 13.5 years (range 0.2–42.0 years). Pulmonary disease, congenital adrenal hyperplasia, and higher maintenance doses of glucocorticoids were significantly associated with +ve COVID-19 (p=0.04, p=0.01, and p=0.001, respectively. In respondents the cumulative incidence of COVID-19^{+ve} during 2020 was 3.1%; greater than the 1.03% worldwide-incidence reported by WHO, by December 31st, 2020. There was a 3-fold (95% CI 2.16-3.98) greater relative risk (RR) of COVID-19 infection and a 23.8- fold (95% CI 20.7-31.2) RR of hospitalization in patients with AI, compared with the global population.

Conclusion: A markedly raised RR of COVID-19 and hospitalization in respondents reporting chronic AI was detected. We found that a diagnosis of congenital adrenal hyperplasia, age>40 years, male gender, pulmonary disease, and higher maintenance doses of glucocorticoids were associated with greatest risk.

KEYWORDS

adrenal insufficiency: incidence COVID-19 SARS-CoV2, COVID-19, adrenal insufficiency, incidence, hospitalization

Introduction

As of July, 2022, there had been > 555 million cases of coronavirus (COVID-19) worldwide. Cases continue to spike with a 7 day average worldwide peak mid-January 2022 of over 3.4 million cases (1, 2). Given the spectrum of presentation from asymptomatic illness to severe disease, variable availability or under-utilization of testing, this may be an underestimate across all populations. Infection, in steroid dependent patients with AI, is the primary driver of adrenal crises and high mortality risk (3–5). This risk has been compounded since 2020 by emotional distress, limited access to medical practitioners associated with pandemic restrictions and staff availability, heightening the need for patient disease knowledge and self- management (4, 6, 7). With the more recent emergence of highly contagious SARS CoV-2 variants such as Omicron and BA.5, coupled with the difficulty predicting future mutations, providing patients with data-derived information to demonstrate level of risk more clearly may be helpful to reinforce the need for ongoing protective behaviors and strategies to mitigate high risk of infection, COVID-19 complications, adrenal crisis and catastrophic outcomes.

Angiotensin-converting enzyme-2 (ACE2) suppression or dysregulation of ACE/ACE2 antagonistic relationship has been proposed as a mechanism for COVID-19 infection susceptibility or severity (8). Ciliated epithelial cells of the respiratory tract are first affected by inhaled virus. Spike proteins of the SARS-Cov-2 attach to ACE2 cell surface receptors and gain ingress into the cell, aided by proteases such as TMPRSS2 and other facilitators. On entry, the protective effects of ACE2 in affected cells are downregulated, allowing unopposed ACE activity (3, 8, 9). Viral replicates attach to ACE 2 receptors in the endothelium of the vasculature and multiple organs such as the hypothalamus, pituitary, thyroid, heart, kidneys, pancreas, testes, adrenals and intestines (3, 10). Older age, male sex, obesity, smoking, ethnicity and comorbidities (for example, diabetes mellitus) have been associated with COVID-19 severity and worse outcomes (10–13) in the general population.

At the onset of the pandemic, leading endocrine societies issued statements of concern for increased risk of COVID-19 infection for patients with known AI (14, 15) However, the impact in patients with pre-existing hypoadrenalism is largely based on known immune suppressive effects of glucocorticoids and assumed risk. The precise risk of COVID-19 infection in patients with AI compared with the general worldwide population and based on factors such as glucocorticoid dose, individual risk behaviors and comorbidities has not been quantified.

We hypothesized that the risk of COVID-19 may be altered by the presence of AI, independent of etiologies and comorbidities, potentially conferring a worse prognosis compared with the general population. We aimed to compare the incidence of COVID-19 infection in individuals with known AI with the worldwide incidence of COVID-19 in 2020 and provide some insight into concomitant diseases or factors that may increase risk. Five groups of patients were included: Primary AI (PAI), secondary AI (SAI), congenital adrenal hyperplasia (CAH), tertiary AI (TAI) patients (a diagnosis of AI with a minimum of 3 months use of long-term glucocorticoid treatment for other than pituitary or adrenal etiologies) and other etiologies (*e.g.* adrenoleukodystrophy). We assessed COVID-19 symptom frequency, testing and diagnosis, age, concomitant disease, glucocorticoid dose, stress dosing, avoidance behaviors, and lingering symptoms on recovery among patients with AI.

Materials and methods

Research ethics, study design, and participants

Survey methodology was selected to recruit the maximum number of respondents worldwide over a short period of time. Given the need for global reach and for maximizing data capture, a questionnaire was developed for electronic distribution.

A 27-item questionnaire, was constructed utilizing a web-based tool (Question Pro.com software Survey Analytics LLC, Austin, TX, US 2002). Closed ended (Yes/No or multiple choice) questions, skip logic (conditional branching) and enforced answer techniques were used to minimize question misinterpretation, survey completion time and survey fatigue, and to eliminate missing data respectively. Items that required clarification, such as glucocorticoid type and dose, year of diagnosis, were also used to support the self-reported diagnosis of AI as a qualification for survey participation. (Appendix 1).

A panel of endocrine nurses, physicians, and patients from the Adrenal COVID Task (ACT) force (representatives from: National Addison's Self Help Group, United Leukodystrophy Foundation, Living with CAH, and the World Alliance of Pituitary Organizations), reviewed and piloted the questionnaire for face content (objective consistency), internal and external validity. Questions were modified as needed and retested. Representatives adjudicating the questionnaire were from multiple countries, including the United Kingdom (UK), United States (US), Europe, Brazil, Australia, and South Africa. Questions were translated into 9 languages for review and publication.

The survey introduction and instructions informed the user of inclusion criteria (individual with AI or a family care-giver, nurse or physician caring for patients with AI) the voluntary nature of participation and anonymity measures. Consent was assumed with data entry. To progress through the questionnaire, participants qualified by adding diagnosis (etiology of AI), date of diagnosis, number of years of treatment, type and dose of glucocorticoids. This information also served to validate adrenal insufficiency status. An e-mail address of the principal

researcher was provided. Institutional ethics review was waived due to the methodology and respondent anonymity.

An open survey methodology was utilized in publishing the questionnaire to allow broader accessibility. Internet access to the survey was distribution world-wide to endocrine medical teams, on social media, on mobile devices, on websites for national and international endocrine nursing societies and advocacy organizations also e-mailed members, including Adrenal Insufficiency United (AIU), the National Addison's Disease Foundation (NADF), Adrenal Alternatives, Pituitary Foundation, Australian Addison's Foundation, CARES Foundation, Magic Foundation, Addison's Brazilian Association, the Australian Pituitary Foundation and World Alliance of Pituitary Organizations (WAPO). Global representation was sought to improve external validity.

Data were collected September 20th, 2020 - December 31st, 2020 and interrogated the period from January 1st, 2020 - December 31st, 2020. Therefore, some participants were asked to recall COVID-19 testing and symptoms from the early part of the year 2020. Given the high public profile and availability of information regarding SARs-CoV2 and COVID-19, memory of these events was assumed to be adequate. A single use identification number was used to anonymize data. Repeated access was exclusively permitted from the same internet protocol address for the purpose of completing a saved, but incomplete questionnaire. Some questions (*e.g.* zip or postal code) were optional and replaced by rural/urban, if the respondent had concerns regarding anonymity).

Questions covered domains of demography, comorbidities, hospitalizations, COVID- 19-related symptoms, self-protective behavior to limit COVID-19 infection, glucocorticoid dose, route of administration, availability of usual glucocorticoids, and COVID-19 screening and outcome. COVID-19^{+ve} respondents were additionally asked if they were treated at home or a hospital, if a stress dose of glucocorticoids was administered, if their glucocorticoid dose was altered during 2020, and if any residual symptoms occurred after their presumed recovery.

Statistics

The dataset was analyzed by group. Group 1: respondents not tested for COVID-19, group 2: respondents who tested negative (-ve) for COVID-19, and group 3: respondents who tested positive (+ve) for COVID-19. Analyses were performed using SPSS27 (Version 27.0. Armonk, NY: IBM Corp) including descriptive statistics, crosstabs, where appropriate, Chi Squared to determine relationships between categorical variables, and ANOVA with *post hoc* Bonferroni correction, to assess the relationship between groups and continuous variables. Multinomial regression analyses were used to predict the influence of multiple independent variables, including symptoms and concomitant diseases on group outcomes. Independent T- tests were undertaken to assess relationships between continuous dependent variables and independent categorical variables and independent samples proportions to compare the proportions in two unrelated groups. A 2x2 contingency table was used to compare risk ratios for global and survey COVID-19 incidence and hospitalizations. Significance was accepted at $p \leq 0.05$.

