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Adrenal insufficiency: diagnostic approaches, treatments, and outcomes, volume II

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Agnieszka Pazderska, Carl D. Malchoff and Elena Valassi

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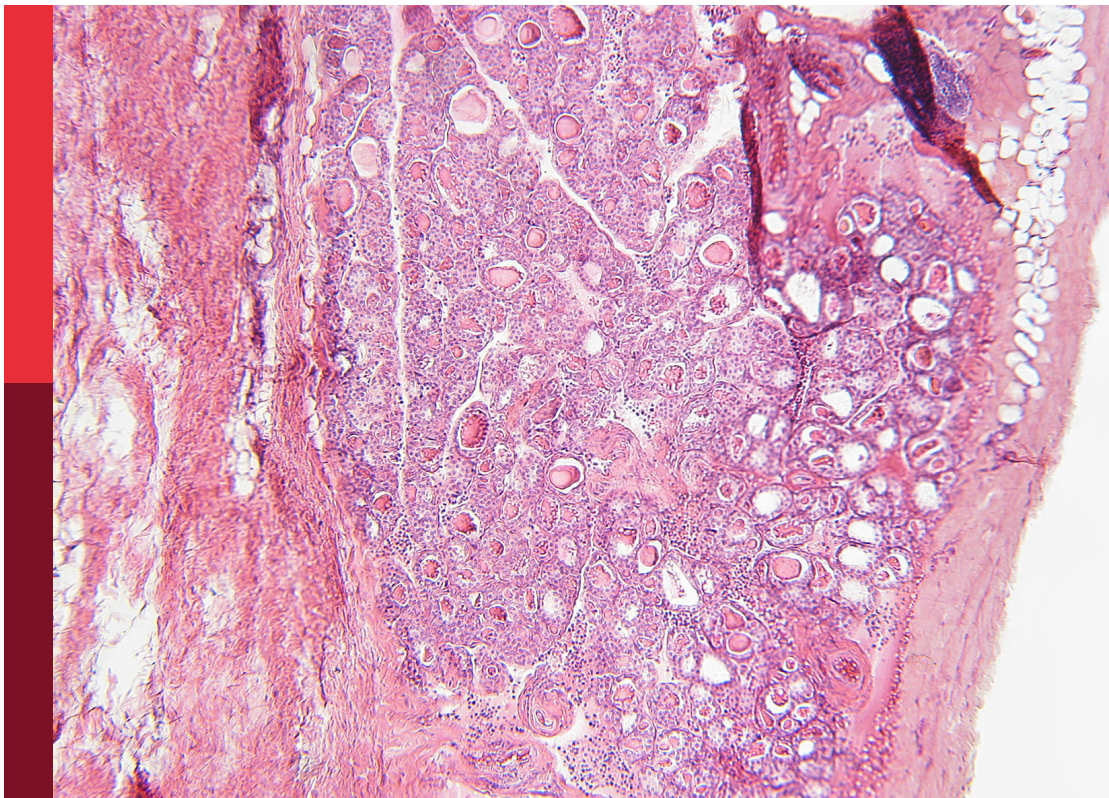

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Adrenal insufficiency: diagnostic approaches, treatments, and outcomes, volume II

Topic editors

Agnieszka Pazderska — St. James's Hospital, Ireland

Carl D. Malchoff — University of Connecticut Health Center, United States

Elena Valassi — International University of Catalonia, Spain

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EDITED AND REVIEWED BY
Henrik Falhammar,
Karolinska Institutet (KI), Sweden

*CORRESPONDENCE
Agnieszka Pazderska
apazderska@stjames.ie

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Editorial: Adrenal insufficiency: diagnostic approaches, treatments, and outcomes, volume II

Agnieszka Pazderska[1,2]*, Carl D. Malchoff[3] and Elena Valassi[4,5]

[1]Department of Endocrinology, St. James's Hospital, Dublin, Ireland, [2]School of Medicine, Trinity College Dublin, Dublin, Ireland, [3]Department of Medicine, University of Connecticut Health Center, Farmington, CT, United States, [4]Endocrinology and Nutrition Department, Germans Trias i Pujol Hospital and Research Institute, Badalona, Spain, [5]Centro de Investigación Biomédica en Red de Enfermedades Raras (CIBERER), Universitat Internacional de Catalunia (UIC), Barcelona, Spain

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adrenal insufficency, corticosteriod, hydrocortisone (HC), adrenal crisis, CIRCI (critical illness related corticosteroid insufficiency)

Editorial on the Research Topic
Adrenal insufficiency: diagnostic approaches, treatments, and outcomes, volume II

Adrenal insufficiency (AI) was first described by Thomas Addison in 1855; it was an invariably fatal condition at the time (1). Despite many advances in recent years, several aspects of the diagnosis and treatment of AI remain challenging. The diagnosis of the disease is often delayed by months resulting in many patients presenting with acute adrenal crisis. Modern cortisol replacement regimes fail to mimic the physiological circadian rhythm of cortisol secretion and currently there are no curative options for primary adrenal insufficiency (PAI). This Research Topic aims to highlight some of the diagnostic and management challenges in adrenal insufficiency and present an overview of the current knowledge and future directions.

In the developed world, autoimmune adrenalitis (PAI) is the most common culprit, with iatrogenic causes also on the rise. In this Research Topic, we present case reports of AI due to use of various medications. Choo et al. report a patient presenting with severe, symptomatic hypercalcaemia due to adrenal insufficiency precipitated by fluconazole and previous exogenous glucocorticoid use. Wu et al. describe two patients presenting with severe symptomatic hypokalemia caused by the use of electronic cigarettes containing etomidate. Both patients presented with hypertension, hypokalemia, low cortisol, high ACTH, low renin, low aldosterone and bilateral adrenal gland thickening on abdominal imaging. Etomidate is known to reduce the synthesis of cortisol, corticosterone and aldosterone by blocking 11β-hydroxylase (CYP11B1). Accumulation of 11-deoxycorticosterone-which has mineralocorticoid activity- can explain the clinical presentation.

Despite advances in glucocorticoid replacement, adrenal crisis remains an issue in patients with AI (2). Patient education and adequate self-management are important aspects in the prevention of adrenal crises. Llahana et al. aim to develop a theory-informed, digital behaviour change intervention (DBCI) to support self-management in patients with AI to complement usual care for such patients. The authors describe the systematic process

they will follow to address determinants of self-management behaviours in patients with AI and subsequently selecting salient behaviour change techniques to use when developing a tailored digital intervention.

In practice, conventional therapy with hydrocortisone or cortisone acetate leads to supraphysiological cortisol peaks which in longer term culminates in complications (3). In an attempt to maintain more physiological cortisol concentrations, a dual release hydrocortisone (DR-HC) preparation, Plenadren®, has been developed. In an observational study, Bioletto et al. attempts to assess the impact of DR-HC on skeletal health by comparing bone-related parameters in patients with PAI treated with conventional therapy (C-GC) (n=13) and DR-HC (n=14) at an equivalent daily dose. Bone turnover markers did not differ significantly between the groups. However, patients treated with DR-HC had higher bone mineral density at lumbar spine and femoral neck, as well as trabecular bone scores, compared to those on C-GC. 3 patients in the C-GC group had vertebral fractures (total of 9 fractures) versus none in the DR-HC group. Overall, the results may suggest a better bone safety profile of DR-HC compared to C-GC.

Wolff et al. discus the genetic underpinning of PAI, including monogenic forms of the disease, as well as discussing the role of genetic polymorphism in the aetiology of autoimmune PAI. The world's first Genome Wide Association Study on autoimmune PAI identified nine genetic regions that were predicted to explain 40% of the genetic susceptibility for autoimmune PAI. Furthermore, the authors discuss how the knowledge of the genetic basis for PAI can be used in the future in predicting the disease susceptibility in high-risk individuals and in helping to identify subjects who may have monogenic forms of the disease. The advancement of the knowledge on how to identify at-risk individuals, paired with the understanding of the autoimmune processes involved in the pathophysiology of the disease may help create targeted interventions designed to prevent PAI development.

The most common aetiology of AI is iatrogenic, with exogenous steroid use being a major contributor (4). However, with the recent rise in opioid use, the inhibitory effects of this class of medications on the hypothalamic-pituitary-adrenal (HPA) axis is increasingly encountered. Patel and Ben-Shlomo discuss diagnostic and management considerations in opioid-induced AI. The authors suggest that all physicians treating patients with chronic opioids should evaluate them periodically for symptoms of adrenal insufficiency. Since many patients who receive chronic opioids can not stop them due to severe pain, screening with AM cortisol is less informative. The authors suggest diagnosis should be confirmed with the high dose (250 μg) $ACTH^{1-24}$ stimulation test, after avoiding administration of opioids for several hours prior to the testing, if possible. Cessation or reduction of opioid doses can lead to recovery of HPA axis; however, the opioid dose at which this occurs remains unknown.

The COVID-19 pandemic which started in December 2019 has seen more than 778 million confirmed cases worldwide (5). In a cross-sectional study, Porntharukchareon et al. evaluated the prevalence of hypocortisolism, diagnosed using low dose (1μg) $ACTH^{1-24}$ stimulation test, three months after radiologically confirmed COVID-19 pneumonia. Of the 41 patients evaluated, eleven (27%) had hypocortisolism. Only five of the eleven patients received dexamethasone for treatment of the acute pneumonia. Increased BMI was identified as a risk factor for hypocortisolism.

Critical Illness-Related Corticosteroid Insufficiency (CIRCI) is a condition describing impairment of the HPA axis developed following critical illness (6). Sobolewska et al. discuss the pathophysiology, diagnostic pitfalls and individualized management of this condition. The current recommendation is to use total serum cortisol measurement <10 μg/dL (276 nmol/L) or change in baseline cortisol of less than 9 μg/dl (248 nmol/l) at 60 min after 250μg $ACTH^{1-24}$ administration, to diagnose CIRCI. However, decrease in cortisol plasma binding protein (CBG) and its binding affinity, often proportional to the severity of the illness, increases the distribution volume for cortisol, thus reducing the incremental response in total plasma cortisol to ACTH injection. This is highly predictive of mortality, but should not be used to identify patients who should be treated with exogenous glucocorticoids. The authors review findings from three major randomised controlled trials (ADRENAL, APROCCHSS and CORTICUS) on corticosteroid use in patients with septic shock and their effect on mortality.

Patients with primary hyperaldosteronism (PA) have increased risk of chronic kidney disease (7). Adrenalectomy is a treatment option for those with unilateral source of aldosterone excess. A significant proportion of patients have a significant eGFR decline after the surgery which is at least partly related to reduction in the hyperfiltration and unmasking of CKD after aldosterone excess has been corrected. Ma et al. report clinical outcomes and changes in renal function after adrenalectomy in patients with PA across different age groups (<40, 40-60, >60 years old). Patients aged <40 years had the highest rate of complete clinical success but the complete biochemical success was similar between the age groups. eGFR declined similarly in all three age groups in the short- and long-term. Preoperative systolic blood pressure, plasma aldosterone concentration and hypertension duration were significant predictors of postoperative renal function impairment.

Fertility and parity in women with PAI is reduced (8). O'Murchadha et al. discuss the current understanding of factors that may affect fertility and parity in women with autoimmune PAI, including premature ovarian insufficiency, co-existence of other autoimmune conditions, the potential role of the impaired adrenal sex-steroid production, and psychosocial factors and libido. The authors summarize studies reporting pregnancy outcomes in women with AI showing higher rates of caesarean section and somewhat increased risk of premature birth in this population.

We trust that this Research Topic provides valuable clinical and scientific updates to inform clinicians on important aspects of managing patients with adrenal insufficiency.

Author contributions

AP: Conceptualization, Writing – original draft. CM: Writing – review & editing. EV: Writing – review & editing.

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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The author(s) declare that no Generative AI was used in the creation of this manuscript.

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EDITED BY
Carl D. Malchoff,
University of Connecticut Health Center, United States

REVIEWED BY
Karin Zibar Tomsic,
University Hospital Centre Zagreb, Croatia

*CORRESPONDENCE
Kuan Swen Choo
choo.kuan.swen@singhealth.com.sg

†These authors share last authorship

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Case Report: Hypercalcemia as a manifestation of acute adrenal crisis precipitated by fluconazole use, and a review of the literature

Kuan Swen Choo [1*], Jielin Yew [1], Eberta Jun Hui Tan [2†] and Troy Hai Kiat Puar [1†]

[1]Department of Endocrinology, Changi General Hospital, Singapore, Singapore, [2]Raffles Diabetes and Endocrine Centre, Raffles Medical Group, Singapore, Singapore

Acute adrenal crisis classically presents with vomiting, altered sensorium, and hypotension. We describe a unique case manifesting with severe hypercalcemia. Addisonian crisis was unusually precipitated by fluconazole use. We reviewed other reported cases and discuss the possible mechanisms of hypercalcemia in adrenal insufficiency. This 67-year-old man presented with fever, cough, and vomiting for 1 week and with anorexia and confusion for 3 weeks. He was hypotensive and clinically dehydrated. Investigations revealed left-sided lung consolidation, acute renal failure, and severe non–parathyroid hormone (PTH)–mediated hypercalcemia (calcium, 3.55mol/L; PTH, 0.81pmol/L). Initial impression was pneumonia complicated by septic shock and hypercalcemia secondary to possible malignancy. He received mechanical ventilation; treatment with intravenous fluids, inotropes, and hydrocortisone for septic shock; and continuous renal replacement therapy with low-calcium dialysate. Although hypercalcemia resolved and he was weaned off inotropes, dialysis, and hydrocortisone, his confusion persisted. When hypercalcemia recurred on day 19 of admission, early morning cortisol was <8 nmol/L, with low ACTH level (3.2 ng/L). Other pituitary hormones were normal. Hypercalcemia resolved 3 days after reinstating stress doses of hydrocortisone, and his mentation normalized. On further questioning, he recently received fluconazole for a forearm abscess. He previously consumed traditional medications but stopped several years ago, which may have contained glucocorticoids. He was discharged on oral hydrocortisone. Cortisol levels improved gradually, and glucocorticoid replacement was ceased after 8 years, without any recurrence of hypercalcemia or Addisonian crisis. Both hypercalcemia and adrenal insufficiency may present with similar non-specific symptoms. It is important to consider adrenal insufficiency in hypercalcemia of unclear etiology.

KEYWORDS

anti-fungal, azole, adrenal insufficiency, iatrogenic Cushing's, hemodialysis, hypothalamic-pituitary-adrenal axis, hypercalcemia

1 Introduction

Acute adrenal insufficiency, or Addisonian crisis, classically presents with nausea, vomiting, altered mental status, and hypotension. Severe hypercalcemia is a rare manifestation. Precipitants of Addisonian crises include concurrent infection, particularly gastrointestinal infections and physical or mental stress. We report a rare case of severe hypercalcemia presenting with acute confusion and shock, precipitated by iatrogenic adrenal insufficiency. The use of fluconazole inhibits adrenal steroidogenesis and aggravated pre-existing adrenal insufficiency, leading to acute adrenal crisis. We review previous reports of fluconazole-associated adrenal insufficiency and the pathophysiology of severe hypercalcemia with adrenal insufficiency.

2 Case description

In September 2010, this 67-year-old man presented with fever, productive cough, and vomiting for 1 week. This was associated with progressive weight loss, poor appetite, lethargy, and increasing confusion over the past 3 weeks. He did not have abdominal pain, chronic cough, night sweats, or altered bowel habit. There was no headache, palpitations, or diaphoresis.

His past medical history included hypertension, hyperlipidemia, and right inguinal hernia, which was operated on 10 years ago. Chronic medications included amlodipine, lisinopril, and simvastatin. He was not on corticosteroids, diuretics, lithium, antacids, calcium, or vitamin D supplementation. Four weeks prior, he underwent saucerization of a right forearm abscess and received a course of antimicrobials. He did not consume alcohol or recreational drug.

On examination, he was confused with a Glasgow Coma scale (GCS) of 14. He was febrile and hemodynamically unstable with blood pressure of 80/50 mmHg, heart rate of 80 beats per minute, and oxygen saturation of 99% on room air. He was clinically dehydrated. Cardiovascular, respiratory, and abdominal examination was unremarkable, with no focal neurological deficit or neck rigidity. He did not have any features of Cushing's syndrome, and there were no hyperpigmented skin creases or buccal mucosa.

Initial investigations showed left upper lobe consolidation on chest radiograph and raised inflammatory markers. Computed tomography (CT) scan of the brain was unremarkable. Biochemical investigation revealed acute renal failure (creatinine at 407 μmol/L, from 84 μmol/L 3 weeks prior) and severe hypercalcemia of 3.55 mmol/L, which was deemed the likely cause of his altered sensorium. Intact parathyroid hormone (iPTH) was low at 0.81 pmol/L (reference: 1.3–7.6 pmol/L). Electrocardiogram revealed normal sinus rhythm with no shortened QTc interval. There was mild hyponatremia (serum sodium of 132 mmol/L) with normokalemia.

The initial impression was pneumonia complicated by septic shock and acute renal failure and hypercalcemia secondary to underlying malignancy in view of his loss of weight. He was immediately initiated on intravenous (IV) 0.9% sodium chloride drip and IV antibiotics (piperacillin/tazobactam and azithromycin) for healthcare-associated pneumonia. Despite 4.5 L of IV fluids, he had refractory hypotension and was put on ionotropic support, followed by IV hydrocortisone of 100 mg every 8 h as per conventional treatment of septic shock (1). He was intubated for airway protection in view of dropping GCS. Subsequently, he underwent continuous renal replacement therapy (CRRT) with low-calcium dialysate for acute renal failure with pulmonary congestion and persistent hypercalcemia.

After 5 days, his hemodynamics and renal function improved, and he was weaned off ionotropic support, hydrocortisone, and CRRT. Despite resolution of pneumonia and normalization of serum calcium, he remained persistently drowsy and febrile. Antimicrobial therapy was escalated to cover for presumptive meningoencephalitis with IV meropenem, vancomycin, and acyclovir. Magnetic resonance imaging (MRI) of his brain was unremarkable, and cerebrospinal fluid (CSF) study was negative for acid fast bacilli, fungal, bacteria, and neurotropic viruses, although electroencephalogram showed severe diffuse encephalopathy with no epileptiform activity. Although his fever resolved and overall condition improved, he remained confused when he was transferred out of the intensive care unit (ICU) on day 17 of admission.

2.1 Work-up for hypercalcemia

His calcium levels (corrected for albumin and ionized calcium) returned to normal after IV fluids, hydrocortisone, and CRRT. Ionized calcium was monitored in ICU during CRRT that can alter total serum calcium, as well as hypoalbuminuria and acid base disturbances. Ionized calcium level remained normal for 12 days after cessation of CRRT and hydrocortisone. However, after transfer out of ICU, hypercalcemia recurred with peak corrected calcium of 3.16 mmol/L on day 19 of admission (Figure 1). He received IV NS hydration and IV pamidronate of 90 mg slow infusion on recurrence of hypercalcemia with little effect.

Initial serum phosphate was high normal at 1.62 mmol/L (reference: 0.65–1.65 mmol/L), and he was vitamin D–insufficient (25-hydroxyvitamin D at 22.6 μg/L). Severe PTH-independent hypercalcemia is most commonly secondary to hypercalcemia of malignancy, and this was consistent with his history of weight loss. However, there was no evidence of malignancy on CT thorax, abdomen, pelvis, and MRI brain. His alkaline phosphatase (ALP) was normal at 58 U/L (reference: 32–103 U/L), which suggested the absence of bone lesions, and myeloma panel was negative. There was no mediastinal enlargement, lymphadenopathy, or hepatosplenomegaly suggesting sarcoidosis or lymphoma on imaging. The endotracheal aspirate and CSF studies were negative for tuberculosis and fungi. Immobilization can cause hypercalcemia in the presence of high bone turnover conditions, but there was no prolonged immobilization prior to admission nor elevated ALP suggesting Paget's disease of the bone or hyperthyroidism. Vitamin A level was sent and returned several weeks later, as low, at 0.2 mg/L (reference: 0.3–0.8 mg/L).

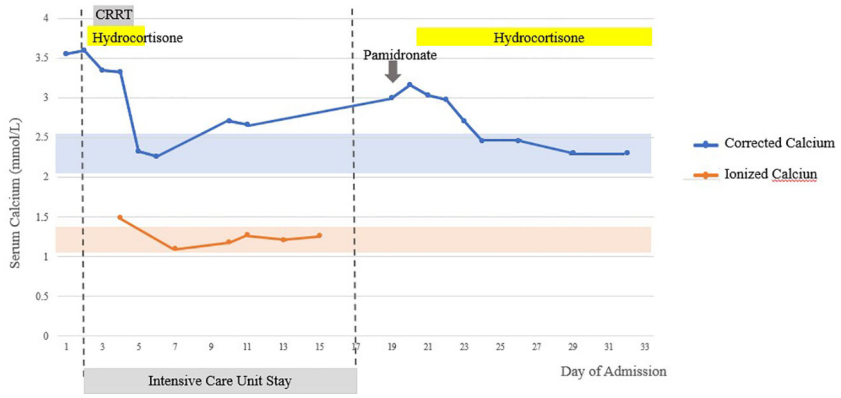

FIGURE 1
Serum-corrected calcium and ionized calcium trend inpatient. Shaded areas represent normal laboratory range.

Although his hypotension and hyponatremia had resolved, a short Synacthen test was performed the following morning, with a blunted response: baseline undetecable, <8 nmol/L; and peak serum cortisol, 12 nmol/L. Serum cortisol had not been assessed prior to hydrocortisone initiation in the ICU. Immediately, IV hydrocortisone of 50 mg every 8 h was reinitiated. Adrenocorticotropic hormone (ACTH) level was low at 3.2 ng/L (reference: 10–60 ng/L), consistent with secondary adrenal insufficiency. His other pituitary hormones were normal: free T4, 13.7 pmol/L; thyroid-stimulating hormone, 2.84 mIU/L; Insulin-like Growth Factor-1 (IGF-1), 62.6 ng/ml; prolactin, 141.8 mIU/L; and total testosterone, 15.4 nmol/L. His calcium level improved markedly after 3 days of IV hydrocortisone, and it was converted to oral hydrocortisone of 20 mg every 8 h and subsequently tailed down to 20 mg daily in divided doses. His altered mentation resolved more gradually, and, by 7 days, he made a complete recovery.