Relative risk (RR) of COVID-19 infection and hospitalization were calculated using a 2x2 table (where cumulative incidence in the COVID-19 group [40/1291] and cumulative incidence in a global group [80,611,600 infected/7.8 billion world population]) and the following formula to calculate a 95% confidence interval (CI):

$$\ln(RR) \pm Z_{critical}\sqrt{\frac{1-\widehat{P}_1}{n_1\widehat{P}_1}+\frac{1-\widehat{P}_2}{n_2\widehat{P}_2}}$$

Ln = natural log; *P*1 = Cumulative Incidence in target population; *P*2 = Cumulative Incidence in global population; n1*P*1 = Number of positive cases in target population; n2*P*2 = Number of positive cases in global population (1, 2).

Results

The survey was viewed 2179 times and opened 2013 times by individuals from 43 countries, with 1291 completions from 37 countries. (Figure 1). Access was permitted to complete an incomplete survey per single Internet Protocol (IP) address. Incomplete surveys accounted for 302 duplicate access attempts from the same IP address without data entry and were deleted. In addition, 101 attempts failed Captcha (excluding robot access) and 19 were for test access and were excluded. The remaining 300 who accessed the survey universally withdrew after capture of referral source and country of origin and prior to any other data entry. Survey completion rate was 81.1%. Surveys were completed by individuals with AI (87.5%), a family member for pediatric cases (12%), and by medical professionals (0.5%). Overall, mean age of subjects was 47.3 years (standard deviation; SD 18.3 years, range 0.25–81.0 years) with 81.3% female. Males were younger than females 43.1 years versus 48.3 years, $p = 0.001$.

Group 1, not tested for COVID-19 (n = 795) constituted 61.6%, group 2 COVID-19^{-ve} (n = 456) 35.3%, and group 3 COVID-19^{+ve} (n = 40) 3.1%, respectively (Figure 1).

Self-reported ethnicity was Caucasian in 931 (72.1%), European (n = 289; 22.4%) Brazilian and/or Hispanic (n = 27; 2.1%) and the remainder 3.3% (n = 43) comprising African, African-American, Asian, mixed ancestry, and any other ethnic group. More respondents identified as Brazilian/Hispanic were found to be COVID-19^{+ve} than Caucasian/European respondents (25.9% vs 2.6%, $p = 0.001$) (Table 1).

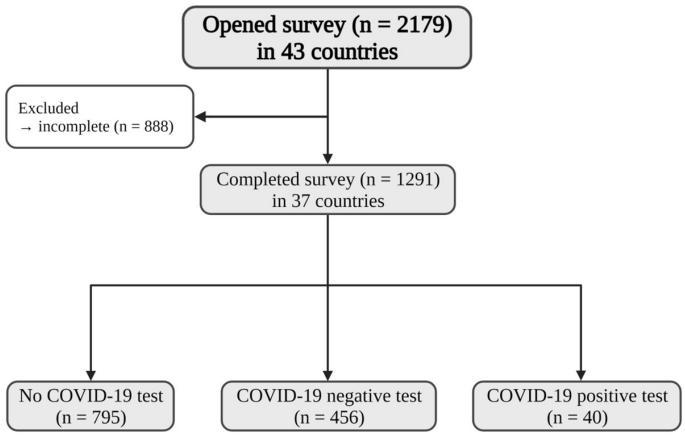

FIGURE 1
Flow Chart – Survey respondent inclusion criteria and COVID-19 status.

Respondents' diagnoses included PAI in 56.1% (n = 724), SAI 28.6% (n = 369), CAH 5.6% (n = 72), TAI 5.4% (n = 70), and other diagnoses 4.3% (n = 56). CAH respondents were significantly younger 23.8 years (p = 0.001) than all other diagnoses. There was a higher proportion of males with CAH. Overall, sex did not differ significantly between diagnoses (Table 1).

The mean age of COVID-19^{+ve} respondents was younger (39.9 years) versus those not tested (48.2 years) or COVID-19^{-ve} respondents (46.5 years) (p = 0.03) (Table 2). However, for the 40 COVID-19^{+ve}, when divided into quartiles based on age (0–19, 20–39, 40–59, ≥ 60 years) the group 40–59 years (n =17; 42.5%) had the highest COVID-19 risk (p = 0.016).

Not surprisingly, COVID-19-related symptoms were more numerous for respondents with positive results, compared with groups 1 & 2 (5.3.vs 3.7 vs 2.6: p = 0.001). On multinomial regression analysis, respondents with a loss of sense of smell and those with fever were 3.2-fold (p = 0.001) and 2.1-fold (p = 0.04) more likely, respectively, to be COVID-19+ve than those either not tested or COVID-19-ve.

Of all the subgroups with AI who were COVID-19^{+ve}, a diagnosis of PAI was reported in 47.5% (n = 19) of respondents, SAI in 22.5% (n = 9), CAH in 20% (n = 8) and TAI in 10% (n = 4) (Table 2).

Patients with CAH (72/1291) demonstrated a higher incidence of COVID-19, compared with other diagnoses [8/72 (11.1%): 6 females/2 males]; (p = 0.001). Respondents were largely female (55), older than males (26.1 years versus 16.4 years respectively [p=0.057]); (range newborn to 60 years), with an average of 19.4 years since diagnosis. The mean glucocorticoid dose was similar for males and females: mean 19.4 mg daily with 77.8% taking fludrocortisone versus 50% (4/8) of those reporting COVID $^{+ve}$ tests.

Comorbidities

Pulmonary disease (asthma, bronchiectasis and pulmonary hypertension) occurred more frequently in respondents who were COVID-19^{+ve}, compared with the remaining groups (p = 0.04). Although numerous other co-morbidities were reported, diabetes, hypertension and cardiovascular disease was not reported with greater frequency in the COVID-19^{+ve} group. (Table 2).

Glucocorticoid replacement

Oral glucocorticoids were used by 97% of respondents; 2% used a continuous or pulsatile subcutaneous pump and 1% utilized subcutaneous injections. Hydrocortisone was used by most respondents (87.7%) and 77% of COVID-19^{+ve} patients. Mean duration of glucocorticoid replacement was 11.3 years (range 0.1 –61.0 years). Daily dosage of glucocorticoids was reported in 1231/1291 (95.4%) respondents. All glucocorticoid doses were converted to hydrocortisone equivalent for comparison: mean (standard deviation; SD) daily dose of 23.02 mg (SD ± 11.5; range 0–150 mg daily). Intriguingly, some patients with SAI and TAI reported taking fludrocortisone (19/20 PAI [95%], 3/8 CAH, 1/9 SAI, and 1/3 TAI) (Tables 1, 2).

Mean daily dose was different among the three groups; (p=0.001) and remained highest for the COVID19^{+ve} group (29 mg daily; SD ± 21 mg). Twenty patients used excessive replacement hydrocortisone equivalent doses (60-150 mg). In group 1, 3 respondents used 150 mg daily, and 6 respondents used between 60 mg and 100 mg daily (1.1%). In group 2, 8 respondents reported a maintenance dose of 60 mg –100 mg

TABLE 1 Baseline demographic characteristics of responders (* p-value < 0.05 considered significant).

	Adrenal insufficiency (AI) aetiology					
	Primary AI	**Secondary AI**	**Congenital adrenalhyperplasia**	**Tertiary AI**	**Other**	***p*-value**
Respondent (n)	724	369	72	70	56	_
Sex: Female/Male (*n*)	590/134	294/74	55/17	61/9	49/7	0.3
Mean age; years (SD)	49.4 (17.1)	47.5 (17.3)	23.8 (17.8)	47.9 (19.2)	48.2 (18.8)	**0.01**
Mean AI duration, years (SD)	12.2 (11.3)	8.1 (8.9)	19.8 (15.3)	7.8 (6.5)	8.9 (9.1)	**0.048**
Ethnicity (n)						**0.003**
Caucasian	514	259	59	55	44	_
European	183	84	5	10	8	_
Hispanic	8	11	3	2	3	_
Other	19	15	5	3	1	_
Protective Behavior; *n* = 1276 (%)						0.4
Low risk	49.6	51.9	50	47.1	39.3	_
Moderate risk	40.1	40.3	40.4	40.6	50	_
High risk	8.1	7.7	6.9	10.1	10.7	_
Mean hydrocortisone dose; mg (SD)	24.1 (12.7)	21.1 (8.2)	19.56 (11.1)	26.2 (13.3)	21.7 (10.0)	**0.002**
Country of Origin (*n*)						**Total**
Great Britain	399	139	25	33	16	612
USA	175	166	36	28	23	428
Australia	40	9	0	1	4	54
Germany	30	12	0	2	4	48
Canada	17	11	2	2	5	37
Ireland	17	10	0	1	1	29
Sweden	15	2	0	0	0	17
Brazil	6	4	7	1	1	19
Spain	6	0	0	0	0	6
Italy	3	0	0	0	0	3
Netherlands	2	3	0	0	0	5
South Africa	2	2	0	0	0	4
Other	16	9	2	0	2	29

Bolded p values indicate significance.

daily (1.8%). In group 3 (COVID- 19^{+ve}) 3 respondents utilized between 80 mg and 100 mg daily (7.5%). After removing outliers from all three groups, there was no difference in mean dose among the groups (p=0.30). Duration of glucocorticoid replacement for AI was not associated with occurrence of COVID- 19 infection (p=1.0). Curiously, two patients noted doubling their regular dose at the start of the pandemic, but this could not be probed further (Table 2).

Pharmacy supply interruptions in 2020 resulted in 129/1291 (10%) of respondents changing maintenance doses and 56 (4.3%) missing doses for up to 3 weeks due to medication non-availability, postal delays, difficulty contacting an endocrinologist or refill refusal by a primary practitioner. In group 3 (COVID-19^{+ve}) 5 respondents (12.5%) reported missing doses lasting from 2 days to 2 weeks and only 2 (5%) reported changing their usual glucocorticoids owing to supply interruptions. The majority 35/40 (87.5%) of COVID-19^{+ve} reported taking a stress dose (at least double) of steroids, with the onset of symptoms relating to COVID-19 (one person was asymptomatic) compared with either 70% not tested or 82.5% COVID-19^{-ve} (p=0.001).

Prevention strategies for COVID-19

The majority (87%) of respondents across all the groups reported limiting SARS-CoV- 2 exposure by social distancing, social isolation or by wearing masks versus 9.1% who did not wear face masks in public. Exposure risk was divided into low, moderate and high-risk behavior for analysis, based on social isolation and mask wearing. In COVID-19^{+ve} respondents, 4 respondents (10%: 2 PAI, 1 SAI, and 1 CAH) indicated they engaged in high- risk behavior (Table 2).

TABLE 2 Respondent Characteristics with Respect to COVID-19 Test Results (*p-value< 0.05 considered significant; NS= not significant).

	Testing Status None	Negative	Positive	% Total	*p-value
Respondent (n)	795	456	40		
Mean age, years (SD)	48.2 (19.1)	46.5 (16.6)	39.9 (18.3)		**0.03**
Sex					0.3
Female (n)	636	382	31	3	–
Male (n)	158	75	9	3.7	0.06
Adrenal insufficiency (AI) aetiology					**0.001**
Primary AI (*n* = 724)	449	256	19	2.6	–
Secondary AI (*n* = 369)	224	136	9	2.4	–
Congenital adrenal hyperplasia (*n* = 72)	46	18	8	11.1	**0.001**
Tertiary AI (*n* = 70)	33	33	4	5.7	–
Other (*n* = 56)	42	14	0	0	–
Mean symptoms; n (SD)	2.6 (2.5)	3.7 (3.1)	5.3 (3.4)		**0.001**
Mean AI duration; years (SD)	12.1 (11.3)	9.1 (9.6)	12.8 (9.8)		0.04
Mean hydrocortisone dose; mg (SD)	22.4 (11.6)	23.5 (9.8)	29 (21.0)		**0.001**
Missed doses (%)	0.034	0.05	0.125		**0.006**
Fludrocortisone (n)	321	466	4		0.5
Ethnicity (n)					0.5
Caucasian	558	340	25	2.7	0.5
European	190	92	7	2.4	0.7
Hispanic	19	12	7	18.4	**0.014**
Other	25	11	1	2.7	0.1
Comorbidities (n)					
Pulmonary	135	103	10		**0.04**
Cardiovascular	140	99	6		0.2
Diabetes	74	37	4		0.7
Gastric	44	39	4		0.09
Rheumatoid	50	43	2		0.1
Renal	33	20	2		0.9
Osteopenia/Osteoporosis	135	66	3		0.2
Pituitary Deficiencies	143	86	6		0.8
Concomitant Comorbidities (n)					0.5
none	255	138	17		
1	275	148	13		
2	153	89	5		
3	70	45	2		
≥ 4	50	37	3		

Bolded p values indicate significance.

Relative risk of COVID-19 and hospitalization in patients with adrenal insufficiency

Overall, 3.1% of 1291 respondents tested COVID-19^{+ve}. Persons per million SARS- COV-2^{+ve} by December 31st, 2020, numbered 10322 (1, 2), representing a 1.03% global annual cumulative incidence in 2020. The RR of infection for respondents in this cohort of AI was 3- fold higher than the world-wide incidence for the same time-period (95% CI=2.16-3.98-).

Of those COVID-19^{+ve} respondents, 75% reported receiving treatment at home, whereas 22.5% (9/40; 4 PAI, 5 SAI; 2 male, 7 female) were hospitalized for respiratory distress, and (5/9; 55.5%) reported pre-existing respiratory disorders. Based on a global population of 7.8 billion (December 31st 2020), with 80,511,600 reported COVID-19^{+ve} and 754,746 requiring hospitalization, the RR of hospitalization in our cohort was 23.78-fold higher than the global population (95% CI=20.69-31.24) (1, 16). ICU admission and ventilator support was reported by two females (5%) ages 51 and 54 years, one patient each from US and UK, respectively. Hospitalization

was required for 4/16 COVID-19^{+ve} women of childbearing age with none reporting pregnancy.

Residual symptoms after COVID-19 infection

Residual symptoms following COVID-19 infection were reported in 15/40 (37.5%) of respondents (Figure 2). SAI and CAH respondents reported residual symptoms more frequently than those with other diagnoses (PAI 3/20, SAI 6/9, CAH 5/8, TIA 1/3, p=0.02).

Discussion

We demonstrated a higher cumulative incidence and RR of COVID-19 infection (3.1% versus 1.3%) and over 23-fold higher rate of hospitalization in patients with AI, compared with background incidence of COVID-19 in 2020. Patients with CAH had higher incidence of COVID-19 infection, with proportionately more males, compared with other AI diagnoses, but none with CAH required hospitalization. Age 40–60 years, pre-existing lung disease, and higher glucocorticoid doses were each independently associated with a higher risk of COVID- 19 infection. Increased symptom burden with loss of smell and fever, were predictors of COVID-19 infection. A greater proportion of COVID-19^{+ve} patients reported working outside the home without social distancing. Although all respondents recovered from COVID-19, 37.5% reported residual symptoms, particularly those with pre-existing lung disease and PAI.

Two studies reported low incidence of COVID-19 infection in patients with AI. A study from Lombardy, Italy, in early 2020 found no increased risk of developing COVID-19 among 279 patients with hypoadrenalism compared with the background population (17). Notably, only 12/279 patients reported COVID-19 testing.A second UK study found 2 COVID-19 $^{+ve}$ cases of 7 patients tested from a pool of 159 patients who were taking replacement steroids (16). These results are at odds with our study, in which we showed a two- fold risk of developing COVID-19. All studies depended on patient recall. In the Carosi study, COVID-19^{+ve} status was assumed based on patient recall of at least 2 symptoms considered highly indicative of COVID-19. Our study differed with respect to: larger sample size; longer duration; and data collected from a larger geographical area. In our study, all COVID-19^{+ve} patients reported testing. Stringent lockdown regulations and differential environmental viral loads as the pandemic progressed may also account for a variable risk of COVID-19. It is notable that the Omicron variant did not emerge until 2021 and therefore is not reflected in either study (18). Our data signals the necessity for careful mitigation

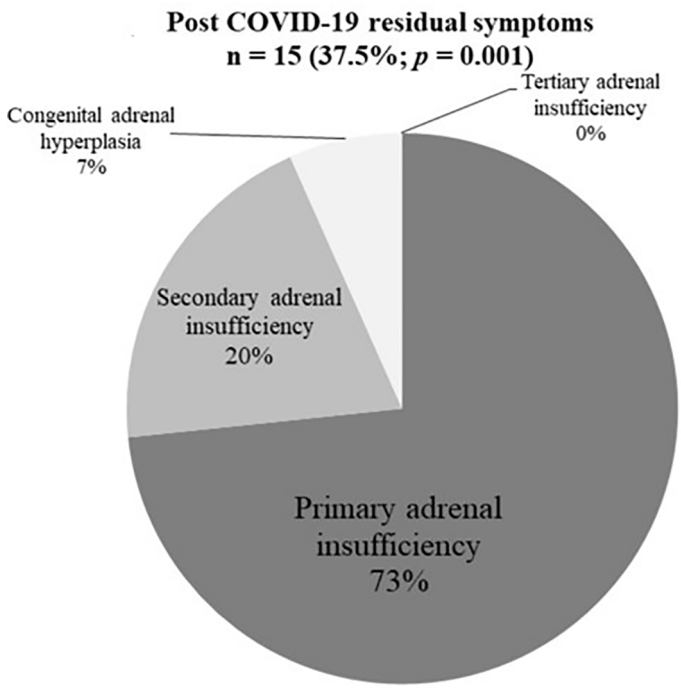

FIGURE 2
Post COVID-19 residual symptoms (n = 15).

strategies, acknowledging that patients with hypoadrenalism and COVID-19 may be at risk for a worse outcome.