On further questioning, he revealed that he previously consumed "Jamu" (traditional Malay medication) to improve vitality but had stopped 5 years prior to admission. "Jamu" are often made up from blends of herbal ingredients. As they are unregulated, they may contain unusually high amount of corticosteroids (2). There was no other exogenous steroid consumption or application. However, he was prescribed oral fluconazole of 200 mg twice daily for 4 weeks for his forearm abscess, as cultures showed "unspecified mould". He had been consuming this up till this admission.

There was no history of previous head trauma, surgery, or radiotherapy. MRI pituitary gland was normal with no adenoma, stalk thickening, or loss of T2 bright spot suggesting lymphocytic hypophysitis. One year later, although all the other anterior pituitary hormones remained normal, serum cortisol remained low, at 15 nmol/L, whereas ACTH increased to 78.2 ng/L, suggesting recovery process of the hypothalamic-pituitary-adrenal (HPA) axis from chronic suppression with exogenous steroid. Serum aldosterone was normal at 17 ng/dl, with plasma renin activity at 4.5 ng/ml/h. Furthermore, previous CT imaging of his abdomen did not show any adrenal masses, hemorrhage, or atrophy.

2.2 Follow-up and outcomes

We established a final diagnosis of adrenal insufficiency secondary to HPA axis suppression from chronic traditional medication use, with Addisonian crisis and hypercalcemia precipitated by fluconazole use. Whereas his ACTH increased after the first year, his cortisol levels rose more gradually. After 8 years, glucocorticoid replacement was completely stopped. He has since been off steroids for 2 years, without any further recurrences of hypercalcemia, or Addisonian crisis since his initial presentation.

3 Discussion

Hypercalcemia, although reported to occur in adrenal insufficiency, is generally uncommon with only 5.5% of patients with primary adrenal insufficiency reported to have hypercalcemia (3) and also seen in patients with secondary adrenal insufficiency. Hypercalcemia in adrenal insufficiency is usually mild to moderate, with the severe hypercalcemia in our patient highly unusual. To our knowledge, this is the first case of severe hypercalcemia occurring due to adrenal insufficiency precipitated by fluconazole use.

3.1 Mechanism of hypercalcemia in adrenal insufficiency

Various mechanisms on how adrenal insufficiency can lead to hypercalcemia have been postulated, including decreased renal calcium excretion, increased bone resorption and less likely, increased gut calcium absorption. In adrenal insufficiency, there is

volume depletion and reduced cardiac contractility, resulting in decreased glomerular filtration rate and reduction in the amount of calcium filtered. Coupled with increased calcium and sodium reabsorption at the proximal tubules, this results in decrease in calcium clearance (4, 5). Patients with primary AI are at greater risk due to concomitant mineralocorticoid deficiency and consequent volume depletion.

Hypercalcemia from increased bone resorption in adrenal insufficiency appears to be mediated by thyroid hormone. In adrenal insufficiency, loss of inhibition of pituitary TSH secretion by glucocorticoid may increase bone resorption and mobilize calcium from bone (6). Thyroid hormone replacement may precipitate hypercalcemic crisis in untreated Addison's disease, possibly through increase cortisol clearance and increase bone resorption (7). In contrast, coexistence of secondary hypothyroidism in hypopituitarism appears to be protective against development of hypercalcemia (8). Our patient was euthyroid, which could have facilitated the development of hypercalcemia. Physiological amount of glucocorticoid may also be necessary for the acquisition and preservation of the differentiated state of osteoblasts (9).

Glucocorticoid inhibits 1α-hydroxylation of vitamin D; hence, there is a consideration whether the lack of cortisol promotes this process and results in increased gut calcium absorption. However, 1,25-hydroxyvitamin D levels have been found to be low in previous reports (5, 10). Calcium-free diet in animal studies and changes in dietary calcium intake did not alter the incidence or degree of hypercalcemia (5, 7), making this mechanism less likely.

3.2 Management of severe hypercalcemia in adrenal insufficiency

Severe hypercalcemia can lead to profound hypovolemia *via* gastrointestinal loss, hypercalcemia-induced diuresis, and nephrogenic diabetes insipidus. This is aggravated by the underlying adrenal insufficiency and profound shock, leading to multiorgan failure. Prompt treatment to lower calcium level, glucocorticoid replacement, and close monitoring for complications are required. Hydration with IV fluids corrects volume depletion and increases glomerular filtration rate and calciuresis when sodium is reabsorbed in exchange of calcium at the proximal tubules (11, 12). Forced calciuresis with loop diuretics is not routinely recommended as it can worsen volume depletion and other electrolytes abnormalities (11). Whereas aggressive volume repletion may improve hypercalcemia, calcium level only normalizes with adequate glucocorticoid replacement therapy, as evident in our patient and previous reports (5, 6, 13).

Drugs that inhibit osteoclastic-mediated bone resorption such as calcitonin, IV bisphosphonates (6, 14, 15), and denosumab can be used to temporarily lower calcium level while adequate glucocorticoid replacement takes effect. Calcitonin has the additional effect of increasing renal calcium excretion, and its rapid onset of action can be beneficial in severe hypercalcemia, but its use is limited due to tachyphylaxis and modest calcium-lowering effect. IV bisphosphonates are more potent in calcium-lowering but are relatively contraindicated in renal impairment due to risk of glomerular sclerosis and acute tubular necrosis (16). Denosumab, which is not renally cleared, can be considered in patients with renal impairment or bisphosphonate-resistant hypercalcemia (17). However, we did not find any reports on denosumab use in hypercalcemia secondary to adrenal insufficiency thus far. Hemodialysis is the most effective and rapid means of improving severe hypercalcemia. It can reduce calcium level by 0.75 to 1.25 mmol/L within hours and should be considered in patients with advanced oliguric renal failure or refractory hypercalcemia (11). It effectively lowered our patient's calcium level but rebounded as glucocorticoid was withdrawn. Our case demonstrated the importance of treating the underlying adrenal insufficiency for resolution of hypercalcemia.

3.3 Fluconazole and adrenal insufficiency

Chronic exogenous steroid use is the commonest cause of adrenal suppression. Even if initial history was unyielding, surreptitious use of steroid in various preparation, including traditional medication, must be sought after. Our patient did not exhibit Cushingoid features. His HPA axis had likely been suppressed for a prolonged period, and the recent use of fluconazole precipitated the adrenal crisis.

Fluconazole is a triazole antifungal with greater affinity for fungal cytochrome P450 compared to mammalian cytochrome P450, making it less toxic and favorable compared to ketoconazole in treatment of fungal infection (18). Ketoconazole is an imidazole antifungal that inhibits side-chain cleavage, 17,20-lyase, and 11β-hydroxylase enzymes in adrenal steroidogenesis pathway and is also used in the treatment of Cushing's syndrome (19). However, the role of fluconazole in affecting steroidogenesis is less clear.

Fluconazole was found to inhibit 11β-hydroxylase and 17-hydroxylase activity in pharmacological doses *in vitro*. Its inhibitory effect on cortisol production was dose-dependent and less potent compared to ketoconazole (20). Reduction in cortisol level has been observed with low doses of fluconazole of 200 mg daily (21, 22). In a study examining the use of fluconazole of 400 mg daily in critically ill patients, basal ACTH, basal cortisol, and ACTH-stimulated cortisol levels were not significantly altered (23). Similarly, fluconazole prophylaxis in critically ill surgical patients did not lower the median plasma cortisol level or result in adrenal dysfunction (24). However, several case reports have described acute adrenal insufficiency in patients receiving fluconazole, in both critically ill and stable patients, which was reversible after cessation of anti-fungal (Table 1). Some of these patients had other precipitating factors for adrenal insufficiency such as previous use of dexamethasone (21, 25), megestrol (25), or combination of ritonavir and fluticasone (26).

In most of these case reports, adrenal insufficiency occurred from 24 h to 68 days after initiation of the drug (25, 32). Most cases

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TABLE 1 Case reports on adrenal insufficiency with triazole antifungals use and the associated calcium level.

	Age/ gender	Antifungal implicated	Evaluation for adrenal insufficiency (AI)	Type of AI	Calcium level	Other relevant history/precipitants	Outcome
Albert et al., 2001 (25)	66, female	Fluconazole of 200–400 mg daily	SST before fluconazole: * B. cortisol of 516 nmol/L * P. cortisol of 773 nmol/L SST after 9 days on fluconazole: * B. cortisol of 408 nmol/L * P. cortisol of 464 nmol/L No ACTH values	Unknown	Not reported	Admitted for septic shock secondary to staphylococcus aureus bacteremia and left knee septic arthritis	Normalization of peak SST response 4 days after stopping Fluconazole: * B. cortisol of 499 nmol/L * P. cortisol of 568 nmol/L * ACTH of 60 ng/L
Shibata et al., 2001 (21)	63, male	Fluconazole of 200 mg daily (days 1–8 of admission) From day 16, Fluconazole restarted at 400 mg daily	SST after 8 days on fluconazole (200 mg daily): * B. cortisol of 276 nmol/L * P. cortisol of 662 nmol/L Patient rechallenged with fluconazole (400 mg daily). SST after 5 days of restarting: * B. cortisol of 166 nmol/L * P. cortisol of 414 nmol/L No ACTH values	Unknown	Not reported	Multiple myeloma Received VAD regimen (vincristine, doxorubicin, and dexamethasone) Underwent peripheral stem cell harvesting after receiving high dose cyclophosphamide	Continued on fluconazole prophylaxis with concurrent corticosteroid supplementation
Krishnan et al., 2006 (18)	38, male	Fluconazole of 400 mg daily	SST before fluconazole: * P. cortisol of 663 nmol/L SST after 2 days on fluconazole: * B. cortisol of 45 nmol/L * P. cortisol of 375 nmol/L No ACTH values	Unknown	Not reported	Hypercapnic respiratory failure secondary to pneumonia	Normalization of peak SST response 10 days after stopping fluconazole: * B. cortisol of 38 nmol/L * P. cortisol of 662 nmol/L
St Clair et al., 2012 (26)	52, male	Fluconazole of 400 mg daily Inhaled fluticasone was stopped concurrently	SST less than 1 week after starting fluconazole and stopping inhaled fluticasone: * B. cortisol of 47 nmol/L * P. cortisol of 353 nmol/L * ACTH of <5 ng/L	SAI (possibly potentiation of fluticasone effect due to ritonavir), aggravated by PAI induced by fluconazole	Not reported	On ritonavir, atazanavir and efavirenz for HIV infection On inhaled fluticasone propionate (250 mg twice daily) for chronic bronchitis	No follow up of the HPA axis reported
Freyer et al., 2022 (27)	70, female	Fluconazole of 400 mg daily	SST after 67 days on fluconazole * B. cortisol of 116 nmol/L * P. cortisol of 353 nmol/L * ACTH of 96.7 ng/L	PAI	Not reported	B-cell acute lymphoblastic leukemia, underwent allogenic hematopoietic cell transplantation	Fluconazole was initially stopped and switched to caspofungin. Fluconazole was later restarted with concurrent hydrocortisone supplementation. SST 31 days after fluconazole was restarted: * B. cortisol of 268 nmol/L * P. cortisol of 339 nmol/L * ACTH of 123.5 ng/L

(Continued)

TABLE 1 Continued

	Age/ gender	Antifungal implicated	Evaluation for adrenal insufficiency (AI)	Type of AI	Calcium level	Other relevant history/precipitants	Outcome
Miller et al., 2017 (28)	63, male	Posaconazole of 300 mg daily	SST after 2 months on posaconazole: * B. cortisol of 52 nmol/L * P. cortisol of 113 nmol/L * ACTH of 154.6 ng/L	PAI	2.13 mmol/L	Chronic myelomonocytic leukemia Had intermittent steroid injections into his spine. Last steroid injection 3 months before presentation	Normalization of peak SST response 1 year after stopping posaconazole: * B. cortisol of 138 nmol/L * P. cortisol of 510 nmol/L * ACTH of 34.1 ng/L
Araque et al., 2020 (29)	65, male	Posaconazole of 500 mg daily	Blood tests done on dexamethasone of 3 mg daily and indeterminate duration of posaconazole: * B. cortisol of 11 nmol/L * ACTH of 3.4 ng/L * Sodium of 130 mmol/L * Potassium of 5.1mmol/L * Renin 16.7 of ng/ml/h * Aldosterone of 1.6 ng/dl	PAI induced by posaconazole, on background of SAI from dexamethasone	Not reported	Hemophagocytic lymphohistiocytosis on dexamethasone	One month after stopping posaconazole, B. cortisol, and ACTH showed recovering HPA axis * B. cortisol of 359 nmol/L * ACTH of 53.9 ng/L
Villar-Prados et al., 2021 (30)	56, male	Posaconazole of 300 mg daily Patient was subsequently switched to fluconazole of 800 mg daily	SST after 5 months on Posaconazole: * B. cortisol of 160 nmol/L * P. cortisol of 198 nmol/L * ACTH of 168 ng/L	PAI	Not reported	Chronic disseminated coccidioidomycosis	SST after 7 months on Fluconazole showed persistent hypocortisolism: * B. cortisol of 5.5 nmol/L * P. cortisol of 22 nmol/L * ACTH of 34 ng/L * Renin of 2.6 ng/ml/h * Aldosterone of <4.0 ng/dl
Nalla et al., 2017 (31)	72, female	Itraconazole of 200 mg daily	Blood tests done after 2 years of itraconazole: * Random cortisol of 4 nmol/L * ACTH of <10 ng/L * MRI pituitary normal	SAI (possibly potentiation of fluticasone effect due to itraconazole)	Not reported	Allergic bronchopulmonary aspergillosis On Seretide evohaler (fluticasone of 500 µg and salmeterol of 50 µg) daily for asthma	No follow up of the HPA axis reported

PAI, primary adrenal insufficiency; SAI, secondary adrenal insufficiency; HPA, hypothalamic-pituitary-adrenal axis; ACTH, adrenocorticotropic hormone; B. cortisol, basal (non-stimulated) cortisol; P. cortisol, peak cortisol response after 250 mcg of Synacthen (cosyntropin) stimulation test; SST, 250 µg of Synacthen (Cosyntropin) stimulation test.

of adrenal suppression were reversible within 5 to 11 days after cessation of fluconazole (18, 21, 25). Unlike the previous cases, our patient remained hypocortisolic for 8 years after stopping fluconazole, which was likely due to prolonged suppression of the HPA axis from exogenous steroid use. HPA axis recovery is known to vary widely due to interindividual susceptibility, dose, and duration of steroid exposure, with recovery in weeks to years, or may not occur at all in some patients (33). Concurrent use of fluconazole during dexamethasone therapy was associated with prolonged HPA recovery (34). This is due to the inhibition of CYP3A4 by fluconazole, which results in decreased metabolism of corticosteroid and increased corticosteroid exposure to the HPA axis (35). Itraconazole is an even more potent inhibitor of CYP3A4, and secondary adrenal insufficiency was reported to occur in a patient on inhaled fluticasone and itraconazole (31). Another triazole antifungal, posaconazole, has also been reported to lead to adrenal insufficiency in two case reports (28, 30).

There have been case reports of hypercalcemia occurring with azole antifungal use, although cortisol levels were not measured, and it is unclear whether hypocortisolism may have contributed to hypercalcemia (36, 37). The authors postulated that hypercalcemia may have occurred due to inhibition of CYP enzymes responsible for the metabolism of vitamin A or *via* PTH-mediated hypercalcemia. Interestingly, fluconazole has also been reported to be used to treat hypercalcemia by inhibiting 1α-hydroxylase. This was used in patients with mutations of CYP24A1 and SLC34A3 gene, leading to persistently raised 1,25-dihydroxyvitamin D, hypercalcemia, hypercalciuria, and nephrocalcinosis/nephrolithiasis (38, 39).

3.4 Conclusion

Adrenal insufficiency is a rare but important cause of severe hypercalcemia. Both adrenal insufficiency and hypercalcemia have similar non-specific symptoms, including anorexia, vomiting, postural giddiness, confusion, and hypotension. Failure to assess cortisol status or serum calcium levels may lead to a delay in diagnosis with fatal consequences. In patients with hypercalcemia of unknown etiology, adrenal insufficiency should be considered. The most common cause of adrenal insufficiency remains exogenous steroid use, and a careful review of drug history is warranted. Finally, although not as potent as ketoconazole, physicians should be mindful of the risk of hypocortisolism when initiating patients on triazole antifungals, which may precipitate an Addisonian crisis.

Author contributions

TP and KC were involved in conception of the manuscript. KC wrote the first draft, and JY and TP wrote additional sections of the manuscript. ET and TP were involved in the clinical care of the patient. 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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EDITED BY
Agnieszka Pazderska,
St. James's Hospital, Ireland

REVIEWED BY
Rosemary Louise Rushworth,
University of Notre Dame Australia, Australia
Roberto Franceschi,
Santa Chiara Hospital, Italy

*CORRESPONDENCE
Sofia Llahana
sofia.llahana@city.ac.uk
sofia@llahana.com

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Using the behaviour change wheel and person-based approach to develop a digital self-management intervention for patients with adrenal insufficiency: the *Support AI* study protocol

Sofia Llahana[1,2]*, Kathleen Mulligan[1,3], Shashivadan P. Hirani[1], Stephanie Wilson[4], Stephanie E. Baldeweg[2,5], Ashley Grossman[6,7], Christine Norton[8], Philippa Sharman[9], Pat McBride[10] and Stanton Newman[1]

[1]School of Health & Psychological Sciences, City, University of London, London, United Kingdom, [2]Department of Diabetes and Endocrinology, University College London Hospitals National Health Service (NHS) Foundation Trust, London, United Kingdom, [3]Community Health Newham, East London National Health Service (NHS) Foundation Trust, London, United Kingdom, [4]Centre for Human Computer Interaction (HCI) Design, School of Science and Technology, City, University of London, London, United Kingdom, [5]Centre for Obesity and Metabolism, Department of Experimental and Translational Medicine, Division of Medicine, University College London, London, United Kingdom, [6]Neuroendocrine Tumour (NET) Unit, Royal Free Hospital, London, United Kingdom, [7]Green Templeton College, University of Oxford, Oxford, United Kingdom, [8]Florence Nightingale Faculty of Nursing, Midwifery and Palliative Care, King's College London, London, United Kingdom, [9]The Addison's Disease Self-Help Group Patient Advocacy Group Bristol, United Kingdom, [10]The Pituitary Foundation Patient Advocacy Group, Bristol, United Kingdom

Introduction: Most patients with Adrenal insufficiency (AI) require lifelong glucocorticoid replacement. They need to increase glucocorticoids during physical illness or major stressful situations and require parenteral hydrocortisone in the event of an adrenal crisis. Patients with AI have impaired quality of life and high mortality; approximately 1 in 6-12 patients are hospitalised at least once/year from a potentially preventable adrenal crisis. Adoption of self-management behaviours are crucial; these include adherence to medication, following "sick day rules" and associated behaviours that aid prevention and treatment of adrenal crisis such as symptom monitoring, having extra tablets, carrying a medical-alert ID and injection kit, and self-injecting when necessary. Current patient education is ineffective at supporting self-management behaviour change or reducing adrenal crisis-related hospitalisations. This research study aims to gain an in-depth understanding of the barriers and enablers to self-management for patients with AI and to develop an evidence-based digital self-management behaviour change intervention.

Methods: The study is conducted in accordance with the MRC Framework for developing complex interventions. Underpinned by the Behaviour Change Wheel (BCW), the Theoretical Domains Framework (TDF), and the Person-

Based Approach, this research will be conducted in two phases: Phase 1 will involve a sequential qualitative/quantitative mixed-methods study involving focus group interviews followed by a cross-sectional survey with patients with AI recruited from patient advocacy groups and endocrine clinics in the UK. Phase 2 will develop the *Support AI*, a website-based digital behaviour change intervention (DBCI) informed by Phase 1 findings to support self-management for patients with AI. The most appropriate behaviour change techniques (BCTs) will be selected utilising a nominal group technique with an Expert Panel of 10-15 key stakeholders. The design of the *Support AI* website will be guided by the Person-Based Approach using an Agile iterative "think-aloud" technique with 12-15 participants over 3 usability testing iterations.

Conclusion: A theory- and evidence-based digital behaviour change intervention will be developed which will be tested in a feasibility randomised trial following completion of this study. The projected benefit includes cost-effective health care service (reduced hospitalisations and demand for specialist services) and improved health outcomes and quality of life for patients with AI.

KEYWORDS

adrenal insufficiency, adrenal crisis, self-management, digital behaviour change intervention, behaviour change wheel, theoretical domains framework, person-based approach

1 Introduction: self-management for patients with adrenal insufficiency

Adrenal insufficiency (AI) is caused by lack or insufficient production of cortisol from the adrenal cortex. Depending on its cause, AI can be "primary" due to adrenal gland failure (most commonly Addison's disease), "secondary" due to conditions affecting the hypothalamo-pituitary axis which fails to stimulate cortisol production, or "tertiary" caused by hypothalamo-pituitary-adrenal axis suppression from chronic treatment with corticosteroids (1, 2). Tertiary AI is usually transient and in more than 75% of patients adrenal function recovers within 6 months of stopping corticosteroids, although it can be life-long (3). Most patients with AI require life-long glucocorticoid replacement therapy, commonly hydrocortisone tablets 2-3 times/day. They need to increase glucocorticoids during illness or major stressful situations and require parenteral hydrocortisone in the event of an adrenal crisis to prevent hospitalisation and death; these are called "sick day rules". Adrenal crisis is a life-threatening acute complication, mainly precipitated by infections, vomiting, diarrhoea, trauma or surgery; patients typically present with profoundly impaired well-being and hypotension, and are often unable to self-inject and may require help from a relative/carer/friend or health professional (4–7).