An endogenous, stress induced increase in adrenal glucocorticoids in the context of infection is a key modulator of cytokine production and adaptive response inhibiting inflammation and helping to avert a cytokine storm (19, 20). However, prolonged critical illness, acute respiratory distress syndrome and sepsis are known to result in relative AI (glucocorticoid deficiency) and treatment with glucocorticoids is recommended (21). In patients with no history of AI requiring hospitalization with COVID-19, a high incidence of AI was found with lower cortisol levels associated with more severe illness (22). The Recovery trial demonstrated decreased COVID-19 related mortality risk and shorter hospitalization in non-AI patients hospitalized with respiratory distress or requiring mechanical ventilation (23, 24). No benefit of glucocorticoid treatment has been found in patients without respiratory compromise.

Long-term and/or high dose glucocorticoids are known to inhibit NK cell activity, resulting in lymphopenia, inhibition of macrophage differentiation and proliferation, and B and T-cell activation, potentially producing a more severe phenotype in AI (19, 25). Olnes et al. (25) demonstrated lymphocyte suppression and persistent NK cell deficiency after a single infusion of hydrocortisone in patients with normal adrenal function. NK cell deficiency has been demonstrated in patients with PAI compared to healthy controls (10, 26, 27). There is little data specific to SAI. Natural killer (NK) lymphocytes directly target antigens and influence B and T-cell responses, limiting viral spread and cell damage (26). As a result, patients with pre-existing AI are more likely to have increased susceptibility to COVID-19, a higher risk of severe infection and hospitalization, particularly in the presence of pulmonary disease as found in our study (10, 26). Early treatment, with higher than physiologic doses (or stress doses) of glucocorticoid, is warranted for patients with AI at the onset of COVID-19 symptoms.

We also found that mean supraphysiologic exogenous doses of maintenance glucocorticoids prior to contracting COVID-19 correlated with a higher rate of COVID-19^{+ve} infection. After removing individuals using doses over 60 mg daily (n=20), there was no difference among the three groups. Prophylaxis with higher doses of glucocorticoids may increase infection risk. Maintaining physiologic dose that mimics normal cortisol production and avoiding periods of relative adrenal insufficiency is recommended.

A multivariate model of rheumatoid arthritis revealed a > 2-fold risk of severe COVID- 19^{+ve}, or death, for patients taking TNF inhibitors plus prednisone, compared with those on conventional disease modifying drugs or non-rheumatoid arthritis controls (28). Similar comorbidities among patients with hypoadrenalism and rheumatoid arthritis render both at enhanced susceptibility to COVID-19 (28).

Interruption in maintenance therapy in 2020 occurred for 10% of respondents and 12.5% of those testing COVID-19^{+ve}. These patients are at-risk for an Addisonian crisis in addition to COVID-19, underscoring the need for an emergency kit and uninterrupted supply of glucocorticoids.

In addition to AI, we found older age (40–60 years), as an independent risk factor, was associated with a higher risk of contracting COVID-19. The literature supports comorbid disease along with advancing age, obesity, diabetes, lung, cardiac and cardiovascular disease (CV) have been associated with higher risk (29, 30). We did not interrogate BMI and although higher levels of CV disease were reported, these were not statistically significant predictors of COVID-19 risk. Exposure risk related to lower frequency of mask use and social distancing was associated with higher frequency of infection, reinforcing the need for self-protective behaviors.

Data at the inception of the pandemic, demonstrated a higher predilection for malescontracting COVID-19 and a 3 fold likelihood of requiring ICU admission with a higher mortality risk (31). Thus, we explored the incidence for males separately. We found male CAH patients at higher risk. It is possible in male patients with CAH, high concentrations of androgens independently suppress immunity and may enhance SARS CoV-2 viral spike binding to ACE2 receptors in the adrenals and testes thereby reducing antigen recognition in the host cell (32). Although our findings accord with the aforementioned data, the relatively small size of respondents with COVID-19, the underrepresentation of males, and the possibility of a type 1 error, demands any conclusions to be considered with caution.

Pulmonary disease was predictive of infection and hospitalization with more severe symptoms regardless of AI etiology. Loss of protective NK cell function is reported in the context of recurrent lung infections, possibly enhancing the risk of infection (10, 26, 27). We did not interrogate smoking, which has been linked to more severe COVID-19. Inhaled steroids for pre-existing asthma and a history of chronic obstructive pulmonary disease and obstructive sleep apnea (diagnosed and undiagnosed) have been reported to increase disease severity (33, 34). For patients with AI, standard mortality ratios are 2.2-8.9-fold higher than the general population, and often as the result of a trivial respiratory infection and impaired innate immunity (35, 36). Respiratory distress was the precipitant for hospitalization in 55.5% of our respondents. ICU admission and ventilator support were reported in only 2/9 (22%) of COVID- 19^{+ve} hospitalized respondents, both of whom reported pre-existing pulmonary dysfunction.

Post viral syndromes are reported after initial recovery from COVID-19 infection (37). Persistent low energy, chronic fatigue, low mood and dizziness were reported, despite physiologic steroid replacement doses.

This is a large multi-national, multilingual survey of patients with AI with COVID-19 status, which was strengthened by large size and global representation, likely improving its generalizability. Multiple AI etiologies were included and

assessed independently, whereas missing data were avoided in the survey design with no response interpretation required. It is acknowledged that numbers of untested patient may include asymptomatic patients or poor access to testing, in which case the incidence of hospitalization would be reduced.

Criticisms of the study design may arise, invoking possible bias from its exclusive internet methodology and exclusion of those without access or who are technically challenged. However, 59.5% of the global population are active internet users which is intriguingly greater than the WHO estimates of fewer than 50% who have access to medical care (www.statistica.com). The use of multiple platforms can minimize bias. Counter to this, our study design avoided face to face transcription and interpretation error. The survey was developed using recommended principles of survey research, including: objective driven, pre- tested, voluntary and widely distributed (38).

Limitations include potential self-selection bias, inability to verify diagnosis and recall bias. We believe that several factors help to minimize these limitations including the wide distribution and the size of the sample in this study and the current emphasis on the patient's disease knowledge, empowerment and need for self-management (4, 6, 7). Direct access to ones own electronic medical records that contain diagnosis and comorbid conditions, plus the availability of medical information on the internet, has resulted in a much more, well informed patient population, regardless of diagnosis. The mortality risk associated with adrenal crisis and COVID-19 are both potential motivators for disease knowledge. However, interrogation of hospital records for mortality data is recommended for future studies. Stress dosing of glucocorticoids during infection is paramount for patient with AI. Although stress dosing was reported in this survey, efficacy in the context of COVID-19 was not evaluated. Interrogation of hospital records is recommended for this purpose in future studies. Likewise regional prevalence of AI and incidence of COVID-19 is not reflected in this data and is recommended for future analysis.

Conclusion

We report a substantially higher incidence of COVID-19 and hospitalization in patients with AI globally compared to the background COVID-19 population. Additionally, premorbid chronic lung conditions, higher maintenance doses of glucocorticoids, a diagnosis of CAH and male sex, were associated with COVID-19. This data supports the need for an uninterrupted supply of glucocorticoids, physiologic maintenance doses, early stress glucocorticoids for acute symptoms of COVID-19, and emphasizes the importance of behavioral protective measures for patients with adrenal insufficiency independent of etiology. Futher studies are recommended to evaluate risk in males with CAH, efficacy of stress steroids in the context of COVID-19 infection and comparison of regional AI prevalence and COVID-19 incidence.

Data availability statement

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

Ethics statement

Ethical review and approval was not required for the study on human participants in accordance with the local legislation and institutional requirements. Written informed consent to participate in this study was provided by the participants' legal guardian/next of kin.

Author contributions

All authors listed have made a substantial, direct, and intellectual contribution to the work and approved it for publication.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Evaluation of plasma ACTH in the metyrapone test is insufficient for the diagnosis of secondary adrenal insufficiency

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Objective: To determine whether a single measurement of ACTH instead of less available in daily practice 11-deoxycortisol assay is sufficient to rule out or confirm secondary adrenal insufficiency (SAI) in the short Metyrapone test.

Design: A retrospective analysis of diagnostic tests (Metyrapone and Synacthen tests) performed at our Center between 2016 and 2018 in patients with suspicion of secondary adrenal insufficiency.

Material and methods: In 103 patients short metyrapone test was performed with assessment of 11-deoxycortisol and ACTH concentration after Metyrapone administered at midnight. In 89 of them short Synacthen (SST) test was also done (1 or/and 250 mcg 1-24ACTH). ROC curves have been performed to evaluate the diagnostic performance of ACTH level in metyrapone test as the predictor of secondary adrenal insufficiency (SAI) analysing sensitivity and specificity for various possible thresholds proposed in literature.

Results: 40 (39%) of examined subjects were diagnosed as SAI, basing on post-Metyrapone 11-deoxycortisol concentration below 70 µg/l. In this group ACTH concentration was 128.1 ng/l (95% CI 96.8-159.4) versus 289.9 ng/l (95% CI 249.1-330.9) in patients with proper adrenal response. There was only a moderate positive correlation between ACTH and 11-deoxycortisol concentrations ($r=0.5$; $p<0.05$). The best cut off value of ACTH in relation to 11-deoxycortisol serum concentrations was 147 ng/l - with sensitivity of 73.2% and specificity 83.9%. However, plasma ACTH was>200ng/ml (the highest threshold proposed in literature) in 8 cases (20%) with positive diagnosis of SAI made on the basis of low 11-deoxycortisole and confirmed in short Synacthen test.

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Conclusion: Our results indicate that for a valuable evaluation of the results of the metyrapone test, the more readily available plasma ACTH assay cannot replace the measurement of 11-deoxycortisol concentrations.

KEYWORDS

secondary adrenal insufficiency, metyrapone test, ACTH, short synacthen test, adrenal insufficiency

Introduction

Diagnosis of adrenal insufficiency (AI) seems to be easy, however, in many cases it is stated only after adrenal crisis, despite obvious symptoms occurring for many months (1–4). Especially secondary forms of disease could be difficult to recognize, as signs and symptoms are non-specific and less pronounced in comparison to primary AI (5–7). On the other hand, misdiagnosed patients with relatively low basal cortisol levels but proper hypophyseal reserve of ACTH are exposed to unnecessary medication with hydrocortisone, which, even in substitutive doses, can increase cardiovascular risk (8, 9).

The most frequently used test in the diagnosis of adrenal insufficiency ACTH stimulation test is a valuable diagnostic tool for primary AI. In this procedure, called the short Synacthen test (SST), serum cortisol concentration is assessed before, 30 minutes, and 60 minutes after intravenous administration of 250 µg synthetic ACTH (1–24 corticotropin, Tetracosactide, Synacthen). Any value ≥ 18 µg/dl defines a proper response of healthy adrenal glands (10). Since after the injection of 250 µg 1-24 ACTH supra-physiological concentration of corticotrophin is achieved, a stimulation with 1 µg dose was proposed. Despite numerous studies, there is still no clear evidence that this variation of the test has an advantage over the "classic" one (11). However, in secondary adrenal insufficiency (SAI) before adrenal atrophy has occurred, the sensitivity of both tests may be too low to recognize the disease (6, 12). The insulin tolerance test is based on the fact that hypoglycemia is one of the most serious physiological stresses which therefore stimulates hypophysis to release a great amount of ACTH and consequently causes an increment in serum cortisol level. This procedure, causing hypoglycemia, is neither pleasant nor safe for the patient, requires physician supervision, moreover is contraindicated in many medical conditions (e.g. in patients with ischemic heart disease, cerebrovascular disease, seizures) (13, 14).

CRH stimulation tests also give false negative results (FN), so can't be recommended as a first-line diagnostic tool (15, 16).

Some clinical centers, including ours, prefer metyrapone tests for the diagnosis of secondary adrenal insufficiency (15–19). The essence of this test is an assessment of the hypophyseal (and consequently adrenal) response to drug-blocking 11 beta-hydroxylase, an enzyme required for the transition from 11-deoxycortisol to cortisol. The administration of metyrapone (in single or repeated doses) causes a decrease in blood cortisol concentration, further incrementing ACTH, and finally the arousal of adrenal steroids synthesis, ending with 11-deoxycortisol, whose level is measured during the test (20). However, the measurement of 11-deoxycortisol concentration is not a standard procedure in most laboratories. Since the essence of the Metyrapone test is to evaluate the corticotropin reserve of hypophysis, the assessment of the ACTH increment would be the ideal single measurement to state the diagnosis. However, recommendations in different guidelines regarding the interpretation of ACTH results in this test are inconsistent. Proposed cut-off values for post-metyrapone ACTH response vary from >75 ng/l (21) by 150 ng/l (22) to >200 ng/l (23).

The aim of the study was the evaluation of ACTH response to single-dose Metyrapone administered at midnight and a comparison of ACTH and 11-deoxycortisol concentrations achieved during the test.

Material and methods

Patients

Data of all patients who underwent a metyrapone test in our tertiary endocrinology center (Department of Endocrinology, Centre of Postgraduate Medical Education, Warsaw, Poland) from January 2016 to December 2018 were collected retrospectively. The reason for the test was the suspicion of secondary adrenal insufficiency stated by general practitioners or endocrinologists in the primary center. Patients were tested by us if previous baseline test results were inconclusive: cortisol levels between 5 and 10 µg/dl in at least two determinations. Since many centers in Poland have problems with ACTH measurement (inadequate sample processing), we did not include results of ACTH concentrations obtained outside our center in the qualification for further studies. If the patient was currently treated with hydrocortisone, the medication was stopped 2 days before the hospitalization.

Metyrapone test

Metyrapone (Metopirone, HRA Pharma Rare Diseases) was administered at midnight in a dose of 30 mg/kg b.w., swallowing with one glass of water; a little snack was also allowed if necessary. Levels of plasma ACTH, serum cortisol, and serum 11- deoxycortisol were measured the next morning, at 8:00 am. The blood samples were collected in a prone position, and 20 mg of Hydrocortisone was taken by the patient immediately after blood donation. Serum cortisol below 5 µg/dl was considered an adequate inhibition of 11 β-hydroxylase.

Additionally, in 89 cases the short Synacthen test (SST) test was performed with 1µg (low dose – LD-SST) or 250µg (high dose – HD-SST) intravenous Synacthen injection. The washout period between Metyrapone and Synacthen tests was 3-14 days. Both LD- and HD-Synacthen tests were carried out between 9:00 am and 10:00 pm. All tests were performed by the same group of well-trained medical personnel.

Laboratory analysis

Serum cortisol concentration was determined using the chemiluminescent immunoassay (CLIA) using the UniCel DxI 600 analyzer (Beckman Coulter, UK). The analytical sensitivity was 0.4 µg/dl (11 nmol/l). The reference range for cortisol determined in the morning (7.00-9.00) is 6.7-22.6 µg/dl (185-624 nmol/l). Conversion factor: µg/dl x 27,8 = nmol/l.

Plasma ACTH concentration was determined, immediately after the blood donation, using the chemiluminescent immunoassay (CLIA) with the LIAISON XL analyzer (DiaSorin, Italy). The analytical sensitivity was 1.6 ng/l. The reference range for ACTH measured in the morning (7.00-9.00) is 4.7 - 48.8 ng/l. Conversion factor: ng/l x 0.2202 = pmol/l.

Sera for assessment of 11-deoxycortisol levels were frozen at -86°C (as we don't have the ability to perform this analysis day-to-day). The determination of the serum 11-deoxycortisol was performed using the radioimmunoassay (RIA) method with kits from Diasource ImmunoAssays (Belgium). The analytical sensitivity was 0.11 ng/ml. Expected values (reference ranges): determination of 11-DOC concentration under baseline conditions (without stimulation) is <0.255 µg/dl (<7.2 nmol/l), while in the level after stimulation with Metyrapon is 7.2 – 22.5 µg/dl (208-649 nmol/l). Conversion factor: 1 µg/dl = 0.2886 nmol/l.

Statistical analysis

Statistical analyses were performed using Statistica software (version 13.1 Dell. Inc. Statsoft). Presented data were expressed as means with a 95% confidence interval. Data distributions were assessed by the W Shapiro-Wilk test. For the comparison of nonparametric single variables, the Mann-Whitney test was used. For nominal variables frequencies were calculated with sensitivity, specificity, positive and negative predictive values, accuracy, and risk ratio with a 95% confidence interval. Frequencies were compared using the Fisher exact test. Correlation between parametric and nonparametric data was assessed by Pearson or Spearman coefficient, respectively.