Abbreviations: AI, adrenal insufficiency; BCTs, Behaviour Change Techniques; BCW, Behaviour Change Wheel; COM-B model, Capability, Opportunity, Motivation – Behaviour model; DBCI, Digital Behaviour Change Intervention; TDF, Theoretical Domains Framework.

The standard mortality rate in patients with AI is twofold compared to the general population (8–10), while 1 in 200 patients die from a potentially preventable adrenal crisis (10–12). As a percentage of total diagnosis, deaths from adrenal crisis in the UK are ten times higher than deaths from insulin-dependent diabetes-related ketoacidosis (13). Between one in 6-12 patients with AI are hospitalised at least once a year following an adrenal crisis episode (11, 14, 15). AI poses a significant health burden on patients, their families, and the healthcare system. A UK study in 2013 estimated an annual cost of illness associated with AI at £39.7 million for approximately 20,000 patients (16). Health care cost for patients with AI is four times higher than for the general population (17). More than 60% of patients with AI have impaired quality of life, 40% take sick leave at least quarterly, and in some countries approximately 25% of patients receive a disability living allowance (18–21). The impact from AI and glucocorticoid treatment sequelae can be minimised significantly, and up to 50% of adrenal crisis-related hospitalisations may be prevented with effective self-management (4). This includes treatment optimisation and improved adherence to daily glucocorticoid replacement therapy (alongside mineralocorticoid replacement therapy for "primary" AI and pituitary replacement therapy for "secondary" AI), appropriate glucocorticoid dose adjustment for "sick days", and timely administration of parenteral hydrocortisone in an adrenal crisis. It also includes adoption of behaviours that aid the prevention or treatment of adrenal crisis, e.g. symptom monitoring, having an extra supply of tablets, wearing a medical-alert ID, carrying a steroid emergency card and an emergency injection kit, and self-injecting when necessary.

These self-management behaviours place a significant burden on patients with AI. Forss et al. reported that 38% of patients with AI found the multiple daily dosing problematic and consequently missed doses (21). Chapman et al. found that only 15% of patients took all their doses as prescribed, 25% of patients took higher doses than advised, while 1 in 25 patients reported prolonged treatment interruptions (22). Non-adherence or interruptions to daily glucocorticoids were found to trigger an adrenal crisis for approximately 5% of patients with AI (14, 23). Glucocorticoid over-replacement exposes patients to medication side effects (21, 22), cardiovascular complications (24), osteoporosis (25), depression and impaired quality of life (20, 21, 26).

Several studies found that 26% – 38% of patients with AI did not adjust their glucocorticoid dose for "sick days" (22, 27–31) and only 60% of patients carried a medical-alert ID (28, 29). Approximately 70% of patients had an emergency hydrocortisone injection kit (5, 15) but only 12% managed to self-inject when they experienced an adrenal crisis (15), while only 19% were trained to self-inject (5). Self-injection reduces the risk of hospitalisations when administered in a timely manner from onset of adrenal-crisis symptoms; 38% of patients who self-injected required hospitalisation versus 73% who were injected by a medical professional due to the delay in waiting for them to arrive (p=0.008) (30).

A recent systematic review of seven patient education studies reported an improvement in patients' knowledge and self-confidence of managing their AI; this was evaluated using patient self-reported diary-based or cross-sectional questionnaire measures. Although the authors of this systematic review claim that their aim was to evaluate behavioural interventions aiming to prevent adrenal crisis, the included studies were not designed as interventions to bring about behaviour change, i.e. to prevent adrenal crisis, and none was a randomised controlled study. All studies were education-based, focusing on increasing patients' knowledge about their condition, with the implicit assumption being that information provision will lead to behaviour change. However, assessment of outcomes such as behaviour change, and frequency of adrenal crisis or hospitalisations was not reported. The authors concluded that there is a need to develop behaviour change interventions to support patients with AI to prevent adrenal crisis (32).

Published studies involving patient with AI are limited in that they lack theoretical underpinning for complex intervention development (33). Therefore, there is a need to develop and test an intervention that adopts a broader self-management approach that goes beyond information provision and is informed by theoretical, evidence-based frameworks. Intervention development should involve a dynamic iterative process with stakeholder input throughout development, and should go beyond a narrow focus of increased effectiveness, paying attention to future implementation in the real world (34, 35).

Annual participation in education programmes has been recommended for patients with AI (1, 4, 36, 37) as only 40% retain all the information in these sessions (38). Uptake of education programmes has not been high, with less than 60% taking up the offer of an education programme. This is mainly due to geographical/time constraints and their "one-size-fits-all" approach to organisation of sessions and their content (37, 39, 40).

Face-to-face programmes require extensive specialist time and resources, e.g. one-hour sessions for individual education (40) and 2-3 hours for small patient group education (30, 37, 39), presenting a financial burden for a health service. Digital behaviour change interventions (DBCI) are increasingly recognised for their value and cost effectiveness in supporting self-management in patients with chronic conditions (41–43). There is currently no published evidence of DBCIs in patients with AI. The present study will develop a DBCI, which in line with the goals set in the United Kingdom "more efficient use of specialist services Five Year Forward View" (44) will harness the potential of technology to enable patients with AI to take an active role in their self-management and to enable more efficient use of specialist services. Delivery of the DBCI through a personalised responsive mobile-optimised website can provide an easily accessible and cost-effective platform to complement usual care for all patients with AI, and in this way reduce inequalities in the treatment they receive.

1.1 Study aim and objectives

This research study aims to develop a theory-informed digital behaviour change intervention (DBCI) to support self-management in patients with AI.

Objectives:

1. Understand the manner and extent to which patients with AI are engaging with the target self-management behaviours.
2. Identify barriers and enablers and measure their impact on self-management behaviours.
3. Explore how and which of these target behaviours can be addressed through a DBCI.
4. Design a theory-informed DBCI that is acceptable to patients and other key stakeholders.

2 Methods and analysis

2.1 Theoretical frameworks underpinning the study

This research study is conducted in line with the *Medical Research Council (MRC) Framework for Complex Interventions* which recommends using a systematic and transparent process that involves the following key elements: Development, Feasibility and Piloting, Evaluation, and Implementation (33). In this protocol we discuss the *Development* element. This initially involves a theoretical understanding of the likely process of behaviour change by drawing on existing evidence and analysis via theoretical frameworks through which the intervention could be developed and modelled. Modelling of the intervention involves

deciding what should be targeted (determinants of behaviour) and how this can be achieved.

Interventions targeted at changing behaviour need to be informed by theoretical, evidence-based frameworks. We will adopt the Behaviour Change Wheel (BCW) framework (Figure 1), developed from 19 existing behaviour change frameworks (45, 46), to underpin the intervention development in 3 stages depicted in Figure 2:

- Stage 1: Understand the behaviour,
- Stage 2: Identify intervention options,
- Stage 3: Identify content and implementation options

At the core of the BCW is the COM-B general model of behaviour which explains how behaviours come about at any particular moment based on an individual's Capability (C) and Motivation (M) and the situations that provide them with the Opportunity (O) to enact or change *Behaviour* (*B*) outlining thus all potential influences on the targeted behaviour (45). The BCW recognises that behaviour change occurs as a result of an interacting system of Intervention Functions, i.e. broad categories of activities aimed at changing behaviour, such as education, persuasion, and Policy Categories, which describe actions on the part of responsible authorities that enable or support the intervention delivery, e.g. service provision, guidelines (45) (Figure 1).

Associated with the BCW and COM-B is also the Theoretical Domains Framework (TDF) comprising of 14 domains, each correlating to a COM-B component, drawn from 33 behaviour change and psychological theories (46, 47). The TDF provides a more granular and deeper exploration of the barriers and enablers to behaviour change. The BCW provides guidance on linking the TDF Domains to the Intervention Functions most likely to be effective in changing the identified target behaviours, and also provides guidance on identifying salient more fine-grained specific Behaviour Change Techniques (BCTs) to deliver the intervention (48).

We will also adopt the Person-Based Approach to design and build the website to deliver the intervention. This approach can provide in-depth understanding of the needs of patients with AI as the intervention users. It can identify the intervention design features that users view as most important and potential usability problems, thus improving acceptability, user engagement and experience, and effectiveness of the intervention (49). A schematic of the study design guided by the BCW and Person-Based Approach is presented in Figure 2.

2.2 Phase 1: developing the intervention context (behaviour change wheel)

2.2.1 Step 1: define the problem in behavioural terms (BCW Stage 1)

The first step of the BCW involves defining the problem behaviours that need to change and the target population involved in the behaviour, i.e. who is performing the behaviours (45, 46). Published evidence described earlier identified that the problem behaviours performed by patients with AI are related to non-adherence to self-management behaviours in treating their AI on a daily basis, during physical or emotional illness, and preventing and treating adrenal crisis.

2.2.2 Step 2: select the target behaviour (BCW Stage 1)

This step will involve generating a "long list" of all the potential behaviours that may influence the target behavioural problem (45, 46) i.e. self-management behaviours, based on existing evidence.

For patients with AI, the target behavioural problem will involve potential behaviours across the three levels of self-management:

1) Daily management of AI and treatment optimisation to minimise adverse effects from over- or under-replacement;

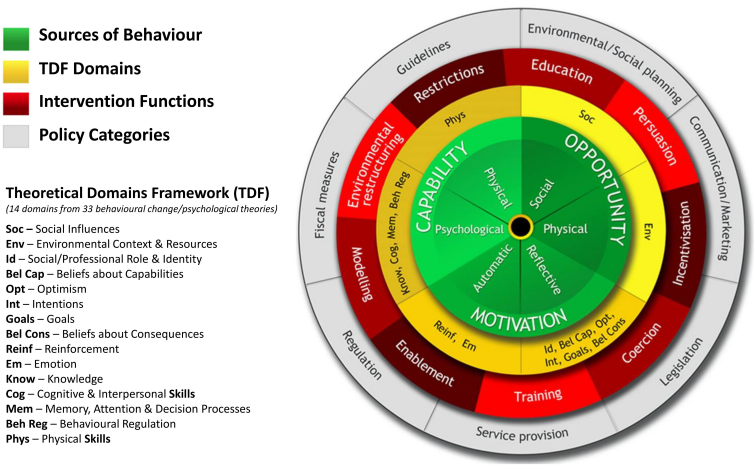

FIGURE 1
The behaviour change wheel and theoretical domains framework. Reproduced from Michie S, Atkins L, West R (2014) The behaviour change wheel: a guide to designing interventions. Silverback Publishing and Ojo SO et al. (2019) BMC Public Health, 19, 1126, doi.org/10.1186/s12889-019-7468-8.

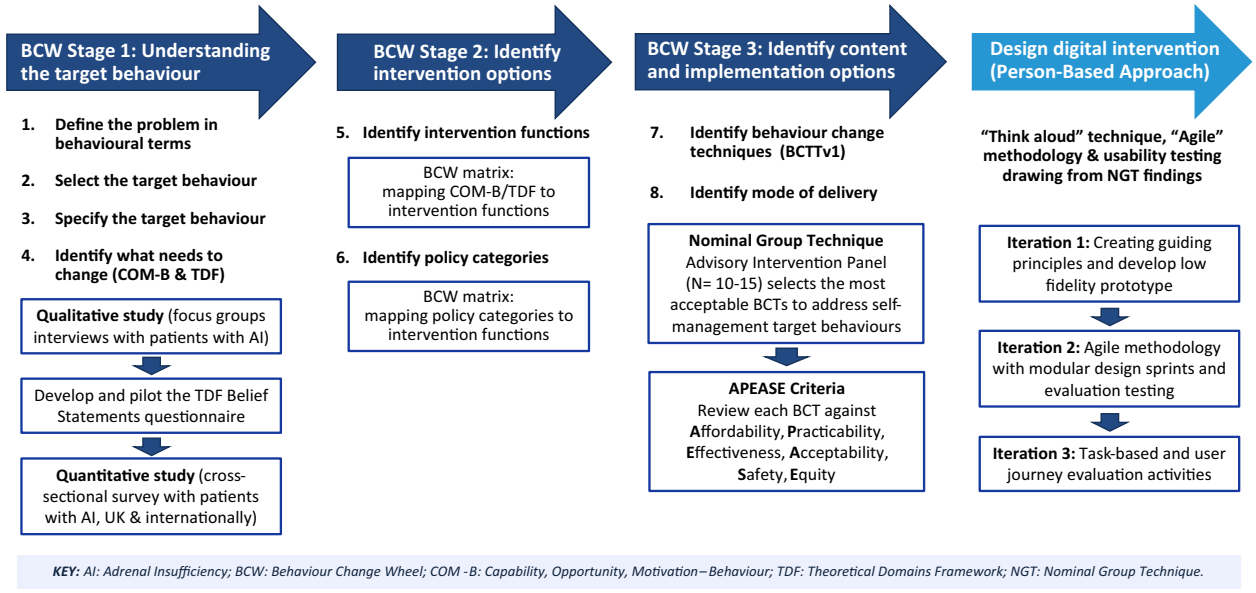

FIGURE 2
The *Support AI* digital behaviour change intervention development process guided by the Behaviour Change Wheel (BCW) and Person-Based Approach. Reproduced from Michie S, Atkins L, West R (2014) The behaviour change wheel: a guide to designing interventions. Silverback Publishing.

2) Recognition of "sick days" and appropriate adjustment of glucocorticoid replacement therapy during physical illness and major stressful situations;
3) Adoption of associated behaviours that aid the timely administration of parenteral hydrocortisone to prevent and treat an adrenal crisis.

The "long list" will then be systematically reduced by considering the possible impact of these behaviours on the target behaviour.

2.2.3 Step 3: specify the target behaviour (BCW Stage 1)

Step 3 will involve specifying the behaviour(s) in appropriate detail and in its context in terms of: *who* needs to perform the behaviour, *what* they need to do differently, *when, where, how often* and with *whom* they will do it (46). In this research we will investigate *what* patients with AI (*who*) need to do differently to improve their self-management behaviours on a daily basis, during "sick days" and to prevent and treat an adrenal crisis (*when, where, how often*), and with *whom* they will need to interact to achieve behaviour change.

2.2.4 Step 4: identify what needs to change (BCW Stage 1)

Step 4 will involve a mixed-methods sequential qualitative and quantitative study to identify what needs to change. The aim of this step is to gain in-depth understanding of the barriers and enablers that people with AI experience to performing the target self-management behaviours (qualitative study); this will inform the development and validation of a TDF-based Belief Statements questionnaire to measure the barriers and enablers to self-management in a wider population of patients with AI (quantitative study). The study design is described separately in the following sections for the qualitative and the quantitative studies.

2.2.4.1 The qualitative study (focus group interviews)

2.2.4.1.1 Methods

We will follow the *Consolidated Criteria for Reporting Qualitative Research (COREQ)* checklist (50) to design and report the *Methods* for the qualitative study. Qualitative methods are recommended for behaviour change intervention development when prior evidence is limited (47). This is also in line with the Person-Based Approach which recommends using qualitative research to elicit user views of the planned behaviour changes and exploration of barriers and facilitators (51).

Focus group interviews with patients with AI will be conducted to gain an in-depth understanding of the barriers and enablers to self-management behaviours and to understand what it will take to change the behaviour (achieve the desired behaviour). The benefit of this method is that group interaction encourages participants to explore and clarify individual and shared perspectives, thus eliciting potential elements that can be incorporated into the digital behaviour change intervention.

2.2.4.1.2 Participant selection

Adopting a purposive sampling, we will conduct 7-10 focus group interviews with patients with AI, stratified by AI type (1 pilot group, 2-3 groups for Primary AI, 2-3 groups for Secondary AI, and 2-3 mixed groups including Tertiary AI). Participants will be recruited via the UK-based patient advocacy groups (PAGs), The Addison's Disease Self-Help Group (https://www.addisonsdisease.org.uk/) and the Pituitary Foundation (https://www.pituitary.org.uk/), using their membership emailing lists, websites, newsletters, and social media;

they have a combined international patient membership (primarily UK) of approximately 3,500 members. These PAGs are also collaborators in this study and their representatives are members of the Study Advisory Group.

2.2.4.1.3 Setting

The PAGs will disseminate the study URL link and QR code populated in Qualtrics®, an online secure platform approved for academic surveys and conforming to GDPR regulations, where prospective participants can access the Participant Information Sheet (PIS), eligibility checklist and electronic consent form. Patients who meet the inclusion criteria and provide consent will be allocated to respective focus groups of 6-8 participants per group. The focus groups will be conducted online using a secure university virtual (*Zoom*) account.

Inclusion criteria:

- Adults aged 18 years of age or over
- Diagnosed with irreversible primary, secondary or tertiary AI and on glucocorticoid replacement therapy
- Having internet access to join the *Zoom* call and being comfortable with taking part in online group discussions

Exclusion criteria

- Unable to communicate in English

2.2.4.1.4 Data collection

An interview guide will be developed based on the COM-B model and TDF domains (46, 47), which will be piloted with the first focus group. Open higher-level COM-B questions on Capability, Opportunity and Motivation will act as filters to potentially relevant TDF domains (47). This will be followed by specific questions and probes within TDF domains to collect data that can address the target behaviours in the digital intervention. An example might be:

- "How would you prevent an adrenal crisis?" (COM-B Capability).
- "How do you recognise symptoms and signs of an adrenal crisis for which you would need a hydrocortisone injection?" (prompt in the TDF domain "Knowledge")

Focus group interviews, lasting 60–90 minutes, will be facilitated by the first author (SL) and another member of the research team and will be video recorded in *Zoom*. The first author will transcribe and de-identify the interviews, adding relevant non-verbal cues and field notes to the transcripts; participants' names will be replaced with a study identifier, e.g. "3P5" for participant number five in the 3rd focus group.

2.2.4.1.5 Data analysis

A "coding guideline" will be developed in discussion with the research team with explicit statements as to how the TDF is to be applied to the qualitative data (47) using example quotations from the pilot focus group interview; SL and KM will also code the pilot interview jointly to minimise potential discrepancies in the analysis approach. Data will be analysed using NVivo_V12 QDAS qualitative data analysis software following each focus group interview using deductive framework analysis (52) and inductive thematic analysis (53) as below:

- Deductively: Data will be coded using the Theoretical Domains Framework V2 (TDF) as the framework for content analysis (47).
- Inductively: After coding data into theoretical domains, themes and belief statements will be generated to describe similar underlying perspectives and beliefs from respondents which reflect barriers and enablers to target behaviours.

Inductive thematic analysis will also be adopted to generate potential new themes for quotations that may not describe self-management behaviours and do not fit into any of the TDF domains. Data analysis and coding will be performed by the first author (SL); KM who is a COM-B and TDF expert will double-code two focus group interviews. Reliability will be calculated using *kappa* score and assessed by concordance of themes. Findings will be discussed and agreed with a third member of the research team (SH).

The TDF belief statements derived from the focus group interviews will inform the development of a TDF questionnaire which will be used to measure the target self-management behaviours in the Quantitative study. An independent TDF expert will map the generated belief statements to TDF domains to test content validity. The TDF questionnaire will also be reviewed by the Patient Advisory Group comprised of 5 patients with AI and will be finalised in consultation with the Study Advisory Group. Focus group participants will be invited to complete the questionnaire in a pilot online survey and to provide feedback that will inform questionnaire revisions for the Quantitative study. Test-retest reliability will be obtained by administering the TDF questionnaire to focus group participants four weeks later.

2.2.4.2 The quantitative study (cross-sectional survey)

2.2.4.2.1 Study design

We will adopt the *STROBE Statement* (54) and will use the *STROBE Checklist* for cross-sectional studies to design and report the *Methods* in this study protocol. The quantitative study will involve a cross-sectional survey aiming to:

- Measure barriers and enablers to self-management and identify associations with determinants of self-management behaviours in patients with AI.
- Describe the patient education, care and support services available for patients with AI and their families to identify potential gaps and unmet needs.

2.2.4.2.2 Setting and participants

Patients with AI will be invited to complete a cross-sectional survey. The data will be primarily collected online using Qualtrics®;

however to ensure inclusivity and accessibility the option for postal survey using a prepaid return envelope will also be available. Participants will be recruited via UK PAGs (patient charities) using the same recruitment strategy as for the qualitative study. An eligibility checklist, relying on patients' self-reporting, will filter participants who meet the inclusion criteria and can proceed with completing the survey. Participants will also be recruited via approximately 15 National Health Service (NHS) endocrine centres invited to the study directly by the research team, via the NIHR Clinical Research Network (CRN) Portfolio, and a call via the *Society for Endocrinology*. Local collaborators (nurse or endocrinologist) in these centres will identify and disseminate the study to potential participants through carrying out a search of patient records based on inclusion criteria.

As the survey will be disseminated via different channels, participants will be advised to complete the survey only once. Cookies will be enabled to prevent participants from completing the survey more than once on the same browser.

Inclusion criteria

- Adult patients aged 18 years or over
- Diagnosed with irreversible Primary, Secondary or Tertiary AI and on glucocorticoid replacement therapy.
- Residing and receiving medical care in the UK.

Exclusion criteria

- Patients with transient AI due to adrenal suppression taking high-dose corticosteroids at the time of study
- Patients not on glucocorticoid replacement therapy

2.2.4.2.3 Variables and *data sources/measurements*

The questionnaire, piloted with focus group participants as described earlier, is expected to take 25-30 minutes to complete and will include:

- The TDF Beliefs Statements questionnaire developed from the focus group interviews in the Qualitative study to measure barriers and enablers to self-management behaviours.
- A battery of questions to collect data on clinical and sociodemographic details, patient education, care and support services available for patients with AI.
- The following validated questionnaires which will be used to assess construct validity of the TDF Belief Statements questionnaire and identify potential associations with the three self-management target behaviours:
  - o The Patient Activation Measure (PAM) (55) to assess the patient's knowledge, skills, and confidence for self-management
  - o The Medication Adherence Report Scale (MARS-5) (56) to assess how patients take their glucocorticoid replacement
  - o The Beliefs about Medicines Questionnaire (BMQ) (57) to assess patients' beliefs and concerns about their treatment
  - o The Brief Illness Perception (IPQ) questionnaire (58) to explore how patients perceive their adrenal insufficiency and treatment.