We performed receiver-operating characteristic (ROC) curves to evaluate the diagnostic performance of ACTH level in the metyrapone test as a predictor of secondary adrenal insufficiency (SAI) analyzing sensitivity and specificity for each possible threshold/cut-off, and we used the area under the ROC curve to express the overall diagnostic accuracy of the index criterion. We have reported also a 95% confidence interval for AUC and p-value. $P < 0.05$ was considered indicative of a statistically significant difference.

Results

Out of the 103 study participants, 11 (10.6%) were males and 92 (89.4%) were females. The mean age was 48.5 years (range 25-88 years), women 48.4 years (range 25-83 years), men 49.4 years (range 29-88 years).

There were 14 patients who were more than 2 months after cessation of prolonged corticotherapy (treated for 6-36 months with 5-7.5 mg of Prednisone daily for rheumatoid arthritis or asthma), 8 were 6-24 months after pituitary neurosurgery, 7 had "empty sella" on MRI and 3 were 6,12 and 24 months after unilateral adrenalectomy due to hypercortisolaemia. The other causes of investigation for adrenal reserve were: hyponatraemia, hypoglycaemia, and hypotonia, muscle pains, lack of appetite, and loss of body weight. Average morning serum cortisol concentration before tests was 8,05 ±0,64 µg/dl and plasma ACTH level was 14,3 ±12,7 ng/l Basal serum cortisol levels were below 10 µg/dl in 75 subjects and in 45 basal plasma ACTH was below 10 ng/l.

Metyrapone test

There were 40 subjects with post-Metyrapone 11-deoxycortisol concentration below 7 µg/dl, so they were classified as patients with confirmed adrenal insufficiency - AI (+). In 22 subjects of this group diagnosis of adrenal insufficiency was additionally confirmed by insufficient cortisol increment in the 30th and 60th minute of the Synacthen test (when we took into account only cortisol in the 30th minute this number increased to 31). In group AI+ concentration of 11-deoxycortisol was 4.74 µg/dl. (95% CI 4.19-5.29) while in "healthy" group, i.e. with 11-deoxycortisol>7 µg/dl – AI(-) was 10.54 µg/dl (95% CI 9.91-11.19)

A decrease of serum cortisol concentration under 5 µg/dl is considered a positive reaction to the test with metyrapone. Mean value of cortisol was 3.2 µg/dl (95% CI 2.9-3.5), whereas in subgroup SAI(+) and SAI(-) the values significantly differ 2.4 µg/dl (95% CI 2.0-2.9) vs 3.7 µg/dl (95% CI 3.4-3.9).

The concentration of post-Metyrapone plasma ACTH for the whole group was 225.6 ng/l (95% CI 194.3-256.9), whereas in group A it was lower: 128.1 ng/l (95% CI 96.8-159.4) vs 289.9 ng/l (95% CI 249.1-330.9) (Table 1).

There was only a moderate positive correlation between ACTH and 11-deoxycortisol concentrations (Pearson r=0.5; p<0.05) and a moderate negative correlation between ACTH and cortisol level in the metyrapone test (Pearson r= - 0.3; p<0.05). There were no significant differences for this calculation in the analysed subgroups.

Analysis using ROC curves revealed that the best cut-off value of post-Metyrapone ACTH in relation to 11-deoxycortisol concentrations was < 147 ng/l - with a sensitivity of 73.2% (predicting SAI) and specificity 83.9% (positive SAI in case of 11-deoxycortisol level under 7.0 µg/dl, negative SAI when 11-deoxycortisol concentrations were at least 7.0 µg/dl). (AUC 0.833; 95% CI 0.749 – 0.917; p=0.00001). The computed risk ratio (RR) was 4.3 (95% CI 1.9 – 9.5), which means that patients with post-Metyrapone ACTH concentration lower than 147 ng/l had 4.3 times the risk of SAI compared to the group of patients who had ACTH level at least 147 ng/l.

Synacthen test

In the beginning of this analysis we have compared cortisol concentrations levels 30 and 60 minutes after Synacthen administration comparing mean values between LD-SST and HD-SST. There was no statistically significant difference (p>0.05) and in further analyses, we treated both types of the test as equivalent (Table 2).

The cortisol concentration increased from baseline to 30 and 60 minutes after injection of Synacthen (p=0.0001 for each). The majority of the examined subjects reached a cortisol peak, at a value greater than 18 µg/dl at 30 minutes. The mean cortisol levels at baseline were 8.2 µg/dl (95% CI 7.4-8.9) at 30 minutes 18.4 µg/dl (95% CI 17.2-19.7) and 21.2 µg/dl (95% CI 19.6-22.8) at 60 minutes. There was a strong positive correlation between 30 and 60 minutes cortisol values after Synacthen injection (Pearson r =0.83; p=0.0001). Further analysis using ROC curves revealed better diagnostic performance for cortisol after 30 minutes (AUC 30' 0.865; 95% CI 0.788 – 0.941) in relation to 60 minutes after Synacthen administration (AUC 60' 0.814; 95% CI 0.725 – 0.904) (Figure 1). Moreover, using 30 minutes cortisol level was resulting in only 9 false negative cases, in opposite to 30 or 60 minutes cortisol level where the number of FN was two times greater, 18 cases.

Additionally, we have compared different cut-off values for cortisol levels 30 minutes after Synacthen administration. Each cut-off value (18 µg/dl, 15.2 µg/dl, and 21.7 µg/dl; our cut off; advised by NHS Gloucestershire hospital and advised by Biochemical Investigations in Laboratory Medicine) was statistically significant, but only 18 µg/dl had the best overall performance (Table 3).

Accuracy of ACTH level in Metyrapone test in predicting SST results

We performed ROC curve analyses with the aim of finding the ACTH cut-off level able to predict an accurate value for SAI (Figure 2).

Post-Metyrapone ACTH levels correlated moderately with the levels of cortisol after Synacthen administration in the analyzed 89 SSTs (Spearman r=0.44 p=0.0012). A ROC curve performed on this data set showed that ACTH<119 ng/l had a sensitivity of 71.4% for predicting failure of the SST (predicting SAI) and specificity of 80.9% for predicting passing the SST (AUC = 0.777; 95% CI 0.663 - 0.981; p=0.000). For this cut-off computed RR was 5.4 (95% CI 1.9 – 15.5), which means that patients with ACTH levels lower than 119 ng/l had 5.4 times the risk of SAI in SST compared to the group of patients who had ACTH levels at least 119 ng/l.

Comparison of performance of different ACTH cut-off values

In Table 4 we are presenting a comparison of the performance of different cut-off values for ACTH in the metyrapone test. We decided to use three cut-offs found in the literature (75 ng/l, 150 ng/l, 200 ng/l) and our estimates based on ROC curves (119 ng/l and 147 ng/l) (shown on scatterplot Figure 3).

TABLE 1 Metyrapone test.

Analyzed group N =103	Mean (95% Cl)	Min	Max	Negative Mean (95% Cl)	Positive Mean (95% Cl)	p-value
11-Deoxycortisol, µg/dl	8.24 (7.53-8.94)	0. 075	17.6	4.74 (4.19-5.29)	10.54 (9.91-11.19)	<0. 0001
ACTH, ng/l	225.6 (194.3-256.9)	2.0	804	128.1 (96.8-159.4)	289.9 (249. 1-330. 9)	<0. 0001
Cortisol, µg/dl	3.2 (2.9-3.5)	0.1	5	2.4 (2.0-2.9)	3.7 (3.4-3.9)	<0. 0001

95% CI, 95% confidence interval; p-value for U Mann-Whitney-test.

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TABLE 2 Difference of cortisol level between LDSST and HDSST after 30' and 60'.

		N	Mean (95% Cl)	Max	p-value
30 minutes			18.4 (17.2-19.7)	34.1	0.1823
	LDSST	30	20.0 (18.2-21.8)	34.1	
	HDSST	59	17.6 (16.0-19.2)	32.6	
60 minutes			21.2 (19.6-22.8)	40.7	0.1151
	LDSST	30	18.9 (17.9-22.4)	40.5	
	HDSST	59	21.8 (19.6-23.9)	40.7	

95% CI, 95% confidence interval; p-value for U Mann-Whitney test; cortisol valuesgiven in µg/dl.