2.2.4.2.4 Bias

Multiple recruitment methods via PAGs and endocrine centres will aim to reduce the response bias often associated with participation from PAG members who are considered more motivated to engage in self-management behaviours compared to the average patient population. Data collection approaches facilitated online and via hard copies can also minimise digital exclusion. In addition, to address recruitment bias and to enable potential generalisability of the findings, we will compare the characteristics of patients responding to the online survey with those of the general population of patients with AI, such as age, sex, AI type, ethnic background, and geographical distribution, which may potentially detect under-representation of some AI patient subgroups. This can further inform the recruitment strategy for the intervention development and usability testing in the next phase of the study.

2.2.4.2.5 Study size

An earlier study recruited 746 patients with AI from PAGs in the UK (59). By extending our study to NHS endocrine centres, we anticipate to recruit approximately 1,500 participants.

2.2.4.2.6 Statistical methods

Quantitative data will be analysed using IBM SPSS Statistics software to determine frequencies, associations and differences between variables and groups, and predictors of self-management behaviours such as adherence to medication and use of preventative measures for adrenal crisis. Qualitative data collected from open-ended questions will be analysed using NVivo using content thematic analysis (53).

2.2.5 Steps 5 and 6: identify intervention functions and policy categories (BCW Stage 2)

Having identified which COM-B components and TDF domains are relevant to the three target self-management behaviours (daily management, "sick days" management, and prevention and treatment of adrenal crisis), we will use the BCW matrix to map the relevant intervention functions such as "education", "enablement", "modelling" (*BCW Step 5*) and policy categories such as "guidelines" and "service provision" to consider what policies in the BCW can support the delivery of the selected intervention functions (*BCW Step 6*) based on links between them to select those likely to change behaviour (46).

2.2.6 Steps 7 and 8: identify behaviour change techniques (BCTs) and modes of digital delivery (BCW Stage 3)

In the final two steps of the BCW process, the TDF domains describing determinants of target behaviours identified in the qualitative research and the selected most appropriate intervention

functions will be mapped to the Behaviour Change Techniques (BCTs) Taxonomy (v1) (48) matrix to identify a preliminary list of salient BCTs (*BCW Step 7*) and to decide on modes of digital delivery (*BCW Step 8*). The TDF domains and respective barriers and enablers to self-management (TDF Belief Statements) will be prioritised based on (47):

- High frequency of specific beliefs (number of quotes) reported in focus group interviews;
- Perceived importance of these beliefs to self-management as rated by focus group participants on a 1 – 10 importance scale in the pilot study;
- Presence of conflicting beliefs indicating whether these act as barriers or enabler to target behaviours, assessed by the polarisation of Likert scale responses (strongly disagree to strongly agree) to the TDF Beliefs Statement questionnaire in the quantitative study.

The key TDF domains will be mapped to specific BCTs which can be used to address barriers and/or enhance enablers associated with the three target behaviours in a given TDF domain. An Expert Panel of 10-15 key stakeholders (patients with AI, endocrinologists, endocrine nurses and HCI (human computer interaction) practitioners) will be formed to discuss the context of the *Support AI* intervention and to select the most salient BCTs to deliver the intervention. Participants will be sent an information package before the meeting outlining the study objectives and a shortlist of proposed BCTs described in lay language accompanied by example applications (concrete strategy for delivering the BCT) for digital delivery, identified by the research team as the most appropriate to deliver the TDF domains and intervention functions. The content development of the example applications of BCTs will be guided by the supplementary materials accompanying the BCTs Taxonomy (48), by educational materials developed by implementation scientists (60), and by published digital self-management interventions used in other chronic conditions similar to AI such as asthma and type 1 diabetes.

A nominal group technique (61) facilitated by the Chief Investigator (SL) and two experts in TDF and BCTs (KM, SH) will guide the discussions to reach consensus on selected BCTs in steps below:

1. Silent generation: Participants will record their individual response on selected BCTs and their respective example application
2. Participants share ideas in a "round robin" fashion by proposing additional applications for BCTs across the TDF domains for each of the three target behaviours: daily management, "sick days" management, and prevention and management of adrenal crisis
3. Clarification of ideas in an open discussion of feedback bringing in examples and ideas from self-management interventions of other chronic conditions where relevant
4. Participants rank their top 5 BCTs to address each of the three target behaviours.
5. The Panel discusses the top ranked BCTs against the APEASE criteria (Affordability, Practicability, Effectiveness, Acceptability, Safety, Equity) (46).

This will be conducted in two sessions with 2 groups, each with 5-7 participants, over a secure Zoom online account. Points 1-4 will be covered in the first session (2 hours); the research team will analyse findings and the group will reconvene 2-3 days later to discuss point 5 (1 hour). A whiteboard will be used to record participants' suggestions and ideas which will be used to generate the context of the *Support AI* website. Findings will be discussed with the Study Advisory Group before moving to Phase 2.

2.3 Phase 2: designing the *Support AI* digital behaviour change intervention

2.3.1 Aim and methods

- To design an interactive and personalised website underpinned by the Person-Based Approach (49) to address the three target self-management behaviours identified in the earlier phase of this research.

The *Support AI* digital behaviour change intervention (DBCI) will be a responsive mobile-optimised website designed in three iterations described below:

2.3.2 Data collection approach

2.3.2.1 Iteration 1

The Person-Based Approach will inform the creation of guiding principles to describe the key intervention design objectives in terms of behaviour change and the key features of the intervention needed to deliver the behaviour change techniques (49), drawing on findings from the Nominal Group Technique to address the three self-management target behaviours. A list of requirements for the website, i.e. what does the website user need to be able to do, will be generated to help web developers to easily recall and refer to features of the intervention identified in the development phase as central to achieving the intervention objectives.

This iteration will involve deciding the content (what information it should contain) and functionality (what the user should be able to achieve) of the website aiming to produce a low fidelity prototype created using prototyping tools such as Axure® or Figma®. The prototype will include sitemaps (hierarchical structure and navigation of the website pages), wireframes (schematic of the general layout of each page content) and user journeys (a visual representation of the actions the user takes when interacting with the website). Five members of the Expert Panel from the Nominal Group Technique will test the low fidelity prototype moderated by the first author and a UX (user experience) designer.

2.3.2.2 Iteration 2

The web developers supporting the research team will use an "Agile" methodology (42) with 2-week design sprints delivering

new modules for review based on the website sitemaps. Adopting a Person-Based Approach and a "think-aloud" technique (49), 12 participants (6 patients, 3 nurses, 3 endocrinologists) will test in real time, using a desktop computer, the high-fidelity prototypes for each module as they are being developed.

This usability testing will evaluate each module for visual design, content and interactivity, iteratively modifying it to optimise user experience. Each "think-aloud" evaluation session will take approx. 60-90 minutes with an estimated total of 12 sessions over 12 weeks (one session per participant). Usability testing will be conducted online and will be video recorded using screen capture.

2.3.2.3 Iteration 3

Following the development of all website modules, 5-7 patients will participate in usability testing of the "alpha version" of the website using "think-aloud" and "screen-capture" techniques as outlined in Iteration 2. Each session, facilitated by the first author and a UX designer, will last approximately an hour and participants will be given specific tasks to complete related to the target behaviours that the *Support AI* intervention aims to address, for example "Find out how to adjust your hydrocortisone tablets if you were ill with the flu".

Participants will be asked to verbalise what they are doing as they perform each task and to provide feedback on features of the website such as navigation, visual design, functionality, efficiency (62). They will also be asked to complete the Single Use Question (SEQ) 7-point Likert scale (1=very difficult to 7 = very easy) to rate the difficulty of performing each specific task (63). The "think aloud" sessions will be recorded, transcribed and analysed using thematic analysis (53) to identify usability problems.

A "rainbow spreadsheet" and "severity scale" (64) designed in advance, will be used during the observations to record usability problems and feedback on website features (suggestions for improvement, good features, participants' reactions). Findings will inform revisions of the website for "beta" testing in a future randomised feasibility study.

2.3.2.4 Study sample

Patients with AI will be recruited from the quantitative study of Phase 1; after submitting the survey, they will receive a link to the Participant Information Sheet (PIS) and electronic consent to for Phase 2. Clinicians for Iteration 2 will be recruited via an open call to members of the *Society for Endocrinology*. Naïve participants will be invited for each iteration to minimise bias from prior exposure to the study.

Inclusion criteria for patient participants

- Adults aged 18 years of age or over and receiving medical care in the UK
- Diagnosed with irreversible AI and on glucocorticoid replacement therapy
- Access to the internet and a computer to conduct the usability testing.
- A basic level of digital literacy and competence with navigating a website.

Exclusion criteria

- Unable to read and communicate in English

Inclusion criteria for clinicians

- Involved in providing care to patients with AI in the UK

3 Ethical considerations and dissemination

3.1 Ethics approvals

Ethics approval was granted by the School of Health Sciences Research Ethics Committee at City, University of London on 6th September 2021 (Reference ETH2021-2215) for the Qualitative Study (focus group interviews) and HRA and HCRW Ethics Approval on 30th March 2022 (IRAS ID: 290622) for the quantitative study and website development.

3.2 Participant anonymity and confidentiality

Participants will be provided with a Participant Information Leaflet (PIS) to inform them of the study objectives, expected time commitments, and that participation is voluntary and they can withdraw at any point without an explanation. Survey completion in Phase 1 will denote consent to participate in the study. The online survey will be anonymous with no IP address tracking. Participants will be advised not to use the survey to request medical assistance. Qualitative data will be de-identified by the first author (SL) before analysis to ensure participant confidentiality. There will be no possibility to identify individuals from the published reports, though participants may be able to recognise their responses in the published quotations.

3.3 Consideration of participant well-being and remuneration

There are minor anticipated risks associated with this study. Participants will be provided with the contact details of the Chief Investigator and collaborators from each patient advocacy group (PAG) and endocrine clinic should they need to ask questions about the study. They will also be given contact details for the Secretary to the Senate Research Ethics Committee at City, University of London, and local National Health Service (NHS) Patient Advice and Liaison Service for any concerns or potential complaints.

The burden for research participants will be to dedicate approximately two hours of their time to take part in the Qualitative study, 25-30 minutes to complete the survey in the Quantitative study (Phase 1), and up to 90 minutes per session for the website usability testing. Participants in the Nominal Group Technique will need to commit up to 5 hours for participation (2 hours preparation and 3 hours for on-line group discussions). The anticipated risk is that participants may withdraw after providing consent. To mitigate this risk, a detailed description of time commitments and expectations for participation will be provided in the PIS and consent form so participants can make an informed decision. Only naïve participants will be allocated to each stage and iteration to avoid research bias from prior exposure to the study and also to minimise participation fatigue.

Participants will be remunerated for their time as per NIHR and INVOLVE guidance (65), i.e. £20 per hour in the form of high-street vouchers. As the cross-sectional survey is anonymous, it is not possible to provide remuneration to individuals. However, a donation of £3.00 per response will be made to the Pituitary Foundation and the Addison's Disease Self-Help Group to reward participation.

3.4 Data management and storage

Data collected from this study in their original form (digital and hard copies) and in the aggregate pool after screening and removing any identifiable details, will be kept for a minimum of 10 years post study completion. Digital data will be stored in a secure password-protected drive and hard copies will be stored in locked research cabinets at the School of Health and Psychological Sciences at City, University of London. Data collection and storage will conform to the University Policy and will be processed in accordance with the Data Protection Act 2018, GDPR and the Data Protection Bill.

3.5 Patient and public involvement and engagement (PPIE)

The PPIE plan for this research conforms to the NIHR INVOLVE National Standards for Public Involvement (66). The Pituitary Foundation and the Addison's Disease Self-Help Group are collaborators in the proposed study and their representatives are members of the Study Advisory Group who will meet approximately twice a year at Study Milestones. A Patient Advisory Group of 5 patients with AI was also formed to advise the research team throughout the study on the conceptualisation, design, delivery and dissemination of findings.

3.6 Dissemination of findings

Findings from the study will be disseminated via scientific meetings, publications in open access peer-reviewed journals and Researchfish® (https://researchfish.com/). The study findings will be disseminated back to participants via the patient conferences, social media and newsletter articles of the participating patient advocacy groups and endocrine centres.

4 Study status

As of March 2023, we have completed data collection and analysis from the Qualitative study which involved 51 patients with AI who took part in 10 focus group interviews. Recruitment for the Quantitative study (cross-sectional survey) commenced in December 2022 and will close in October 2023.

5 Discussion and anticipated outcomes

Self-management for patients with AI involves complex behaviours across three levels: 1) daily management of the condition and replacement therapy, 2) adjustment of glucocorticoids during "sick days" and 3) adoption of associated behaviours to prevent and treat an adrenal crisis. However, as evidenced by the existing literature, traditional strategies for patient education about these behaviours are not effective. This is the first research study to develop an evidence- and theory-based intervention for patients with AI focusing on behaviour change by targeting the behaviours most relevant to self-management. In line with the MRC guidelines (33) for the design and evaluation of complex interventions such as behaviour change, this research protocol describes the rationale underpinning the development process and maps the intervention components to theory and outcome, providing a fully transparent step by step project design and replicable research methodology.

We have described the systematic process which we will follow using the Behaviour Change Wheel (45, 46) to address determinants of self-management behaviours in patients with AI by qualitatively and quantitively analysing sources of behaviour underpinned by the COM-B/TDF model, linking to the most appropriate intervention functions and policy categories, and subsequently selecting salient behaviour change techniques to use when developing a tailored digital intervention. Adopting a Person-Based Approach (49) and UX (user experience) Design principles with iterative usability testing, we aim to build a digital intervention that is acceptable, engaging, appealing, and easy to use for patients with AI.

In addition, "self-management strategies" is one of the clinical questions being addressed in the National Institute for Clinical Excellence (NICE) guidelines on the management of adrenal insufficiency currently being developed with expected publication in 2024 (67). Given the limited empirical research on self-management in patients with AI, the findings from this study can make a significant contribution to developing evidence-based NICE Guideline recommendations. Future implementation of the *Support*

AI intervention in clinical practice can complement usual care to improve patient health outcomes and reduce demand on specialist services.

Author contributions

SL conceptualised the study, developed the study methodology and drafted the manuscript. SN provides oversight and mentorship for the research activity planning and execution. KM, SH, SW, SB, AG, CN, and SN contributed to the development of the study methodology and provided critical review to the initial manuscript. PS and PM provided PPIE guidance and contributed to the study design. 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

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EDITED BY
Elena Valassi,
International University of Catalonia, Spain

REVIEWED BY
Filippo Ceccato,
University of Padua, Italy
Pietro Locantore,
Catholic University of the Sacred Heart, Italy

*CORRESPONDENCE
Roberta Giordano
roberta.giordano@unito.it

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Bone safety of dual-release hydrocortisone in patients with autoimmune primary adrenal insufficiency

Fabio Bioletto[1], Marco Barale[1], Mirko Parasiliti-Caprino[1], Jacopo Giannelli[1], Lorenzo Campioni[1], Vincenzo Cappiello[1], Maria Chiara Di Carlo[1], Ezio Ghigo[1], Massimo Procopio[1] and Roberta Giordano[2*]

[1]Division of Endocrinology, Diabetes and Metabolism, Department of Medical Sciences, University of Turin, Turin, Italy, [2]Department of Clinical and Biological Sciences, University of Turin, Turin, Italy

Background: Conventional glucocorticoids (C-GC) replacement regimens have a detrimental effect on skeletal health in patients with adrenal insufficiency (AI), ultimately leading to an increased fracture risk. The novel dual-release hydrocortisone (DR-HC) formulations are characterized by a more favourable safety profile on various clinical endpoints. Data comparing the impact of C-GC and DR-HC on bone, however, are scarce.

Methods: Twenty-seven patients with autoimmune primary AI (PAI; 13 treated with C-GC and 14 treated with DR-HC) were evaluated to compare bone-related parameters between the two treatment groups.

Results: No significant differences between the two treatments groups were observed with respect to bone turnover markers. Patients treated with C-GC showed a lower bone mineral density (BMD) at lumbar spine (LS; 0.791 ± 0.195 vs. 0.942 ± 0.124 g/cm^2, p=0.025) and at femoral neck (FN; 0.633 ± 0.114 vs. 0.716 ± 0.088 g/cm^2, p=0.045). Moreover, they were characterized by a lower trabecular bone score (TBS; 1.236 ± 0.035 vs. 1.383 ± 0.030, p=0.004) and by a higher mean number of vertebral fractures per patient (0.75 vs. 0 fractures, p=0.002). TBS was the best predictor of fracture risk, with a pseudo-R^2 of 0.593; moreover, at mediation analysis, it was able to fully explain the observed detrimental effect of C-GC, compared to DR-HC, on fracture risk.

Conclusions: These results suggest that DR-HC is associated with less bone-related complications compared to C-GC in patients with PAI. Moreover, TBS seems to play a pivotal role in the mediation of the relationship between glucocorticoid treatment regimens and fracture risk.

KEYWORDS

primary adrenal insufficiency, bone turnover markers, bone mineral density, trabecular bone score, glucocorticoid replacement therapy, dual-release hydrocortisone

Introduction

Patients affected by adrenal insufficiency (AI) require the chronic administration of a glucocorticoid replacement therapy (1). Although the aim of replacement therapies is to simulate as much as possible the physiological cortisol secretion (1), conventional glucocorticoid (C-GC) replacement regimens with hydrocortisone or cortisone acetate are not able to fully mimic the normal hormonal rhythm, inducing serum cortisol peaks beyond physiological levels (2, 3). This determines a mild but persistent glucocorticoid excess with a poor diurnal exposure-time profile (4, 5), which in the long-term leads to detrimental effects at different levels. Patients with AI, in fact, present an increased morbidity and mortality compared to the general population, mainly due to a worse metabolic profile and to a higher risk of cardiovascular diseases (6–9).

The exogenous administration of systemic glucocorticoids exerts a detrimental effect on bone metabolism, ultimately leading to an increased fracture risk, which is related both to the dose and to the duration of the ongoing glucocorticoid treatment (10–14). From a pathophysiological point of view, these adverse effects are mostly due to the persistent suppression of bone formation, which is determined by a direct inhibitory effect of glucocorticoids on osteoblast differentiation and function (11, 15). Nevertheless, an early but transient increase in bone resorption is also present, due to an initial stimulation of the differentiation, maturation and survival of osteoclasts (11, 15).

Even though the alteration of bone metabolism is mainly reported in case of glucocorticoid treatments for immunosuppressive and anti-inflammatory purposes, a long-term replacement therapy with C-GC regimens has been demonstrated to exert a negative impact on skeletal health as well, though to a lesser extent (16, 17). A decrease in bone mineral density (BMD) and an increase in fracture risk have been reported in adult patients receiving C-GC replacement therapy for either primary adrenal insufficiency (PAI), secondary adrenal insufficiency (SAI) or congenital adrenal hyperplasia (CAH) (16–18); moreover, a correlation between these outcomes and the cumulative glucocorticoid dose has been observed (19, 20).

In recent years, a dual-release hydrocortisone (DR-HC) preparation (Plenadren®) was developed to maintain cortisol levels in a more physiological range, better reproducing the endogenous cortisol rhythm both when evaluated by serum cortisol (21, 22) and by salivary cortisol (23) levels. Compared to C-GC, this formulation has been demonstrated to have a more favourable clinical profile, with better outcomes with respect to blood pressure control, glucose metabolism, lipid metabolism, body weight, and health-related quality of life (22, 24–27).

Data about the impact of DR-HC on bone metabolism, however, are scarce. In a recent study by Frara et al. (28), performed on patients with SAI, a shift from C-GC to DR-HC was associated with a significant increase in BMD values at lumbar spine and femoral neck. Similar results were demonstrated by Guarnotta et al. (29) in a cohort of patients with PAI, in which a treatment with DR-HC was associated with a more favourable clinical profile in terms of BMD, bone turnover markers and, possibly, fracture risk.

Further data are thus required to better establish the safety profile of DR-HC, compared to C-GC, on skeletal health. Based on this background, we designed a clinical study aimed at comparing bone-related parameters between patients with PAI treated either with DR-HC or with C-GC replacement therapies. Both biochemical and imaging parameters were assessed, and possible mediators of the relationship between glucocorticoid treatment regimens and fracture risk were analysed.

Methods

Patient selection

Twenty-seven patients with PAI of autoimmune aetiology were evaluated between September 2020 and August 2021. Twenty-one were affected by autoimmune polyendocrine syndrome (APS) type 2, one by APS type 4, and five by autoimmune isolated Addison's disease.

The following exclusion criteria were applied: (a) APS type 1; (b) hypo- or hyperparathyroidism; (c) hyperthyroidism; (d) end-stage chronic kidney disease; (e) current or previous treatment with anti-osteoporotic drugs (apart from calcium and vitamin D supplementation).

All pre-menopausal women were studied in the early follicular phase. Female patients with primary ovarian insufficiency were under appropriate replacement therapy at the time of the study, as well as male patients with primary hypogonadism. Patients with autoimmune hypothyroidism were under appropriate treatment with levothyroxine at the time of the study. Patients affected by vitamin D insufficiency received adequate supplementation.

The following parameters were evaluated for each patient: age, sex, disease duration, current treatment duration (defined as the duration of therapy with the current formulation and dosage), hydrocortisone equivalent (HCeq) dose, smoking habit, weight, body mass index (BMI), creatinine, fasting glucose, HbA1c, serum and urinary calcium, serum and urinary phosphate, parathyroid hormone (PTH), 25-OH vitamin D (25(OH)D), bone alkaline phosphatase (BAP), osteocalcin, urinary crosslinks, bone mineral density (BMD) at lumbar spine (LS), femoral neck (FN) and total hip (TH), trabecular bone score (TBS) at LS, and number of vertebral fractures at vertebral morphometry.