The highest sensitivity - 71.4% was noted for ACTH=147ng/l, 150 ng/l and 200 ng/l, while specificity was higher for both 147ng/l and 150ng/l in comparison with 200 ng/l 83.9% vs 69.4%. The highest specificity for predicting passing the metyrapone test had 75 ng/l 100%, but at the same time in 26 cases the test result would be false negative. The NPV, PPV, ACC and risk ratio for 147 ng/l, 150 ng/l and 119 ng/l were comparable 82.5%, 75.0%, 79.6% and 4.3 (95% CI 1.9-9.5) vs 79.5%, 86.7%, 81.6% and 4.2 (95% CI 1.9-9.1) respectively (Table 4). A comparison of estimated in this study cut-off values is presented in a scatterplot in Figure 4.

Discussion

The proper diagnosis of adrenal insufficiency is essential In any case. Undiagnosed adrenal insufficiency or delayed diagnosis may lead to a life-threatening adrenal crisis (1, 2). On the other hand, unnecessary lifelong steroid therapy in patients with false positive diagnoses of adrenal insufficiency could be very harmful, as it can enhance cardiovascular risk increasing the possibility of premature death (8, 9, 24). All of our patients have been treated with hydrocortisone in the past or currently, some of them without significant improvement in their general condition and

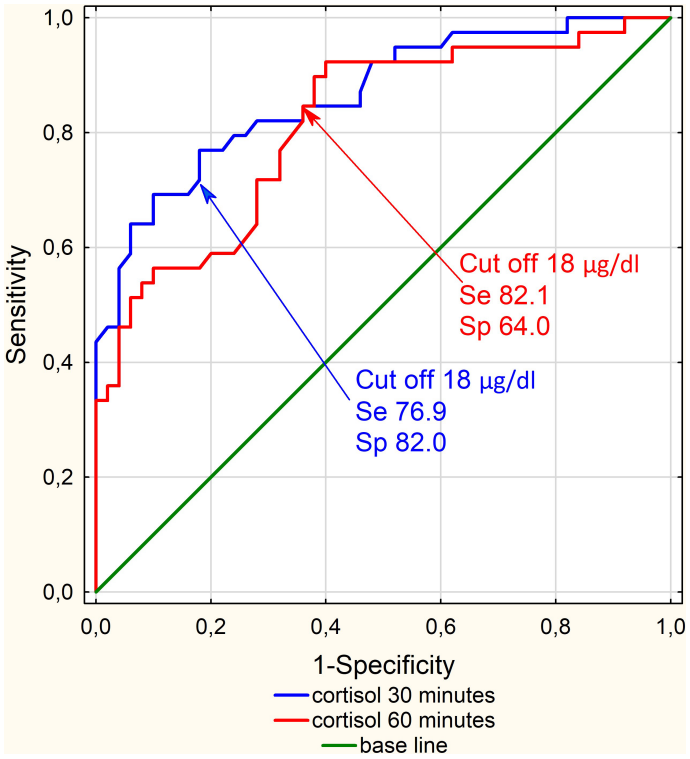

FIGURE 1
ROC curves for Cortisol after Synacthen administration - 30 minutes (AUC 30' 0.865; 95% CI 0.788 – 0.941) in relation to 60 minutes (AUC 60' 0.814; 95% CI 0.725 – 0.904) N=89.

TABLE 3 Comparison of different cortisol cut off values in SST.

Analyzed group								
N=89	**Cut off [µg/dl]**	**Se**	**Sp**	**NPV**	**PPV**	**ACC**	**RR (95% Cl)**	**p-value**
30 minutes	18	76.9	82.0	82.0	76.9	79.8	4.3 (95% CI 1.8-10.04)	0.0000
30 minutes NHS Gloucestershire hospital	15.2	46.2	96.0	69.6	90	74.1	2.9 (95% Cl 1.3-6.6)	0.0000
30 minutes Biochemical Investigations in Laboratory Medicine	21.7	97.4	34.0	94.4	53.5	61.8	9.6 (95% Cl 1.2-74.9)	0.0001

Se, senistivity; Sp, specificity; NPV, negativepredictive value; PPV, positivepredictive value; ACC, accuracy;RR, risk ratio; 95%CI, 95%confidence interval; p-value for Fisher exact test.

quality of life. After very thorough testing, we were able to confidently rule out adrenal insufficiency in as many as 63 of the 103 patients studied (61%).

In patients with secondary adrenal insufficiency, especially in states of incomplete ACTH deficiency, the "gold standard" - Synacthen short test can give false-negative results (25, 26). The use of 1 µg instead of 250 µg of Synacthen was initially thought to be a better procedure (more physiologic blood concentrations of 1-24ACTH (27), but some later studies have not confirmed this approach (28). In our material, there was a group of 18 patients who did not respond adequately to Metyrapon (their 11-deoxycortisol levels were below 7.0 µg/dl), although a diagnosis of adrenal insufficiency was initially ruled out after 1 and 250 µg of Synacthen. Such cases are reported in the literature and should be taken into account in the individual approach to the patient (23, 24).

This discrepancy between Synacthen and Metyrapon (or insulin) test results is mainly expected in patients in the first months after pituitary surgery (20). In our cases, this may be due to a partially preserved corticotropic reserve, when the adrenal cortex does not atrophy due to partial (but insufficient under stress) ACTH secretion. The adrenal glands then respond properly to high concentrations of 1-24ACTH.

Another proposed approach is to assess cortisol levels only at 30 minutes after Synacthen administration. In our study indeed we have found better diagnostic performance for cortisol after 30 minutes compared to 60 minutes after Synacthen administration. Using only 30 minute cortisol levels yielded only nine false-

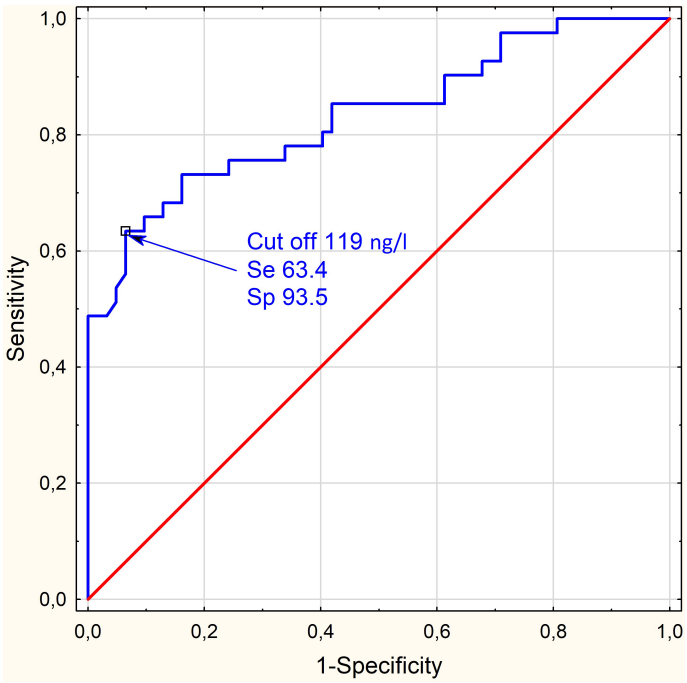

FIGURE 2
A ROC curve: ACTH<119 ng/l after Metyrapone had a sensitivity of 71.4% for predicting SAI (diagnosed in the SST) and specificity of 80.9% (AUC = 0.777; 95% CI 0.663 - 0.981; p<0.0001). N=103.

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TABLE 4 Comparison of different ACTH cut off values (from literature and estimated in our patients).

Analyzed group							
N=103	**Se**	**s**	**NPV**	**PPV**	**ACC**	**RR (95% Cl)**	**p-value**
ACTH 75	36.6	100	70.5	100	74.8	3.4	0.0000
ACTH 150	73.2	83.9	82.5	75	79.6	4.3 (1.9-9.5)	0.0000
ACTH 200	73.2	69.4	79.6	61.2	70.9	3.0 (1.4.6)	0.0004
ACTH 119	63.4	93.5	79.5	86.7	81.6	4.2 (2-9.1)	0.0000
ACTH 147	73.2	83.9	82.5	75	79.6	4.3 (1.9-9.5)	0.0000

Se, senistivity; Sp, specificity; NPV, negativepredictive value; PPV, positivepredictive value; ACC, accuracy; RR, risk ratio; 95%CI, 95%confidence interval; p-value for Fisher exact test.

negative results, compared to 30 and 60 minute cortisol levels where the number of FN was two times greater. However, a retrospective study of as many as 804 patients with adrenal insufficiency did not find this difference (29). The appearance of false-negative SST results, regardless of how it was performed and interpreted, makes one remember the option of performing a test with Metyrapon - which for the diagnosis of secondary adrenal insufficiency may be more useful than Synacthen (30).

Testing with Metyrapone is fairly easy, but in our opinion requires hospitalization. Extremely low levels of cortisol in the blood can lead to malaise and even fainting before blood is drawn, so our patients are tested after one night in the hospital and in a prone position. In 50 healthy volunteers, tested on an outpatient basis, plasma ACTH rose to 64-907 ng/l by the Metyrapone test, however, 7 of them did not respond to the drug with an adequate reduction in serum cortisol levels (21). It was interpreted as probably due to incompliance with the intake of tablets (the drug was taken at home). In our study all 103 patients had adequate post-Metyrapone cortisol levels – good compliance is a bonus of hospitalization.

In none of the 103 subjects in the described group metyrapone caused serious side effects. However, the study is retrospective, and in our clinic if there are side effects of metyrapone, the test is stopped and the doctor on duty gives the patient 50 mg of hydrocortisone intravenously. Such a patient is therefore not included in the statistics, because he does not complete the test. After reviewing the records from 2016-2018, we found 2 patients in whom the test was discontinued due to nausea and vomiting after metyrapone administration.

In countries where metyrapone is not readily available, an alternative could be a test with osilodrostat, which acts at the

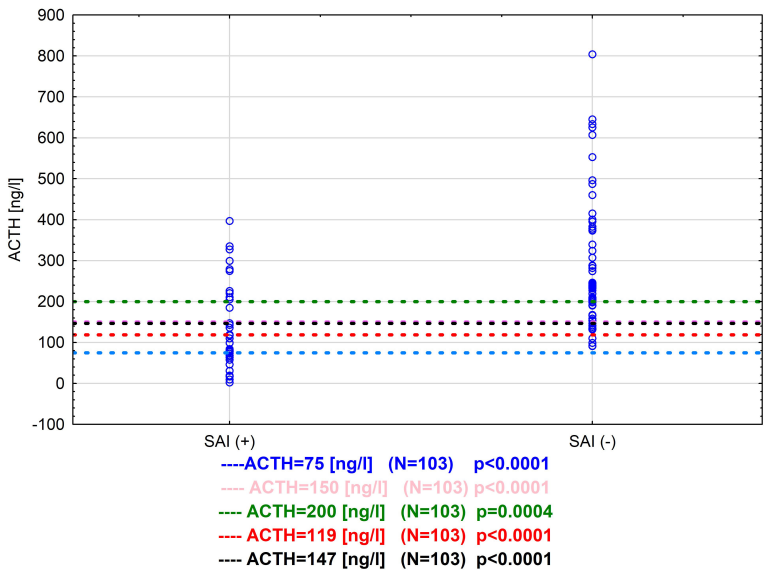

FIGURE 3
Comparison of different cut off values for ACTH in metyrapone test for our tested group (from literature and estimated in our patients) N=103.

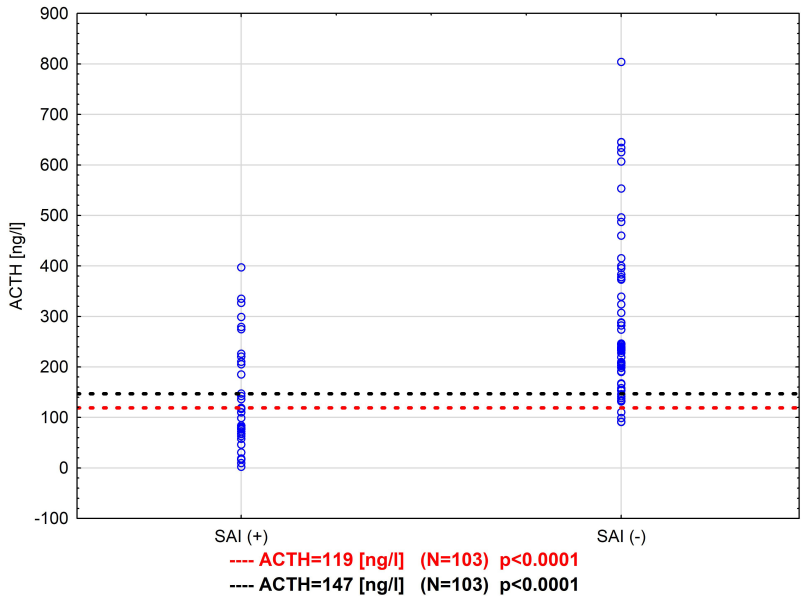

FIGURE 4
Comparison of different cut-off values for ACTH in the metyrapone test - two thresholds were found in two ways: SAI diagnosed by SST (ACTH = 119 ng/l) and by metyrapone test (ACTH = 147 ng/ml) N=103.

same stage of cortisol synthesis as metyrapone. However, it has not yet been determined what dose of osilodrostat should be used with such a test. A very wide range of doses has been described for the treatment of hypercortisolemia with this new drug, so further research is needed to determine what dose should be used to achieve suppression of cortisol synthesis after a single administration of Osilodrostat.

The best ACTH cut-off value in SAI patients confirmed by the SST was surprisingly low (119 ng/l). This is most likely due to the fact that SST gives truly positive results in advanced disease, when the pituitary reserve is very significantly reduced. The best cut-off value of ACTH in relation to 11-deoxycortisol serum concentrations after Metyrapone administration was estimated at 147 ng/l - with a sensitivity of 73.2% and specificity of 83.9%. However, plasma ACTH was >200ng/l (the highest threshold proposed in literature) in 8 cases (20%) with a positive diagnosis of SAI made on the basis of low 11-deoxycortisole and confirmed with the short Synacthen test. Such results would be expected in patients with secondary adrenal insufficiency after treatment with high doses of glucocorticoids. In these cases, an increase in ACTH concentrations was observed first, and the adrenal function returns later (31). In our eight cases in which the 11-deoxycortisol response to Metyrapone was poor, despite high ACTH after Metyrapone, only three were after treatment with high doses of GCS, and two were after unilateral adrenalectomy due to hypercortisolemia (the same mechanism of secondary adrenal insufficiency as after GCS). In these patients, adrenal function did not return during follow-up.

As the above summary and our experience show, fairly good results for sensitivity and specificity of the test fail in some cases, regardless of the cut-off adopted.

Conclusions

The diagnosis of adrenal insufficiency should be carried out extremely carefully, and no single test seems to be conclusive for every case. Although the metyrapone test has proven to be a valuable tool in the diagnosis of secondary adrenal insufficiency, the determination of ACTH concentrations alone seems insufficient. A more difficult-to-obtain assessment of 11-deoxycortisole concentration values is necessary.

Data availability statement

The original contributions presented in the study are included in the article/supplementary material. Further inquiries can be directed to the corresponding author.

Ethics statement

The studies involving human participants were reviewed and approved by EC at Medical Center of Postgraduade Education.

Written informed consent for participation was not required for this study in accordance with the national legislation and the institutional requirements.

Author contributions

LP - conception of work, data collection, article writing; MR - consultations of work shedule and conception, patients management; BM - Statistical analyses; DL - conducting examinations in the clinic; KN - conducting examinations in the clinic; AŁ-S - conducting examinations in the clinic; PG - hormonal analyses; WZ - Head of Clinic. All authors contributed to the article and approved the submitted version.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

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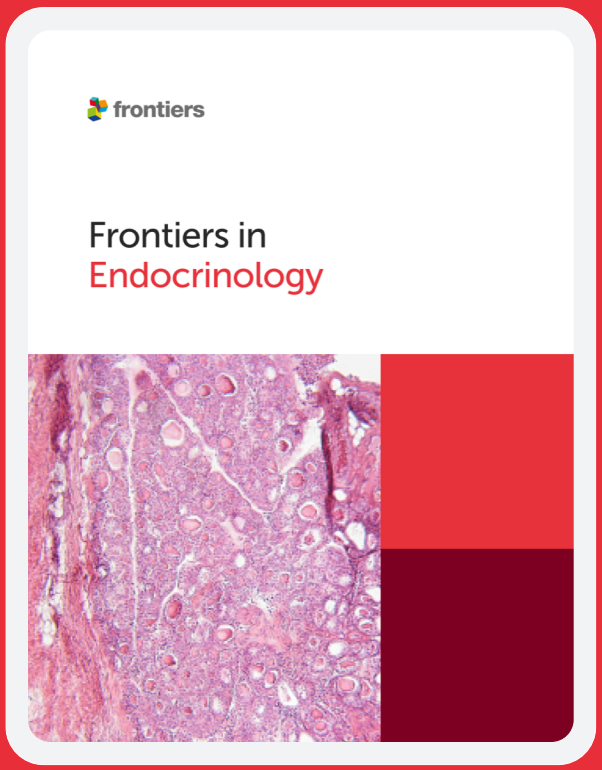

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