The study was approved by the local Ethics Committee and was in accordance with the principles of the Declaration of Helsinki. Written informed consent was obtained from all included patients.

Analytical methods

Blood samples were taken in the morning between 8 and 9 am, after an overnight fast. Serum PTH (ng/L), BAP (μg/L) and osteocalcin (μg/L) were tested by chemiluminescence immunoassay (CLIA; Liason Analyzer, DiaSorin, Saluggia, Italy). Serum 25(OH)D (μg/L) was tested by chemiluminescence microparticle immunoassay (CMIA; Alinity system, Abbott Laboratories, Chicago, Illinois, USA). Urinary crosslinks (nmol/

mmol of creatinine) were tested by crosslinks method (Chromsystems, Grafelfings, Germany) with fluorescent detection based on high performance liquid chromatography (HPLC; Prominence System, Shimadzu Corporation, Kyoto, Japan). All other biochemical variables were assayed in plasma, serum or urine using standard methods.

BMD, TBS and vertebral morphometry analysis

BMD was measured by dual energy x-ray absorptiometry (DXA) at L1-4 LS, FN and TH, using a Hologic QDR 4500 densitometer. Fractured vertebrae were excluded from the calculation of BMD and its derived parameters. The coefficient of variation of BMD measurements was equal to 1.0% at all examined sites. T-scores were calculated by comparing BMD results with those obtained in a sex-matched Caucasian population at peak of bone mass. Z-scores were calculated by comparing BMD results with those obtained in an age and sex-matched Caucasian population. TBS was evaluated from LS DXA scans using the TBS iNsight® software (version 3.0.2.0, Medimaps SASU, Pessac, France). The coefficient of variation of TBS measurements was equal to 1.2%. Vertebral morphometry was performed on lateral spine images obtained by DXA scan; conventional spinal radiographs (T4-L4) in the lateral and the anteroposterior projections would have been performed in case on unclear or inconclusive findings at lateral spine DXA imaging (30), but this was not necessary for any patient in the present study.

Statistical analysis

Continuous data were summarised using mean and standard deviation (SD) for normally distributed variables, and median and interquartile range (IQR) for non-normally distributed variables. Count data were summarised using the mean number of observed events per patient. Categorical data were summarised using percent values. Normality of continuous variables was assessed through Kolmogorov-Smirnov test. Differences between treatment groups were evaluated by Student t-test or by Mann-Whitney U-test for continuous variables, by exact Poisson regression for count variables, and by Fisher's exact test for categorical variables. The same tests have been applied to evaluate differences between patients with and without vertebral fractures.

All the bone-related parameters that were significantly different between treatment groups at univariate analysis were further analysed by analysis of covariance (ANCOVA) if continuous-type, or by multivariable exact Poisson regression if count-type; adjustments for age, disease duration, current treatment duration, HCeq dose, 25(OH)D levels, and HbA1c levels were performed.

Univariate and multivariable exact Poisson regressions were used to further define the most relevant factors associated with vertebral fractures, considering as potential predictors the glucocorticoid treatment regimen (C-GC vs. DR-HC), together with all the other parameters found to be significantly associated with fractures at univariate analyses. Finally, a mediation analysis was performed in order to assess whether the impact of glucocorticoid treatment regimen on fracture risk could be explained by one or more of the bone-related parameters that have been analyzed; a mediation model aims to uncover and elucidate the mechanism that lies beneath an observed relationship between an independent variable and a dependent variable via the inclusion of a third variable, known as a mediator variable (31, 32); through this approach, the total exposure-outcome effect is decomposed into direct and indirect effects, the first one being the direct exposure-outcome effect, while the second being the combination of exposure-mediator and mediator-outcome effects, as visually represented by direct acyclic graphs (31, 32); with regard to this study, thus, the total effect of glucocorticoid treatment regimen on fracture risk was decomposed to assess whether this relationship was significantly mediated by other measured variables.

A cut-off of 0.05 was adopted for the definition of statistical significance. Statistical analysis was performed using STATA 17 (StataCorp, College Station, Texas, USA).

Results

General characteristics of the study population

Thirteen patients were treated with C-GC replacement therapy (4 patients with cortisone acetate, divided in two or three doses; 9 patients with hydrocortisone, divided in two or three doses). Fourteen patients were treated with DR-HC (single dose in the morning). In addition, all patients were treated with fludrocortisone (0.025-0.1 mg/day in the morning). All patients reported adequate adherence to prescribed treatments. The main anthropometric, clinical and biochemical characteristics of the patients are reported in Table 1.

Comparing patients treated with C-GC and those treated with DR-HC, those belonging to the first group showed a tendency towards an older age (59.5 ± 11.4 vs. 49.8 ± 15.0 years, p=0.070), while no differences were found in terms of sex (23.1 vs. 35.7% of males, p=0.678), disease etiology/subtype (isolated PAI or APS, p=1.000), disease duration (15.5 ± 12.9 vs. 17.8 ± 9.4 years, p=0.631), current treatment duration (5.0 [IQR: 3.6-8.4] vs. 7.0 [IQR 4.0-8.0] years, p=1.000), and HCeq dose (20.0 ± 3.7 vs. 22.1 ± 3.8 mg/day, p=0.149) (Table 1). Weight, BMI and fasting glucose were comparable between groups, while HbA1c was higher in patients treated with C-GC than in those treated with DR-HC (46.1 ± 13.0 vs. 35.3 ± 7.1 mmol/mol, p=0.012) (Table 1).

Comparison of bone-related parameters between C-GC and DR-HC replacement therapies

With respect to bone-related biochemical parameters, no differences could be observed between patients treated with C-GC

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TABLE 1 Clinical characteristics of patients treated with C-GC therapy versus patients treated with DR-HC formulation.

Parameter	C-GC therapy (n = 13)	DR-HC therapy (n = 14)	p-value
Age (years)	59.5 ± 11.4	49.8 ± 15.0	0.070
Male sex (n, %)	3 (23.1)	5 (35.7)	0.678
Disease etiology/subtype			1.000
Isolated AD (n, %)	2 (15.4)	3 (21.4)	
APS type 2 (n, %)	11 (84.6)	10 (71.4)	
APS type 4 (n, %)	0 (0.0)	1 (7.1)	
Disease duration (years)	15.5 ± 12.9	17.8 ± 9.4	0.631
Current treatment duration (years) [a]	5.0 [3.6-8.4] [a]	7.0 [4.0-8.0] [a]	1.000 [a]
HCeq dose (mg/day)	20.0 ± 3.7	22.1 ± 3.8	0.149
Current smoking (n, %)	0 (0.0)	0 (0.0)	NA
Weight (kg)	62.6 ± 11.9	65.7 ± 10.0	0.483
BMI (kg/m^2)	24.3 ± 4.3	23.6 ± 2.7	0.637
Creatinine (mg/dL)	0.88 ± 0.20	0.78 ± 0.21	0.225
Glucose (mg/dL)	90.8 ± 31.7	79.4 ± 17.3	0.252
HbA1c (mmol/mol)	46.1 ± 13.0	35.3 ± 7.1	**0.012**
Calcium (mmol/L)	2.37 ± 0.12	2.38 ± 0.30	0.932
Urinary calcium (mmol/24h)	3.16 ± 1.60	3.54 ± 2.11	0.606
Phosphate (mmol/L)	1.15 ± 0.22	1.17 ± 0.16	0.740
Urinary phosphate (mmol/24h)	19.00 ± 8.40	20.61 ± 10.21	0.660
PTH (ng/L)	27.9 ± 7.2	24.5 ± 8.7	0.270
25(OH)D (µg/L)	39.0 ± 8.1	33.3 ± 11.4	0.151
BAP (µg/L)	12.4 ± 4.1	10.8 ± 2.6	0.255
Osteocalcin (µg/L)	21.9 ± 3.2	25.5 ± 6.5	0.083
Urinary crosslinks (nmol/mmol of Cr)	26.6 ± 9.1	29.8 ± 17.1	0.556
Lumbar spine T-score	-2.01 ± 1.51	-1.10 ± 1.04	0.080
Total hip T-score	-1.47 ± 0.89	-1.26 ± 0.62	0.489
Femoral neck T-score	-1.85 ± 1.02	-1.39 ± 0.67	0.167
Lumbar spine Z-score	-1.13 ± 1.20	-0.44 ± 0.97	0.122
Total hip Z-score	-0.86 ± 0.77	-0.70 ± 0.70	0.586
Femoral neck Z-score	-1.10 ± 0.50	-0.65 ± 0.78	0.099
Lumbar spine BMD (g/cm^2)	0.791 ± 0.195	0.942 ± 0.124	**0.025**
Total hip BMD (g/cm^2)	0.752 ± 0.128	0.820 ± 0.091	0.128
Femoral neck BMD (g/cm^2)	0.633 ± 0.114	0.716 ± 0.088	**0.045**
Trabecular bone score	1.236 ± 0.035	1.383 ± 0.030	**0.004**
Prevalence of vertebral fractures (%) [b]	25.0	0	0.085
Mean n. of fractures per patient [b,c]	0.75 [c]	0 [c]	**0.002** [c]

[a]Data reported as median [IQR] and tested for differences with Mann-Whitney U-test due to the non-normal distribution of the considered parameter according to Kolmogorov-Smirnov test; [b] Missing data in one patient treated with conventional glucocorticoid therapy; [c] Count-type data tested for differences by exact Poisson regression.

25(OH)D, 25-OH vitamin D; AD, Addison's disease; APS, autoimmune polyendocrine syndrome; BAP, bone alkaline phosphatase; BMD, bone mineral density; BMI, body mass index; C-GC, conventional glucocorticoid; Cr, creatinine; DR-HC, dual-release hydrocortisone; eGFR, estimated glomerular filtration rate; HCeq, hydrocortisone equivalent; n, number; NA, not applicable; PTH, parathyroid hormone.

Bold values indicate statistically significant results.

and those treated with DR-HC. In particular, the two treatment groups presented with comparable levels of serum and urinary calcium, serum and urinary phosphate, 25(OH)D, and PTH (Table 1). A borderline-significand trend towards lower osteocalcin levels in the C-GC group could be observed (21.9 ± 3.2 vs. 25.5 ± 6.5 µg/L, p=0.083); the other evaluated bone turnover markers (i.e., BAP and urinary crosslinks) were similar between the two groups (Table 1).

Considering DXA evaluation, patients treated with C-GC showed a lower BMD at LS (0.791 ± 0.195 vs. 0.942 ± 0.124 g/cm^2, p=0.025) and at FN (0.633 ± 0.114 vs. 0.716 ± 0.088 g/cm^2, p=0.045), while only a non-significant trend could be observed at TH (0.752 ± 0.128 vs. 0.820 ± 0.091 g/cm^2, p=0.128) (Table 1). At TBS analysis, lower values were observed in patients treated with C-CG compared to those treated with DR-HC (1.236 ± 0.035 vs. 1.383 ± 0.030, p=0.004) (Table 1). Vertebral fractures were observed in 3 patients treated with C-GC, with a total number of 9 fractures (one patient with 2 fractures, one with 3 fractures, one with 4 fractures); conversely, no vertebral fractures were observed in patients treated with DR-HC (p=0.002 for difference between groups) (Table 1).

All parameters found to be significant at univariate analysis were further evaluated by taking into account the main possible confounding factors (i.e., age, disease duration, current treatment duration, HCeq dose, 25(OH)D level and HbA1c), using ANCOVA for continuous-type data and multivariable exact Poisson regression for count-type data (Table 2). The difference between groups in LS-BMD remained statistically significant after accounting for almost all confounders (p=0.045 adjusted for age; p=0.024 adjusted for disease duration; p=0.047 adjusted for HCeq dose; p=0.047 adjusted for 25(OH)D; p=0.008 adjusted for HbA1c), with the exception of current treatment duration, for which a borderline significance was nevertheless observed (p=0.052) (Table 2). The difference between groups in FN-BMD remained statistically significant when accounting for disease duration (p=0.021) and HbA1c (p=0.032), but the significance was lost when adjusting the analysis for age (p=0.108), current treatment duration (p=0.135), HCeq dose (p=0.082), and 25(OH)D (p=0.107) (Table 2). The difference between groups in TBS remained statistically significant in all adjusted analyses (p=0.023 adjusted for age; p=0.007 adjusted for disease duration; p=0.020 adjusted for current treatment duration; p=0.007 adjusted for HCeq dose; p=0.011 adjusted for 25(OH)D; p=0.006 adjusted for HbA1c) (Table 2). The same held true for the difference in the mean number of fractures per patient (p=0.029 adjusted for age; p=0.013 adjusted for disease duration; p=0.004 adjusted for current treatment duration; p=0.004 adjusted for HCeq dose; p=0.008 adjusted for 25(OH)D; p=0.002 adjusted for HbA1c) (Table 2). Further analyses accounting for multiple confounders at the same time were not feasible due to the limited sample size.

Prediction of fragility fractures

Regardless of the treatment, patients with vertebral fractures were characterized by a significantly longer disease duration (30.7 ± 10.1 vs. 15.0 ± 10.2 years, p=0.021), lower LS T-score (-3.20 ± 1.92 vs. -1.32 ± 1.18, p=0.022), lower FN T-score (-2.73 ± 0.12 vs. -1.49 ± 0.83, p=0.018), lower FN-BMD (0.545 ± 0.013 vs. 0.695 ± 0.102 g/cm^2, p=0.019), and lower TBS (1.098 ± 0.096 vs. 1.343 ± 0.117, p=0.002) compared to those without vertebral fractures (Table 3).

Among the parameters that significantly differed between patients with and without vertebral fractures, TBS was the one that showed the best predictive performance at univariate Poisson regression, with a pseudo-R^2 of 0.593. The performance of TBS in fracture prediction could not be improved by adding to the model any of the other variables that were significant at univariate analysis, which all lost significance when adjusted for TBS values (p=0.667 for disease duration; p=0.625 for LS T-score; p=0.625 for FN T-score; p=0.625 for FN-BMD), while TBS always maintained a significant association with the outcome (p<0.01 in all models).

Finally, the hypothesis of a mediating effect of TBS in the relationship between glucocorticoid treatment regimen (C-GC vs. DR-HC) and fracture risk was tested in a mediation analysis model. The retrieved results showed that, in our cohort, the detrimental effect of C-GC on fracture risk was entirely explicable through the

TABLE 2 Adjusted comparison between treatment groups of bone-related parameters that were significantly different at univariate analysis.

Dependent variable	Crude p-value for difference between treatment groups	Adjusted p-value for difference between treatment groups					
		Adjustment for age	Adjustment for disease duration	Adjustment for current treatment duration	Adjustment for HCeq dose	Adjustment for 25(OH)D levels	Adjustment for HbA1c levels
Lumbar spine BMD (g/cm^2)	0.025	0.045	0.024	0.052	0.047	0.047	0.008
Femoral neck BMD (g/cm^2)	0.045	0.108	0.021	0.135	0.082	0.107	0.032
Trabecular bone score	0.004	0.023	0.007	0.020	0.007	0.011	0.006
Mean n. of fractures per patient	0.002	0.029	0.013	0.004	0.004	0.008	0.002

25(OH)D, 25-OH vitamin D; BMD, bone mineral density; n, number.

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TABLE 3 Comparison of the clinical characteristics of patients with and without vertebral fractures, irrespective of the ongoing glucocorticoid treatment regimen.

Parameter	Patients without vertebral fractures (n = 23)	Patients with vertebral fractures (n = 3)	p-value
Age (years)	52.7 ± 14.3	67.7 ± 4.2	0.087
Male sex (n, %)	7 (30.4)	1 (33.3)	1.000
Disease etiology/subtype			0.562
Isolated AD (n, %)	4 (17.4)	1 (33.3)	
APS type 2 (n, %)	18 (78.3)	2 (66.7)	
APS type 4 (n, %)	1 (4.3)	0 (0.0)	
Disease duration (years)	15.0 ± 10.2	30.7 ± 10.1	**0.021**
Current treatment duration (years) [a]	6.0 [3.4-8.0] [a]	9.7 [3.0-32.0] [a]	0.322
HCeq dose (mg/day)	21.6 ± 3.7	20.0 ± 0.0	0.456
Current smoking (n, %)	0 (0.0)	0 (0.0)	NA
Weight (kg)	64.6 ± 11.1	64.0 ± 11.5	0.927
BMI (kg/m^2)	24.0 ± 3.4	24.4 ± 5.4	0.845
Creatinine (mg/dL)	0.80 ± 0.18	1.00 ± 0.34	0.115
Glucose (mg/dL)	84.9 ± 26.6	85.0 ± 24.9	0.994
HbA1c (mmol/mol)	40.3 ± 11.4	44.0 ± 16.5	0.614
Calcium (mmol/L)	2.39 ± 0.24	2.26 ± 0.09	0.341
Urinary calcium (mmol/24h)	3.43 ± 1.76	2.06 ± 2.41	0.233
Phosphate (mmol/L)	1.19 ± 0.18	1.01 ± 0.22	0.119
Urinary phosphate (mmol/24h)	19.64 ± 8.78	20.17 ± 15.98	0.929
PTH (ng/L)	25.2 ± 7.6	33.9 ± 10.1	0.083
25(OH)D (µg/L)	35.4 ± 10.6	41.4 ± 8.8	0.353
BAP (µg/L)	11.6 ± 3.6	12.3 ± 2.2	0.740
Osteocalcin (µg/L)	24.3 ± 5.6	19.5 ± 3.1	0.160
Urinary crosslinks (nmol/mmol of Cr)	29.8 ± 14.1	20.8 ± 6.2	0.293
Lumbar spine T-score	-1.32 ± 1.18	-3.20 ± 1.92	**0.022**
Total hip T-score	-1.32 ± 0.75	-1.93 ± 0.57	0.190
Femoral neck T-score	-1.49 ± 0.83	-2.73 ± 0.12	**0.018**
Lumbar spine Z-score	-0.62 ± 0.98	-1.80 ± 1.77	0.085
Total hip Z-score	-0.75 ± 0.76	-0.97 ± 0.25	0.629
Femoral neck Z-score	-0.80 ± 0.72	-1.27 ± 0.25	0.285
Lumbar spine BMD (g/cm^2)	0.889 ± 0.168	0.740 ± 0.212	0.170
Total hip BMD (g/cm^2)	0.802 ± 0.113	0.688 ± 0.039	0.101
Femoral neck BMD (g/cm^2)	0.695 ± 0.102	0.545 ± 0.013	**0.019**
Trabecular bone score	1.343 ± 0.117	1.098 ± 0.096	**0.002**

[a]Data reported as median [IQR] and tested for differences with Mann-Whitney U-test due to the non-normal distribution of the considered parameter according to Kolmogorov-Smirnov test
25(OH)D, 25-OH vitamin D; AD, Addison's disease; APS, autoimmune polyendocrine syndrome; BAP, bone alkaline phosphatase; BMD, bone mineral density; BMI, body mass index; n, number; Cr, creatinine; eGFR, estimated glomerular filtration rate; HCeq, hydrocortisone equivalent; n, number; NA, not applicable; PTH, parathyroid hormone.
Bold values indicate statistically significant results.

mediation of a reduced TBS; in fact, the direct path from the treatment regimen to fracture risk lost any statistical significance (p=0.875); on the other hand, the indirect path passing through TBS showed a significant mediating effect of this variable (p=0.004 for the association between glucocorticoid treatment regimen and TBS; p<0.001 for the association between TBS and vertebral fractures) (Figure 1).

Discussion

In this study, we compared the impact of two different glucocorticoid replacement regimens (C-GC vs. DR-HC) on skeletal health in a group of patients with PAI. Both biochemical and imaging parameters were assessed, and possible mediators of the relationship between glucocorticoid treatment regimens and fracture risk were analysed. Overall, our data seemed to suggest a more favorable bone-safety profile in patients treated with DR-HC; in fact, these patients displayed higher BMD values, higher TBS values, and a lower fracture rate compared to those treated with C-GC. Given the observational nature of this study, a possible role of confounding variables cannot be excluded in this regard; although not significantly, patients treated with C-GC were older than those treated with DR-HC, and this may have contributed to the differences in bone health parameters between the two groups; overall, however, our findings were essentially confirmed also after adjusting for age, disease duration, current treatment duration, HCeq dose, 25(OH)D levels and HbA1c.

To the best of our knowledge, only two studies provided, so far, a comparison between C-GC and DR-HC with respect to bone-related endpoints. The first one, published in 2018 by Frara et al. (28), was a retrospective cohort study which enrolled 14 patients with SAI who were shifted from C-GC to DR-HC upon clinical decision; this therapeutic change was associated with a significant increase in LS-BMD and FN-BMD after a follow-up of 24 months; no data on other parameters, such as bone turnover markers, TBS or fractures, were available. The second one, published in 2022 by Guarnotta et al. (29), was a retrospective cohort study which enrolled 70 patients with PAI, half of which were shifted from C-GC to DR-HC upon clinical decision; after a follow-up of 60 months, patients who switched to DR-HC displayed a significant increase in BAP, LS-BMD and FN-BMD; on the other hand, patients who continued on C-GC displayed a significant decrease in BAP, osteocalcin and LS-BMD, together with an increase in vertebral fracture rate. Overall, these results are in line with those observed in our study; however, some elements of novelty of our research should be underlined.

From a pathophysiological point of view, glucocorticoid-induced osteoporosis (GIO) is characterised by a preferential loss of trabecular bone, with a lesser impact on cortical bone; as a consequence, the spinal column is the skeletal site that is most affected, with a preferential loss of BMD at LS and a more prominent increase in vertebral fracture risk (33, 34). Overall, however, the increase in fracture risk in patients treated with chronic systemic glucocorticoids is only partially related to the observed changes in BMD (35–37). Although a reduction in BMD is present, in fact, this parameter alone has a poor discriminatory capacity in predicting fracture risk in the setting of GIO (35–37). The mechanisms underlying this BMD-independent increase in fracture risk are related to a glucocorticoid-induced impairment of bone microarchitecture, with a greater decrease in bone quality rather than in bone density (38–40).

In recent years, alternative parameters of skeletal fragility have been sought, in place of BMD, for a better fracture risk assessment

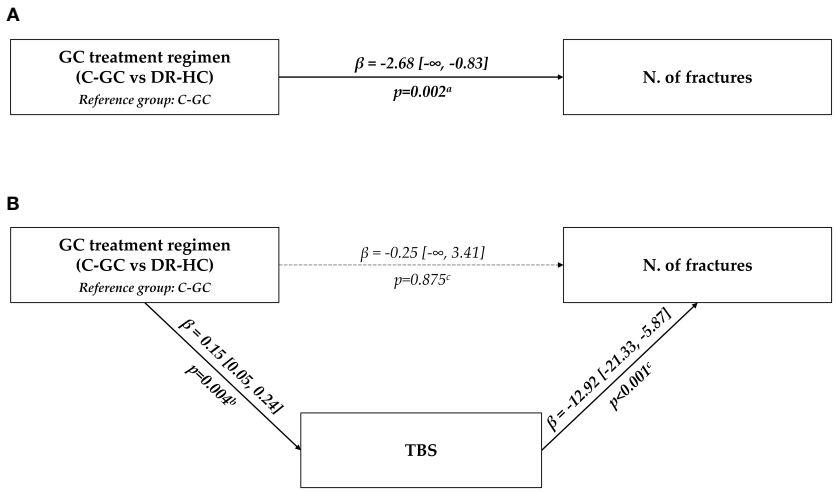

FIGURE 1
Graphical representation of the association between glucocorticoid treatment regimen (C-GC vs. DR-HC) and fracture risk. The upper panel **(A)** shows the results of the univariate analysis; the lower panel **(B)** shows the results of the multivariable mediation analysis, considering TBS as a mediating path. [a]Univariate exact Poisson regression; [b]Linear regression; [c]Multivariable exact Poisson regression. C-GC, conventional glucocorticoid; DR-HC, dual-release hydrocortisone; GC, glucocorticoid; N, number; TBS, trabecular bone score.

in patients with primary and secondary osteoporosis (41–47), including GIO (38, 39, 48). Among these, TBS has emerged as a novel index of bone micro-architectural health and skeletal fragility (41, 42). TBS is a qualitative index that captures the mean rate of gray-level variations in LS DXA images; higher TBS values reflect a better trabecular bone microstructure, while lower TBS values indicate a trabecular microstructure impairment (41, 42). Compared to BMD, TBS is able to better characterize bone fragility in GIO, demonstrating a higher discriminatory capacity in the estimation of fracture risk (49, 50). Within the setting of AI, however, data are scarce; to our knowledge, only one study has to date evaluated TBS values in patients with AI (51); in this study, TBS was negatively correlated with age and disease duration, but no data were available comparing TBS between patients treated with C-GC and DR-HC replacement therapies, nor about its possible role as a predictor of fracture risk (51).

In our study, in agreement with previous literature (28, 29), we observed that DR-HC was associated with higher BMD values compared to C-GC, both at LS and FN. Our study, however, was the first to evaluate the possible role of TBS in this setting, with promising results. The evaluation of TBS represented a key point for a finer assessment of skeletal fragility in the enrolled cohort. In fact, the difference between treatment groups in terms of TBS was stronger, and TBS was the only DXA-derived parameter that strictly remained statistically significant in all adjustments performed by ANCOVA.

The key role of TBS in our study, however, became even more evident in the analysis of the predictors of vertebral fractures. Overall, our data showed a significant difference in terms of vertebral fracture risk between the two treatment groups, with a significantly higher number of fractures in patients treated with C-GC. This result is in line with the one reported by Guarnotta et al. (29), who reported an increase in vertebral fracture rate in patients treated with C-GC but not in those treated with DR-HC. When evaluating the possible predictors of vertebral fractures, TBS was the one that displayed the strongest association with the outcome, with a pseudo-R^2 of 0.593. Notably, none of the other considered variables could improve this performance when added to the model, thus emphasizing the pivotal role of TBS for the assessment of fracture risk in this clinical setting.

Moreover, a mediation analysis model was used to analyze the possible mediating role of TBS in the association between glucocorticoid treatment regimens (C-GC vs. DR-HC) and fracture risk. In this analysis, the total effect of the glucocorticoid treatment regimen on fracture risk was partitioned into direct and indirect effects, considering TBS as a possible mediator. Interestingly, the whole detrimental role of C-GC on fracture risk could be explained by the indirect path passing through TBS, while the direct path from the treatment regimen to fracture risk lost any statistical significance. In other words, TBS was able to capture the entire association between the glucocorticoid treatment regimen (C-GC vs. DR-HC) and fracture risk: the treatment regimen had a significant impact on TBS, and, in turn, TBS had a significant impact on fracture rate, but no significant direct (i.e., not TBS-mediated) impact of the treatment regimen on fractures could be detected.

With respect to biochemical parameters, in our study, bone turnover markers did not significantly differ between the two treatment groups. Nevertheless, a borderline-significant trend towards lower levels of osteocalcin in patients treated with C-GC could be seen. This difference is in line with the findings by previous authors (22, 29), and suggests a stronger inhibitory effect on bone formation exerted by C-GC compared to DR-HC.

Our study had some limitations. First, it was a proof-of-concept study with a limited sample size; the obtained results should be considered as preliminary and need to be confirmed in larger cohorts. Second, it had a cross-sectional design, and did not evaluate the effect of treatments over time; this permits to infer associations, but it does not allow to establish a link of causality. Third, the assignment to treatment regimen was not randomized and, thus, even if we evaluated the influence of possible covariates by multivariable analyses, the presence of a residual confounding cannot be excluded.

In conclusion, our study suggests a better safety profile of DR-HC, compared to C-GC, in terms of bone-related complications in patients with PAI. These results are in line with the more favorable pharmacological profile of DR-HC, which is able to better reproduce the endogenous cortisol rhythm, thus leading to less side effects related to glucocorticoid excess. Notably, TBS seems to play a pivotal role in the mediation of the relationship between glucocorticoid treatment regimens and fracture risk. This finding is coherent with the specific impairment of bone microarchitecture that is determined by glucocorticoid excess, which affects bone quality rather than bone density.

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 humans were approved by “Comitato Etico Interaziendale - AOU Città della Salute e della Scienza di Torino”. The studies were conducted in accordance with the local legislation and institutional requirements. The participants provided their written informed consent to participate in this study.

Author contributions

FB contributed to data analysis and manuscript writing. MB and MP-C contributed to manuscript review and editing. JG, LC, and MCDC contributed to data collection. VC contributed to data collection and manuscript writing. EG and MP supervised the manuscript drafting. RG contributed to work conceptualization

and final draft supervision. All authors approved the manuscript in its final form. All authors contributed to the article.

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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

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EDITED BY
Agnieszka Pazderska,
St. James's Hospital, Ireland

REVIEWED BY
Stefan Fischli,
Luzerner Kantonsspital, Switzerland
Giorgio Radetti,
Ospedale di Bolzano, Italy

*CORRESPONDENCE
Bergithe E. Oftedal
bergithe.oftedal@uib.no

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Autoimmune primary adrenal insufficiency -current diagnostic approaches and future perspectives

Anette S. B. Wolff[1,2], Isil Kucuka[1] and Bergithe E. Oftedal[1,2]*

[1]Department of Clinical Science, University of Bergen, Bergen, Norway, [2]Department of Medicine, Haukeland University Hospital, Bergen, Norway

The adrenal glands are small endocrine glands located on top of each kidney, producing hormones regulating important functions in our body like metabolism and stress. There are several underlying causes for adrenal insufficiency, where an autoimmune attack by the immune system is the most common cause. A number of genes are known to confer early onset adrenal disease in monogenic inheritance patterns, usually genetic encoding enzymes of adrenal steroidogenesis. Autoimmune primary adrenal insufficiency is usually a polygenic disease where our information recently has increased due to genome association studies. In this review, we go through the physiology of the adrenals before explaining the different reasons for adrenal insufficiency with a particular focus on autoimmune primary adrenal insufficiency. We will give a clinical overview including diagnosis and current treatment, before giving an overview of the genetic causes including monogenetic reasons for adrenal insufficiency and the polygenic background and inheritance pattern in autoimmune adrenal insufficiency. We will then look at the autoimmune mechanisms underlying autoimmune adrenal insufficiency and how autoantibodies are important for diagnosis. We end with a discussion on how to move the field forward emphasizing on the clinical workup, early identification, and potential targeted treatment of autoimmune PAI.

KEYWORDS

primary adrenal insufficiency (PAI), glucococorticoids, 21-hydroxylase autoantibodies, Addisons disease, Genetic causes of PAI

1 Adrenal insufficiency

Adrenal glands regulate functions such as metabolism, blood pressure and the stress response by producing hormones from the two main parts it consists of: cortex and medulla. The adrenal cortex is responsible for producing glucocorticoids, mineralocorticoids, and adrenal androgens whereas the medulla produces adrenaline and noradrenaline, also referred to as catecholamines (Figure 1). Glucocorticoids, primarily cortisol, also known as the stress hormone, have numerous effects on

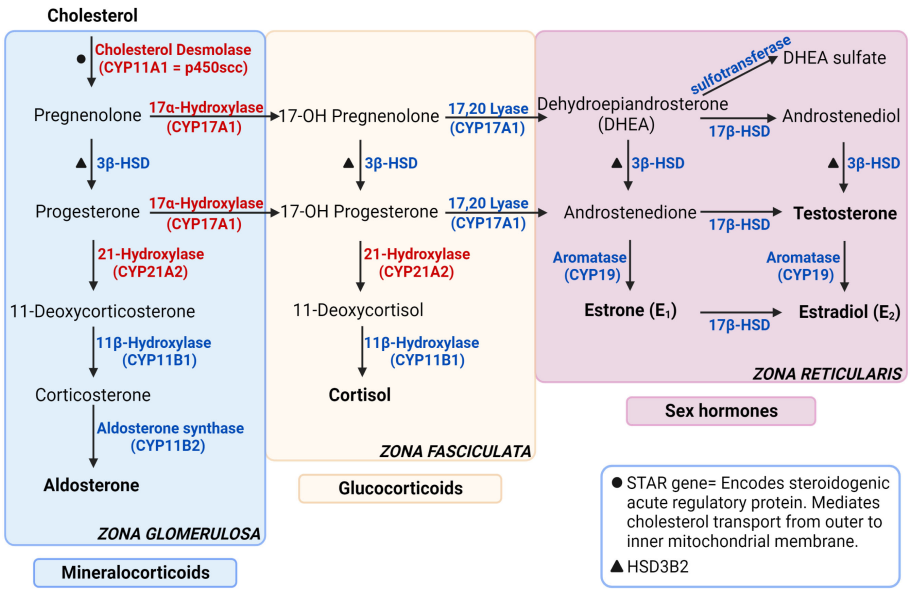

FIGURE 1
Steroidogenesis pathway. The enzymes specified are encoded by the genes stated in parenthesis under respective enzymes. The genes that are not stated in parenthesis are represented with symbols. The enzymes in red are targets for autoantibodies in autoimmune primary adrenal insufficiency. Figure created in BioRender.com. Modified from (1).

metabolism. It increases gluconeogenesis in liver, elevates blood sugar levels by inhibiting glucose intake into the cells and by suppressing insulin secretion, regulates the body's stress response, increases blood pressure and heart rate, and suppresses inflammation (1). Mineralocorticoids, primarily aldosterone, maintain salt-water balance and regulate blood pressure by increasing sodium and water reabsorption into circulation and potassium excretion from kidneys. Adrenal androgens are dehydroepiandrosterone (DHEA), dehydroepiandrosterone sulfate (DHEAS) and androstenedione.

Adrenal insufficiency (AI) is the condition where the glucocorticoids are not produced adequately because of a decreased function of adrenal glands (2). There are 3 main types of adrenal insufficiency: primary (adrenal), secondary (pituitary) and tertiary (hypothalamic). Tertiary AI is often incorporated in Secondary AI category without additional distinction. Primary AI (PAI), also known as Addison's disease, is a rare disease with a prevalence of approximately 100 per million in Europe (3–5). PAI is caused by damage or malfunction of the adrenal glands, resulting in insufficient production of cortisol, aldosterone, and DHEA. In secondary adrenal insufficiency, inadequate adrenocorticotropic hormone (ACTH) levels cause reduced stimulation of adrenal glands leading to decreased glucocorticoid secretion. Adrenal insufficiency caused by exogenous steroid treatment is a common type of AI. Exogenous glucocorticoid treatment suppresses the hypothalamic-pituitary-adrenal axis by negative feedback mechanisms, leading to low corticotropin-releasing hormone (CRH), ACTH and inadequate cortisol production (Figure 2) (6, 7).

The most common cause of primary adrenal insufficiency is autoimmunity; this type is often called Autoimmune Addison's Disease, or simply autoimmune PAI. Other causes include, but are not limited to, congenital adrenal hyperplasia, surgical removal of adrenal glands (bilateral adrenalectomy), damaged adrenal glands as a result of infection or hemorrhage and more recently, as an adverse event of cancer immune checkpoint inhibitor therapy (8) (Table 1). Autoimmune PAI can appear as an isolated manifestation but is in >60% of cases seen concomitantly with other autoimmune disorders in autoimmune polyglandular syndromes (APS) (9). A recent study comprising > 22 million persons in the United Kingdom revealed an increased risk of autoimmune PAI connected with almost any other autoimmune disease, either linked to joint genetic risk factors, or the commonly used treatment for several autoimmune disorders with high doses of glucocorticoids (10).

2 Clinical overview

Clinical manifestations of PAI can be grouped depending on the deficient hormones. Glucocorticoid deficiency related symptoms are muscle and joint pain, weakness, anemia, loss of appetite, weight loss, and low blood pressure. Maintenance of blood glucose concentration is damaged, causing hypoglycemia, exhaustion, and fatigue. In addition, patients may present with slightly elevated levels of the thyroid stimulating hormone (TSH). Hypercalcemia can also be seen in PAI, but it is uncommon (2, 11). Hypercalcaemia is presumably due to increased bone resorption caused by raised TSH commonly seen in PAI due to the lack of the inhibitory effect of glucocorticoids on pituitary TSH secretion (12). Symptoms related to low Aldosterone levels include hyponatremia, hyperkalemia, salt craving and abdominal pain. Serum electrolyte disturbances lead to dizziness, nausea, vomiting and low blood pressure. Symptoms like low energy, reduced sexual responsiveness,

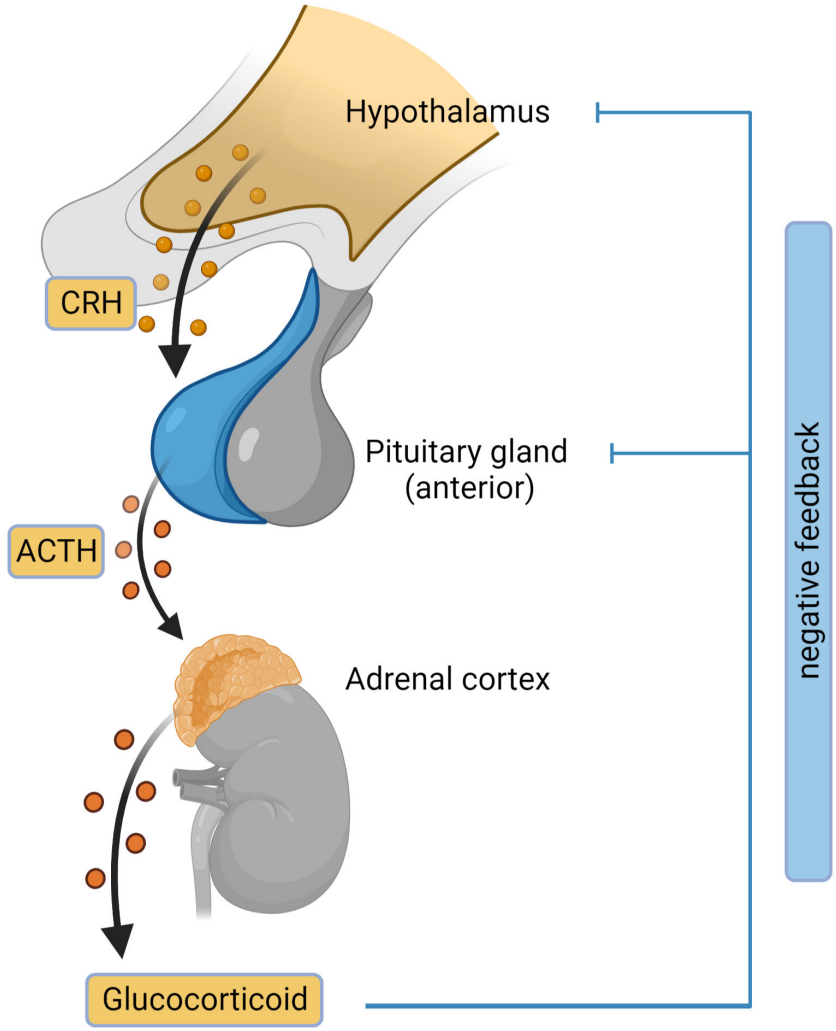

FIGURE 2
Hypothalamic-pituitary-adrenal (HPA) axis. The outline of the HPA axis between the hypothalamus, pituitary gland and the adrenal cortex is shown, including the negative feedback-loop. CRH, corticotropin-releasing hormone; ACTH, adrenocorticotropic hormone. Figure created in BioRender.com.

lack of libido in women, and erectile disfunction in men are associated with deficiency of adrenal androgens. Other symptoms of PAI include dry skin and patchy hyperpigmentation in oral mucosa and areas with increased friction such as palmar creases, axillary region, and dorsal foot.

2.1 Diagnosis

Suspicion of adrenal insufficiency through clinical manifestations is the first step to diagnosis. Clinical presentation of patients is often non-specific, which may lead to delayed diagnosis. Common symptoms and findings include weight loss, fatigue, postural hypotension, and hyperpigmentation; the latter stands out as notably specific to primary adrenal insufficiency. In routine laboratory assessments, hyponatremia, hyperkalemia, and hypoglycemia increase clinical suspicion of PAI. Next step for suspected PAI is assessing the adrenocortical function through the diagnostic test: paired measurement of serum cortisol and plasma ACTH. Morning cortisol concentration <140 nmol/L and an ACTH concentration twice the upper reference limit verifies a PAI diagnosis (2).

In ambiguous cases with morning cortisol >140nmol/L, cosyntropin stimulation test (also known as ACTH 1-24 stimulation test or short synacthen test) is performed. Cosyntropin, a synthetic form of ACTH, stimulates secretion of adrenocortical hormones from the adrenal glands. The principle of cosyntropin stimulation test is to measure plasma cortisol concentrations before and after injecting cosyntropin. A healthy person is expected to have >500 nmol/L of plasma cortisol concentration 60 minutes after 250µg cosyntropin injection. The first blood sample taken before the cosyntropin injection is to measure the baseline plasma cortisol levels. After injection, at 30 and 60 minutes, blood samples are taken for cortisol assessment. The threshold in this test at 60 min. is 500 nmol/L, defining cortisol concentrations <500 nmol/L as adrenal insufficiency (13). Low-dose

TABLE 1 Causes of primary adrenal insufficiency.

Aetiology	Example
Autoimmunity	Autoimmune primary adrenal insufficiency Autoimmune polyendocrine syndrome type-2 Autoimmune polyendocrine syndrome type-1
Infection	Tuberculosis (*Mycobacteria*) Septic shock, Meningococcal sepsis Bacteria (*Neisseria meningitidis, Haemophilus influenzae, Pseudomonas aeruginosa*) Virus (*HIV, Herpes Simplex Virus, Cytomegalovirus*) Fungi (*Pneumocystis jirovecii*)
Tumour	Primary tumours Metastatic cancers Adrenal lymphoma
Infiltration	Amyloidosis Haemochromatosis Sarcoidosis Histiocytosis
Adrenal Hemorrhage	Anti-phospholipid syndrome Disseminated intravascular coagulation Anticoagulant therapy
Trauma/ Surgery	Tumour surgery Bilateral adrenalectomy (e.g. Cushing's syndrome) Radical nephrectomy
Genetic	Congenital adrenal hyperplasia Congenital lipoid adrenal hyperplasia Adrenoleukodystrophy (X-linked) Kearns-Sayre Syndrome Smith-Lemli-Opitz Syndrome ACTH resistance (familial glucocorticoid deficiency type 1) (familial glucocorticoid deficiency type 2)
Adrenal Dysgenesis	Adrenal hypoplasia congenita
Medication	Ketoconazole, Fluconazole, Etomidate, Mitotane, Aminoglutethimide, Metyrapone, Rifampin, Phenytoin, Phenobarbital, Mifepristone.

(1μg) ACTH stimulation test has also been proposed as an alternative diagnostic test for adrenal insufficiency. Although the low-dose test is closer to physiological ACTH secretion levels in comparison to 250μg stimulation test, studies have shown that it is not superior in diagnostic accuracy to the standard test (14, 15). Low-dose test is prone to be affected by technical difficulties of the process such as dilution of the preparation, precise measurement, and injection (15). Even small volume loses can lead to significant differences in the ACTH dose delivered and affect the results. Because of these differences, although the low-dose test can also be used in clinical practices, 250 μg ACTH stimulation test remains as the gold standard test and is recommended. In addition to cortisol and ACTH, plasma renin and aldosterone levels are assessed. Low aldosterone and high renin concentrations (or high plasma renin activity) indicate mineralocorticoid deficiency (2).

After a PAI diagnosis is made, further investigation starts to determine the cause of adrenal failure. In Western countries, where other than autoimmune causes of PAI are uncommon, serum 21-hydroxylase antibodies should be assessed. Positive autoantibody evaluation confirms the diagnosis as autoimmune primary adrenal insufficiency (autoimmune PAI) and requires screening for autoimmune comorbidities such as autoimmune thyroid disease, coeliac disease, and type 1 diabetes. The monogenic APS-1should be considered in patients under 20 years old (6, 16). In patients with negative autoantibody test, the next step is CT imaging of the adrenal region, which could diagnose infections, hemorrhage, or infiltrations such as tumors in the area. In male patients with negative 21-hydroxylase autoantibody tests, very long chain fatty acid (VLCFA) levels should be measured in serum to eliminate adrenoleukodystrophy (2, 13). Patients who present with suspected adrenal crisis should start treatment as quickly as possible, without being delayed by diagnostic assessments.

2.2 Treatment

The principle of adrenal insufficiency treatment is replacement of deficient hormones (Table 2). The main goals of the treatment are to improve the life quality of patients, reaching optimum glucocorticoid regimen, avoiding comorbidities caused by glucocorticoid over-replacement such as metabolic syndrome, and preventing mortality associated with adrenal crisis. In PAI, patients need replacement of both glucocorticoids and mineralocorticoids (2, 17). The standard medication of glucocorticoid treatment for patients with AI (primary and secondary) is hydrocortisone or cortisone acetate (6, 18, 19). Patients with primary AI additionally take fludrocortisone treatment for mineralocorticoid deficiency. Unrestricted sodium intake and avoiding salt craving are also parts of the mineralocorticoid substitution therapy. These patients are allowed to use salt without restrictions and recommended to consume salty food as needed (11).

Adrenal androgen replacement is not a part of the standard treatment, even though it is deficient in adrenal insufficiency along with glucocorticoids and mineralocorticoids. Female patients taking optimized glucocorticoid and mineralocorticoid replacement may still present with persistent low energy and/or lack of libido. In these cases, adrenal androgen replacement can be considered. The positive effects of DHEA treatment have shown to be minor; but subjective health status, mood and libido were found to be improved with treatment doses between 10mg- 25mg daily (6). Due to DHEA being converted to estrogen after intake, it poses a risk of several estrogen associated diseases such as venous embolism, cardiovascular disease, and estrogen-sensitive cancers. The risk is currently unassessed and long-term safety data are lacking, and DHEA replacement is not recommended for routine use in a recent guideline by the Endocrine Society (6, 20).

3 Genetics in adrenal diseases

With the Human Genome Project and the emerge of whole exome or genome sequencing and vast amounts of genome association studies (GWAS) that are available and affordable, prediction of disease is not only based on gender, age, known family history and biochemical patterns and markers anymore, but potentiate prediction of disease in more unbiased ways (21). A number of genes are known to confer early onset adrenal disease in monogenic inheritance patterns, including genetic defects in

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TABLE 2 Standard substitution doses of glucocorticoid and mineralocorticoid treatment in adrenal insufficiency.

Hormone replacement therapy	Treatment
Hydrocortisone*	Tablets Adults (18 years or older) : 10-25 mg daily dose (in 2-4 doses) Examples: 10mg + 5mg + 5mg, **7.5mg + 5mg + 2.5mg,** **10mg + 5mg + 2.5mg,** **10mg + 10mg,** **10mg + 5mg + 5mg,** **10mg + 5mg + 5mg + 5mg** Children-Adolescents (up to 18): 8-10 mg/m^2 (in 3-4 doses, 50-66% in morning dose) Modified release Adults (18 years or older) : 15-25 mg once daily Capsules Children-Adolescents (up to 18): 8-10 mg/m^2 (in 3-4 doses, 50-66% in morning dose)
Fludrocortisone**	(*Only given in primary adrenal insufficiency*)! Adults (18 years or older) : 0.05-0.20 mg once daily Children with 6-17 years of age: 0.075-0.100 mg/m^2 once daily Children with 1-12 years of age: 0.100-0.150 mg/m^2 once daily Infants (up to 2 years of age): 0.150 mg/m^2 once daily

*Hydrocortisone for glucocorticoid substitution, **Fludrocortisone for mineralocorticoid substitution. Treatment of adrenal crisis is not included in the table.

enzymes of adrenal steroidogenesis (e.g., *CYP21A1* and *CYP11A1*), the ACTH receptor (*MC2R*) and molecules involved in establishing the mitochondrial redox potential like nicotinamide nucleotide transhydrogenase (*NNT*) and thioredoxin reductase 2 (*TRXR2*) amongst others. Yet other genes include steroidogenic acute regulatory protein (*StAR*), *NR5A1*/steroidogenic factor-1, and *NR0B1* (DAX-1) (22–24). Figure 3 summarizes some of the genetic defects that lead to congenital adrenal hyperplasia.

The etiological basis of the most common form of adrenal insufficiency in the western world, the autoimmune, has been linked to genetic variation in addition to unknown environmental factors (27). Skov and collaborators recently showed, by analyzing 29 twin pairs with PAI, a concordance for monozygotic twins of 0.71 and a heritability of 0.97, revealing that PAI is a highly genetic disorder (28). Informative syndromes caused by errors in essential immune genes like *AIRE* (autoimmune polyendocrine syndrome type 1, APS-1) and *NFKB2* (DAVID syndrome) causing autoimmune disease in the adrenals either as a primary or secondary, further throw light on parts of the pathogenesis of autoimmune PAI. Interestingly, rare mutations in several other immune genes have also recently been found to be likely causes for an autoimmune pathology of the adrenals, e.g., *RAG1, TNFAIP3, LAT* and *IKZF2* (29). Numerous candidate studies performed in the pre-genomic era further pointed to "PAI-associated genes", i.e. polymorphisms that are significantly more (un)common in patients than in healthy controls, revealing several "known autoimmunity genes" to also confer risk for PAI, including *HLA, CTLA4, BACH2* and *PTPN22* (30–34).

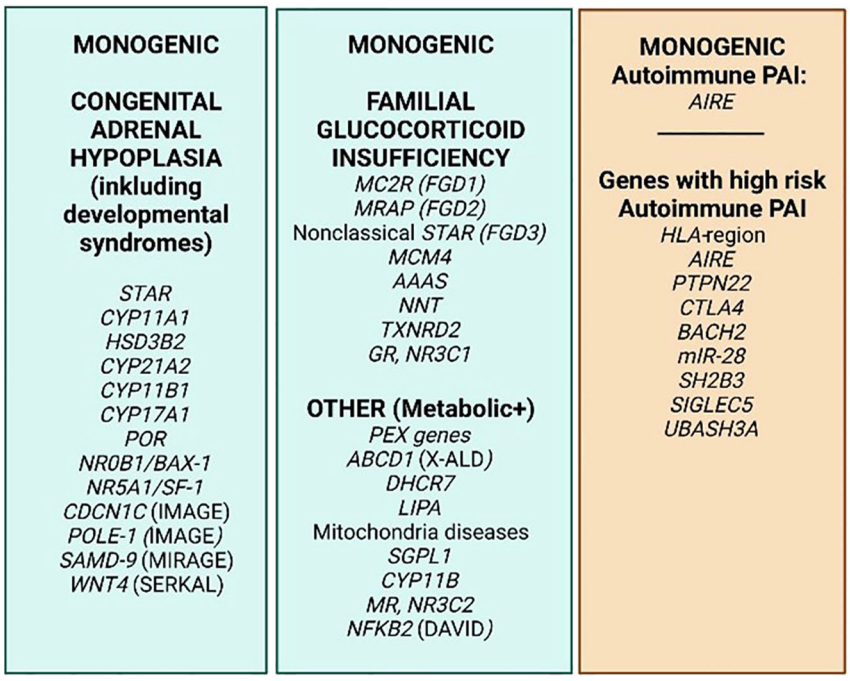

FIGURE 3
PAI genetics. The most common monogenic genetic causes of PAI (green) and 8 genes/gene regions where specific variants cause extra risk for autoimmune PAI in a polygenic setting on a genome–wide basis (orange). Based on http://www.icped.org/, and (25, 26).

3.1 Genome wide association study for autoimmune PAI

With a few exceptions, autoimmune PAI does not follow a Mendelian inheritance pattern, and is considered a complex genetic disorder. However, twin studies (28) and observation of familial clustering (3, 35) confirm the high heritability of PAI, prompting further studies. We recently performed the world's first GWAS on autoimmune PAI including only ~1200 patients, but which succeeded to elucidate nine genetic regions with quite remarkable risk potential for PAI (21) (Figure 3). The success criteria relied on carefully phenotyped patients, high quality DNA and stringent criteria for inclusion, i.e., positivity for autoantibodies against one of the key enzymes for adrenal hormone synthesis, 21-hydroxylase (21OH). Indeed, the elucidated genes were predicted to cause 40 percent of the genetic risk traits for autoimmune PAI with the absolutely highest risk depending on the HLA-region (36). Several of the risk genes revealed were previously confirmed to confer risk for PAI, e.g., *BACH2, PTPN22* and *CTLA4*. Other genes were newly identified, including *LPP, BACH2, SH2B3, SIGLEC5, UBASH3A*, and intriguingly two coding SNPs in *AIRE*. Hence *AIRE* is a risk gene both in a monogenic and polygenic setting for PAI and was also recently discovered as conferring risk towards two other organ specific autoimmune disorders, namely type 1 diabetes, and pernicious anemia (37, 38). Indeed, our research shows that dosage of AIRE probably has an impact on the development of autoimmune disease (35, 39).

3.2 Interpreting and expanding the genetic knowledge in PAI

So how can we use this information? Eriksson and colleagues estimated with a linear model that the odds ratio for PAI more than doubled with every additional PAI risk allele when analyzing 479 Swedish PAI patients and found the subject's age at disease onset to depend on their combined risk allele load. On average, subjects with more than nine risk alleles acquired PAI more than eight years earlier than subjects with less than five risk alleles, but no single loci could be associated to age of onset (40). Based on the risk alleles revealed in the GWAS, we established a polygenic risk score (PRS) for autoimmune PAI, revealing an average score for patients more than 1,5 SD higher than healthy controls (41). The PRS was tested in 18 pediatric patients and identified three cases of monogenic PAI which had clearly low PAI PRS-scores compared to the autoimmune patients (41). Hence, this technique might be applied to point to patients with a likely monogenic, rather than polygenic, cause of adrenal failure; i.e. direct who should be screened for mutations based on next generation sequencing efforts. The PRS can furthermore be applied to cohorts at special risk for autoimmune adrenal failure (e.g., registries of other endocrine autoimmune conditions or families with high burden of endocrine autoimmunity, or cancer patients who receive immune checkpoint therapy), providing likelihood-measures for development of PAI.

4 Autoantibodies in autoimmune PAI

Due to their deficiency in proper immunological tolerance mechanisms, B cells and plasma cells from PAI patients typically produce autoantibodies directed against intracellular enzymes in the affected organs. These organ-specific autoantibodies are excellent markers for autoimmune disease in the organ which they are expressed. Since autoantibodies often precede clinical symptoms, they are assayed at a routine basis (42–44).

4.1 Autoantibodies in PAI

Patients with pathogenic *AIRE* mutations are found to have high levels of circulating autoantibodies against cytokines, in particular interferon (IFN) omega (45–47) and interleukin (IL-)22 (48, 49). Neutralizing antibodies against IFN alpha and omega have also been detected in a number of monogenic immunodeficiencies (50–54). In a study by Sjøgren et al., 675 PAI patients were screened for autoantibodies against IFN-omega and IL-22, detecting 29 positive patients; 4 new APS-1 cases and 11 variants in immune genes in 8 patients suggesting these autoantibodies to be present in rare, genetic causes of PAI and endocrine autoimmunity (29).

More commonly, organ-specific autoantibodies are detected in PAI. The adrenal, steroidogenic P450 superfamily autoantigens 21-OH, 17-a-hydroxylase (17-OH) and side-chain cleavage enzyme (SCC) catalyze chemical reactions required for production of steroid hormones like aldosterone and cortisol (21-OH), progesterone (17-OH) and pregnenolone (SCC). Autoantibodies against SCC is a valid marker for primary ovarian insufficiency (POI), defined as menopause before 40 years of age, within the PAI cohort. POI is more commonly found among women with PAI than in non-autoimmune subjects, where 10.2% of women were diagnosed with POI in a Norwegian PAI cohort (55). SCC had the highest accuracy detecting POI, and the accuracy did not increase compared to combining the results from SCC and 17-OH autoantibody testing, suggesting that young PAI women should be routinely tested for autoantibodies against SCC as a predictive marker for POI.

21-OH is a steroid enzyme exclusively located in the adrenal cortex. It is a microsomal cytochrome P450 enzyme with two main functions; converting 17-hydroxyprogesterone to 11-deoxycortisol and progesterone to deoxycorticosterone. Like other microsomal P450s, the enzyme accepts electrons from a NADPH-dependent cytochrome P450 reductase, thus reducing molecular oxygen and hydroxylating the substrate. With its central role in the adrenal, autoantibodies targeting 21-OH is a hallmark of autoimmune PAI.

4.2 Autoantibodies targeting 21-OH and their diagnostic and prognostic value in autoimmune PAI

The autoimmune nature of PAI is supported by the presence of autoantibodies recognizing 21-OH (21OH-Abs), found in most of

the patients at diagnosis (56). The autoantibodies targeting 21-OH was first described in 1992 by Winqvist et al., identified as a major autoantigen in autoimmune PAI (57). These antibodies have since then been shown to be highly specific for autoimmune PAI in several patient cohorts (44, 58–61), and found present in 80–90% of patients with PAI in cross-sectional studies after exclusion of known non-autoimmune causes (62, 63). Hence, it is a reliable marker used in the diagnostic work up of PAI (14). Conversely, 21OH-Abs can also be found in individuals with normal adrenal function, where their presence hints to a future risk of developing overt PAI (64, 65). However, it has been shown that pre-clinical adult patients with 21-OH Abs only have a cumulative risk of about 20% of developing overt PAI if adrenal function is normal at the start of the observation (64), although these studies were clearly unpowered. However, combined with the genetic risk factors, these autoantibodies can be used to identify future PAI patients in the early stages of the disease (Figure 4).

Data from a small patient cohort indicates that the frequency of anti-21OH is higher shortly after diagnosis (>95%) (67), and it has been reported to decrease with increasing disease duration reaching about 50% positivity after 20 years (67, 68). We have previously investigated the levels of 21-OH Abs in a longitudinal fashion and found them to be remarkedly stable, where >90% of the patients being positive 30 years after diagnosis (61). Even though the levels of 21-OH Abs declined with increasing disease duration, it only rarely reached negativity. Importantly, a negative 21-OH Abs result does not exclude autoimmunity if assayed many years after disease onset. We found the 21-OH Abs indices to be affected by factors like age at diagnosis, sex, type of Addison's disease (isolated vs autoimmune polyendocrine syndrome type I or II) and mostly the risk HLA genotype in the patients. These autoantibodies are also useful markers within cohorts with increased risk of developing PAI, like newly diagnosed APS-1 patients, where it is an excellent marker for predicting PAI (69, 70).

5 Autoimmune reaction towards adrenal gland and 21-OH

Identification of the main autoantigen (as determined by binding of autoantibodies) also points to mechanisms behind the autoimmune destruction of the adrenal glands. But as 21-OH is an intracellular enzyme, it is unlikely that the 21-OH Abs directly mediate their destruction, and they are probably rather a sign of ongoing tissue destruction and ongoing antigen presentation (71). Several lines of study support this notion. Firstly, during pregnancy a mother with PAI will transfer 21-OH Abs to the child but the disease is not transmitted (72). In line with this, histological studies of adrenal glands from deceased Addison's disease patients show significant mononuclear cell infiltration into the adrenal gland (73). An ongoing immune reaction was further shown to be likely by Freeman et al. three decades ago, using Peripheral Blood Mononuclear Cells (PBMCs). PBMCs from PAI patients proliferated in response to adrenal proteins while control PBMCs did not (74). Also using PBMCs, Bratland et al. found that their proliferation and production of interferon-gamma in response to 21OH was significantly higher in patients compared to healthy controls. Furthermore, the 21OH-specific production of interferon-gamma was enhanced in the presence of 21OH autoantibodies. They also showed mature dendritic cells to be superior antigen-presenting cells in invoking cellular responses to 21OH and linked their findings to the high-risk HLA genotype for Addison's disease, DRB1*0301-DQ2/DRB1*0404-DQ8 (71).

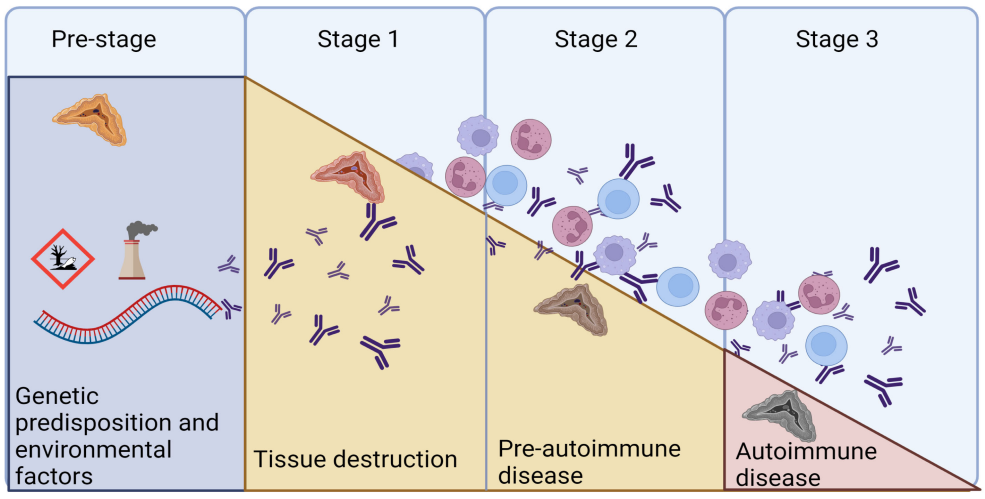

FIGURE 4
The natural cause of progression of autoimmune diseases. We are today posed to identify the genetic predisposition in AAD, and to provide replacement therapy in Stage 3, when overt disease is evident. Autoantibodies against 21OH can appear as early as in the pre-stage, as markers of the autoimmune reaction. Modified from (66) and created in BioRender.com.

Two immunodominant peptides have been found to be recognized by CD8+ T cells. Rottembourg et al. identified circulating T cells specific for a dominant 21-OH peptide in a significant proportion of HLA-B8+ patients. They further identified these responses to target the HLAB8-restricted octameric sequence EPLARLEL (position $21OH_{431-438}$), and most of the responding patients carried the HLA-B*0801 allele (75). A comprehensive peptide mapping was introduced by Dawoodji and colleagues using 18mer overlapping synthetic peptides spanning the entire 21-OH protein. They demonstrated that T cells from PAI individuals, unlike healthy controls, responded to the pool of 21-OH peptides. The responses were dominated by MHC class I restricted CD8+ T cells and focused on immunodominant regions on 21-OH, specifically an HLA-A2-restricted epitope (LLNATIAEV, position $21OH_{342-350}$), and demonstrated the ability of a $21OH_{342-350}$-specific CD8+ T cell clone to lyse LLNATIAEV-pulsed target cells by granzyme B release (76). Following up on these results, Hellesen et al. confirmed a higher frequency of HLA-A2 restricted LLNATIAEV specific cells in patients with PAI than in controls. These cells could also be expanded *in vitro* in an antigen specific manner and displayed a robust antigen specific IFN-γ production. In contrast, only negligible frequencies of EPLARLEL-specific T cells were detected in both patients and controls with limited IFN-γ response. However, significant IFN-γ production was observed in response to a longer peptide encompassing EPLARLEL, $21OH_{430-447}$, suggesting alternative dominant epitopes. Accordingly, we discovered that the slightly offset ARLELFVVL ($21OH_{434-442}$) peptide is a novel dominant epitope restricted by HLA-C7 and not by HLA-B8 as initially postulated (77).

6 Suggested clinical workup, early identification, and potential targeted treatment of autoimmune PAI

6.1 Identifying people at risk for autoimmune diseases

Evaluation of genetic risk factors and predictive autoantibodies are merely signs of an increased risk of developing disease. For PAI, there is currently no therapy except replacement of the missing corticosteroids, and curing therapeutic solutions in the future would rely on early identification of persons at risk of developing disease. Population screening has been done for several years, exemplified by the screening of newborns for the type 1 diabetes (T1D) genetic risk factor HLA-DQB1 in Finland as part of the Finnish Diabetes Prediction and Prevention Project (DIPP) (78). Here they follow newborn children every 3-6 months and had in 2016, after a decade of implementing the program, numerous examples of maturation and a healthy outcome, as well as cases reflecting the progression toward T1D (79). HLA-DQB1 is also a risk factor in PAI, celiac disease and autoimmune thyroid disease. This is also reflected by presence of additional autoimmune disease and disease-specific autoantibodies when T1D is diagnosed, and 1% were found to be 21OH Abs positive among a cohort of 491 children diagnosed with T1D (80). Indeed, a study on >22 million inhabitants of the United Kingdom recently reported that PAI is the autoimmune disorder which is most often seen together with additional autoimmune manifestations (10). This suggests that a broader autoantigen-screening might be beneficial for these patients, with the potential to identify isolated cases of other autoimmune diseases than T1D. Combined with PRS for the different diseases, this might pose an effective way to identify people at risk. Notably, it will be an ethical challenge to communicate results of a hypothesizing risk that spans birth through to adulthood is difficult. Further, the progression from detectable autoantibodies to disease will be highly variable, and demanding for the health care system, the patients, and their families. For T1D, the FDA recently approved teplizumab as the first disease-modifying therapy in type 1 diabetes. Teplizumab is a humanized anti-CD3 monoclonal antibody found to delay the onset of stage 3 type 1 diabetes and is approved for use in adults and children aged 8 years and older in people with multiple autoantibodies and dysglycaemia (stage 2 type 1 diabetes). This gives good reasons for identifying people at risk for early follow-up and gives hope that targeted therapy of organ-specific autoimmune diseases is a future possibility also for other diseases than T1D.

6.2 Suggested clinical work-up and potential for targeted treatment

The last decades have provided new insight into the underlying genetic risk factors and the immune cells that contribute to adrenal tissue destruction. Environmental factors are difficult to determine in rare diseases, but involvement of viruses has been suggested to play a part (81). Still, PAI patients are diagnosed when most of the adrenal cortex is destroyed, representing the end stage of an autoimmune inflammation taking place over months and years, and several patients are still diagnosed during Addisonian crisis where the outcome might be fatal.

Interestingly, it was recently found that as much as 30% of all PAI patients have some residual hormone-producing capacity (82), which could represent a window of opportunity to rescue adrenal function, but there are still several hurdles to overcome to provide early diagnosis and targeted treatment of PAI. Although some information has been gathered on the natural course of the autoimmune destruction by observing decline in hormone level, details on how the autoimmune reactivity against the adrenal is triggered and evolves are completely unknown and this information is needed for intervention at an early stage to be successful. We also lack in-depth information about the immune system in patients with PAI. Mapping the pathogenic pathways can point to which drugs that could be effective in halting the autoimmune reaction. Identifying the exact epitopes in the autoantigens associated with adrenalitis can help develop peptide or mRNA vaccines or chimeric antigen receptor T regulatory cells (CAR Tregs) to induce immune tolerance.

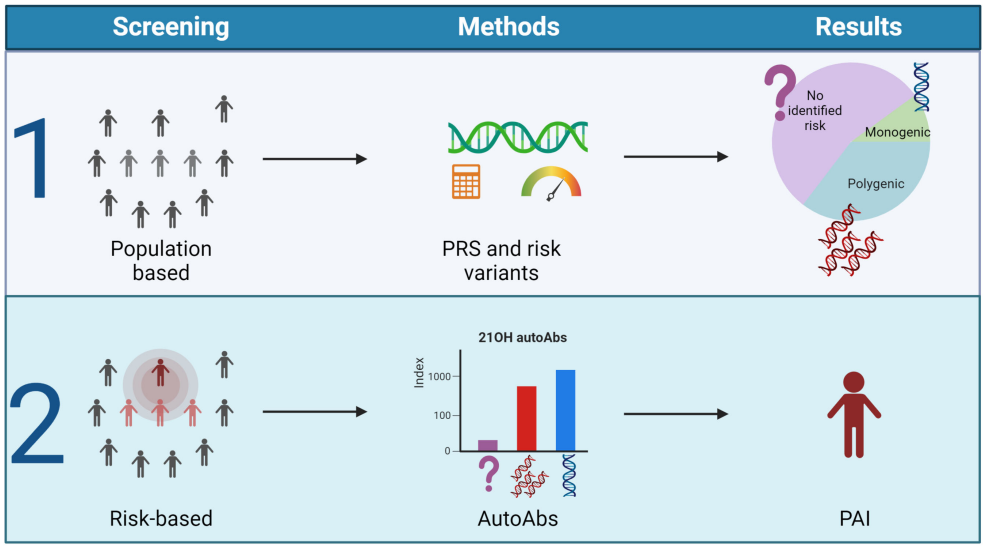

FIGURE 5
Outlined screening possibilities for PAI. The figure suggests two different strategies for early identification of PAI. Population-based screening dependent on polygenic risk scores and genetic screening of risk variants (1) or 21-OH autoantibody measurements in high-risk groups either identified by genetic screens or family history (2). Figure created in BioRender.com.

To conclude, the underlying genetic landscape in PAI is emerging, a prerequisite for future genetic population screens. These will be powerful tools together with autoantibodies targeting 21OH to identify patients earlier before the adrenal tissue is destroyed (Figure 5). This will be a crucial step to enable targeted therapy for this disease.

Author contributions

AW: Conceptualization, Writing – original draft, Writing – review & editing. IK: Conceptualization, Writing – original draft, Writing – review & editing. BO: Conceptualization, Writing – original draft, Writing – review & editing.

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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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EDITED BY
Elena Valassi,
International University of Catalonia, Spain

REVIEWED BY
Sylvère Störmann,
Ludwig Maximilian University of Munich, Germany

*CORRESPONDENCE
Anat Ben-Shlomo
anat.benshlomo@cshs.org

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Opioid-induced adrenal insufficiency: diagnostic and management considerations

Erica Patel[1] and Anat Ben-Shlomo*[,1,2]

[1]Division of Endocrinology, Diabetes & Metabolism, Department of Medicine, Cedars-Sinai Medical Center, Los Angeles, CA, United States, [2]Multidisciplinary Adrenal Program, Department of Medicine, Cedars-Sinai Medical Center, Los Angeles, CA, United States

The dramatic rise in opioid use over the last two decades has led to a surge in their harmful health effects. Lesser known among clinicians is the impact of opioids on the endocrine system, especially with regard to cortisol. Opioids can suppress the hypothalamus-pituitary-adrenal (HPA) axis and may result in clinically significant adrenal insufficiency, especially in those treated at higher doses and for a longer time. A high clinical suspicion is necessary in this population for early diagnosis of opioid-induced adrenal insufficiency (OAI). Diagnosis of OAI is challenging, as the symptoms are often vague and overlap with those due to opioid use or the underlying pain disorder. Traditional assays to diagnose adrenal insufficiency have not been widely studied in this population, and more investigation is needed to determine how opioids might affect assay results. Once a diagnosis of adrenal insufficiency has been made, glucocorticoid replacement in the form of hydrocortisone is likely the mainstay of treatment, and effort should be made to taper down opioids where possible. Cortisol levels should be retested periodically, with the goal of stopping glucocorticoid replacement once the HPA axis has recovered. In this review, we provide context for diagnostic challenges in OAI, suggest diagnostic tools for this population based on available data, and offer recommendations for the management of this disorder. There is a paucity of literature in this field; given the widespread use of opioids in the general population, more investigation into the effects of opioids on the HPA axis is sorely needed.

KEYWORDS

Adrenal insufficiency, opioids, cortisol, hydrocortisone, narcotics, adrenal insufficiency

Introduction

The inhibitory effects of opioids on the hypothalamic-pituitary-adrenal (HPA) axis in humans were established years ago (1). However, surprisingly, this fact is frequently underappreciated among clinicians (2). Given the escalating 'opioid epidemic,' which has tragically claimed an increasing number of lives over the past two decades (3), there is a pressing need for clinicians to pay closer attention to the suppressive effects of opioids on the HPA axis.

Both acute and chronic opioid treatments have been shown to inhibit adrenocorticotrophic hormone (ACTH) and cortisol production (4, 5), elevating the risk of opioid-induced adrenal insufficiency (OAI). This effect might occur through a secondary mechanism, possibly involving inhibition of corticotrophin-releasing hormone (CRH) action on pituitary corticotrophs (6). Although not every reduction in cortisol is clinically relevant and warrants full workup for OAI, a high index of suspicion is prudent for patients treated with high-dose opioids and/or for prolonged periods (7). It is worth noting that prolonged opioid treatment was not shown to inhibit aldosterone in humans (5).

Data on the prevalence of OAI are primarily derived from small, retrospective studies that lack uniformity in setting, opioid dose, duration, or route of administration (Table 1). Additionally, not all studies employ rigorous or updated diagnostic testing for adrenal insufficiency. Clinical practice guidelines for management of hypopituitarism recommend screening for adrenal insufficiency with morning cortisol [AI is more likely if <3 mcg/dL (100 nmol/L)], and ACTH$^{1-24}$ (250 mcg) stimulation testing for confirmation. Peak cortisol ≤18 mcg/dL (500 nmol/L) at 30 or 60 minutes after ACTH$^{1-24}$ stimulation is diagnostic for adrenal insufficiency according to older cortisol assays (13). However, according to recent study, a lower threshold of <14-15 mcg/dL (386.3-413.9 nmol/L) at 30 minutes post stimulation should be used for validation of adrenal insufficiency (14). Only one study utilized a lower cortisol cutoff of 14.7 mcg/dL (405 nmol/L) measured at 25-70 minutes after ACTH$^{1-24}$ stimulation in a retrospective evaluation demonstrating OAI in 4% of 75 patients on chronic opioid treatment (11). Moreover, diagnostic criteria or OAI are not uniformly applied across studies. In a systematic review and meta-analysis evaluating endocrine effects of opioids, only 8 of 21 studies assessing the effects on the HPA axis used stimulation testing to measure cortisol, and these studies used insulin-induced hypoglycemia and ACTH$^{1-24}$ stimulation testing using the older cortisol cutoffs or other assays such as CRH, metyrapone, and yohimbine. They calculated prevalence of OAI to be 5-42% with weighted mean percentage of 15% (6). In a prospective cross section study of 102 patients receiving opioids and referred to a pain rehabilitation clinic, 9% (n=9) were diagnosed with OAI, but criteria included clinical presentation, morning cortisol ≤10 mcg/dL (277 nmol/L), ACTH ≤15 pg/ml (3.3 pmol/L), and dehydroepiandrosterone sulfate (DHEAS) ≤25 mcg/dL (86.7 nmol/L), as well as peak cortisol level of 16-17 mcg/dL (441.4-469 nmol/L) during an ACTH$^{1-24}$ stimulation test in 3 patients (8), which is higher than the new cortisol cutoff suggested. Given the high rates of opioid use in the community, the number of patients at risk for OAI and its associated morbidity and mortality is substantial.

TABLE 1 Prevalence of OAI Among Patients Receiving Chronic Opioid Therapy.

	N	Inclusion Criteria*	MME dose, median mg (range)	Diagnostic Criteria	Prevalence of OAI
Cross-Sectional Studies					
Li, 2020 (8)	102	Intermittent or continuous opioid use for the last 90 days	60 (3-840)	Serum ACTH <16 pg/mL (3.5 pmol/L), morning cortisol < 11 mcg/dL (303.5 nmol/L), DHEAS < 26 mcg/dL (90.1 nmol/L), DHEAS <26 mcg/dL, ACTH$^{1-24}$ stimulation test with peak cortisol < 18 mcg/dL (496.6 nmol/L)	9% overall; 6% based on cortisol, serum ACTH, and DHEAS, 3% also based on stimulation test
Lamprecht, 2018 (9)	40	Opioid use >6 months, ≥25 mg MEDD	74 (25–265)	ACTH$^{1-24}$ stimulation test with peak cortisol cortisol < 18 mcg/dL (496.6 nmol/L), overnight metyrapone stimulation test (30 mg/kg)	cortisol < 18 mcg/dL (496.6 nmol/L)
Gibb, 2016 (10)	48	Opioid use >6 months	68 (40-153)	ACTH$^{1-24}$ stimulation test with peak cortisol <15.6 mcg/dL (430 nmol/L), 8 AM cortisol <3.62 mcg/dL (100 nmol)	6.25% based on stimulation test, 8.3% with low morning cortisol
Retrospective Studies					
Colling, 2022 (11)	75	Opioid use >30 days	83 (23-193)	ACTH$^{1-24}$ stimulation test with two different peak cortisol cutoffs: 1. with peak cortisol <14.7 mcg/dL (405 nmol/L) 2. <18.1 mcg/dL (500 nmol/L)	4% with <14.7 mcg/dL (405 nmol/L) 18.7% with <18.1 mcg/dL (500 nmol/L)
Merdin, 2016 (12)	20	≥25 mg MEDD for ≥1 month for cancer pain and VAS <2	180 (10-420)	Serum cortisol < 4.3 mcg/dL (118.6 nmol/L)	cortisol < 4.3 mcg/dL (118.6 nmol/L)
Abs, 2000 (5)	73	Intrathecal opioid therapy	4.8 (0.6-15)	24h UFC <20 mg/L, ITT <180 µg/L	19.7% based on 24h UFC, < 20 mcg/dL (551.8 nmol/L) ITT peak cortisol < 18 [not 180] mcg/dL [not mcg/L] (496.6 nmol/L)

*Patients with noncancer pain unless otherwise noted.
ACTH, adrenocorticotrophic hormone; DHEAS, dehydroepiandrosterone sulfate; ITT, insulin tolerance test; MEDD, morphine equivalent daily dose; MME, morphine milligram equivalent; OAI, opioid-induced adrenal insufficiency; UFC, urine [not urinary] free cortisol ; VAS, visual analog scale.

This article suggests best practices for diagnosis and management of OAI in current clinical practice and identifies key areas in which further study of this often-overlooked disorder can improve patient outcomes.

Diagnostic considerations in OAI

Signs and symptoms of OAI are nonspecific, and include fatigue, lack of appetite, weight loss, gastrointestinal symptoms such as nausea, vomiting, diarrhea, mood changes, signs such as orthostatic hypotension, biochemical findings such as hyponatremia, and impaired quality of life; moreover, they may overlap with symptoms of opioid treatment and the disorders causing pain for which opioids are prescribed (11).

Once OAI is clinically suspected, the question of which assay to use remains. The cutoffs recommended by the Endocrine Society guidelines (13) for confirmation of adrenal insufficiency from other causes, i.e., screening morning cortisol <3 mcg/dL (100 nmol/L) and baseline cortisol ≥18 mcg/dL (500 nmol) at 30 and 60 minutes after $ACTH^{1-24}$ stimulation, have not been prospectively validated in opioid-treated patients. The refined cutoff for newer immunoassays and mass spectrometry, i.e., 14-15 mcg/dL (386-414 nmol/L) 30 minutes after stimulation (14), was retrospectively assessed in OAI (11) but prospective validation has not been conducted. This is a crucial issue, as the cutoffs established for central adrenal insufficiency might not be applicable to OAI. For example, morning total cortisol threshold levels were established in outpatients without specific consideration of pain, yet pain, as with all other stressogenic conditions, stimulates the HPA axis (15), skewing accurate assessment of cortisol level independent of any effect from the opioids. In addition, both pain and opioids have been shown to alter the circadian rhythm that controls the HPA axis (16), which, in turn, suggests the need for a different cutoff for morning cortisol screening tests particularly among hospitalized patients (11).

Even if it were possible to control for the effects of pain and opioid use on tests that rely on total cortisol levels, multiple other factors that may co-exist in opioid-treated patients might affect their reliability in confirming a diagnosis of secondary adrenal insufficiency regardless of the etiology (17). The limited sensitivity and specificity of these tests have been described in patients with conditions affecting total circulating protein (albumin and corticosteroid binding globulin), including kidney and liver diseases and critical illnesses (18), and a meta-analysis of studies evaluating diagnostic performance of the $ACTH^{1-24}$ stimulation test for secondary adrenal insufficiency shows high specificity but low sensitivity even in unselected patients (19).

Considering the known challenges with current diagnostic assessments for adrenal insufficiency, and given the lack of optimal validation in patients with suspected OAI, we suggest the following diagnostic approach for patients treated with long-term, high-dose opioids. These patients should be periodically assessed for new symptoms suggestive of OAI, such as fatigue, reduced appetite and weight loss, gastrointestinal disturbance, mood changes, hypotension, and hyponatremia, while also recognizing that similar adverse effects may be due to opioid use itself or the disease that causes the pain for which they receive treatment with opioids (20). If the patient manifests symptoms of adrenal insufficiency, we suggest the clinician refer the patient to an endocrinologist for further evaluation. The endocrinologist should carefully review the patient's medical history to address the potential contribution of any other medications that might affect total cortisol levels, including any glucocorticoid formula (glucocorticoid injections are often used for the treatment of pain); antifungals or other steroidogenesis inhibitors; somatostatin analogues; or drugs that change the activity of CYP3A4, an enzyme responsible for the metabolism of cortisol. All other possible etiologies for adrenal insufficiency resulting from infections, bilateral bleeding, tumors, surgery and autoimmune disorders should be considered as well.

Patients with intense pain often cannot stop taking opioid medications, which would allow the HPA axis to recover from an acute opioid suppression and for morning cortisol to reach the true endogenous levels. Therefore, screening with morning cortisol is less likely to be informative in cases of suspected OAI. Rather, we suggest the pharmacological high-dose (250 mcg) $ACTH^{1-24}$ stimulation test be administered in all suspicious cases. Importantly, given the rapid effect of acute opioid administration on the HPA axis, it is reasonable to avoid administration of opioids for several hours prior to testing and for as long as tolerated by the patient in an attempt to allow for the true endogenous cortisol levels to manifest. For further confirmation, we also suggest measuring cortisol at the two established time points of 30 and 60 minutes after stimulation. Failure to exceed a threshold of 14-15 mcg/dL (386-414 nmol/L) at 30 minutes and/or failure to exceed the threshold of 18 mcg/dL (496.6 nmol/L) at 60 minutes in the setting of long-term, high-dose opioid use supports a diagnosis of OAI. Baseline clinical evaluation and measurement of cortisol levels can be considered before initiating opioid treatment to better assess patients during follow up for the development of OAI.

The combination of morning cortisol, low-normal to low ACTH, and low DHEAS has been proposed as a possible diagnostic marker for OAI (8), but its role might be limited. The challenges of relying on morning cortisol in screening for OAI were discussed above. Measurement of ACTH is not utilized in the initial diagnosis of adrenal insufficiency, but can be an indicator of where along the HPA axis the deficiency is located (21). Low ACTH in the presence of a failure to pass the $ACTH^{1-24}$ stimulation test is indicative of secondary adrenal insufficiency, which supports the diagnosis of OAI due to a central cause; however, opioid receptors are abundant on the adrenal glands as well (22) and the presence of a combined primary (which will cause high ACTH level) and secondary (causing low ACTH level) adrenal insufficiency resulting in low-normal ACTH level had never been studied. Nevertheless, if results of the $ACTH^{1-24}$ stimulation test support a diagnosis of adrenal insufficiency, ACTH levels should be measured to distinguish between primary adrenal insufficiency at the level of the adrenal glands or central adrenal insufficiency at the level of the pituitary or the hypothalamus (21). DHEAS largely originates in the adrenal gland, has a long half-life of approximately 24 hours, and is regulated by ACTH (23). Accordingly, it may reflect ACTH

deficiency and has been studied as a potential additive biomarker to ACTH$^{1-24}$ stimulation in the diagnosis of adrenal insufficiency (24, 25). However, DHEAS levels decrease with age (26) and with chronic diseases such as diabetes mellitus (27), autoimmune disease (28), and cancer (29), and the lack of OAI-specific, age-specific reference ranges all may limit its use. Nevertheless, as access to an endocrinologist and ACTH$^{1-24}$ stimulation testing may not be readily available to all clinicians, the combination of low/low-normal morning cortisol, DHEAS, and ACTH levels several hours after the last dose of opioids may be used as a preliminary screening tool for OAI.

Management considerations in OAI

After establishing a diagnosis of OAI, treatment decision-making remains challenging, as there are no large, prospective, randomized, placebo-controlled clinical trials of patients diagnosed according to accepted standards to offer guidance on optimal approaches and outcomes.

To date, the only prospectively designed study included 17 patients treated with mean morphine milligram equivalent (MME) dose of ≥20 mg/day for at least 4 weeks for noncancer pain who were diagnosed with hypocortisolism based on plasma cortisol levels ≤12.7 mcg/dL (≤350 nmol/L) using the cold pressure test (CPT), in which exposure to cold stimulates nociceptive neuronal pathways that activate the HPA axis (30). Patients received hydrocortisone replacement (10 mg/m^2/day given in 3 daily divided doses) or placebo for 28 days, then switched to the opposite treatment after 10-day washout. Health status questionnaires completed before and after each 28-day treatment period showed that treatment with hydrocortisone resulted in better outcomes on the SF-36 bodily pain and vitality domains, as well as greater improvements in general activity, mood, and work on the Pain Interference Score and on pain response threshold and tolerance on the CPT. No improvement was noted on the AddiQoL survey specific to symptoms of adrenal insufficiency, or on the Brief Pain Inventory-Short Form survey or the Pain Severity Score survey (30). Although these data suggest that hormone replacement in OAI could positively impact overall patient outcomes, the CPT is not used in standard endocrinology practice to diagnose adrenal insufficiency and the 12.7 mcg/dL (350.4 nmol/L) cortisol threshold has never been validated using the gold-standard insulin tolerance test or the ACTH$^{1-24}$ stimulation test for this purpose.

Nevertheless, results of a larger retrospective study in 40 patients with more rigorously defined OAI (peak cortisol level ≤18 mcg/dL [497 nmol/L] after 250 mcg ACTH$^{1-24}$ stimulation) who were treated with higher doses of opioid (median MME dose 105 mg/day [range, 60-200 mg/day]) for a longer period of time (median duration 60 months [range, 3-360 months]) also suggests clinical benefit with glucocorticoid replacement (31). Comparing symptoms reported before and after treatment, fatigue improved in 48% of patients, weight loss in 41%, abdominal pain and nausea in 25%, and headache in 17%. Notably, 38% ultimately tapered and/or stopped opioids, and HPA axis recovery was evident in 70% of these patients on biochemical follow up (31). Reports of individual patients with OAI treated with hydrocortisone describe similar benefits (22).

Considering the scarce data available on OAI treatment, we suggest a treatment approach that aims to replace the deficient endogenous cortisol and provide symptomatic relief. Thus, in cases where OAI is strongly suspected based on clinical and biochemical presentation, treatment with hydrocortisone should be considered following the Endocrine Society guidelines of 15-20 mg per day, divided into 2-3 doses, with the highest dose taken in the morning (13). As long-term treatment with opioids does not adversely affect plasma renin activity and aldosterone production (32),

TABLE 2 Efficacy of Glucocorticoid Treatment in Patients With OAI.

	Study Type	N	Opioid Use*	OIA Assessment	Intervention	Outcomes	Follow-up
Nenke, 2015 (30)	Randomized, double-blind, placebo-controlled crossover	17	MEDD ≥20 mg for ≥4 weeks (47% 20-50 mg, 53% >100 mg)	Plasma cortisol ≤12.7 mcg/dL (350 nmol/L) after cold pressor test	28 days of 10 mg/m^2/d oral hydrocortisone TID or placebo, 2-week washout, then crossover for 28 days	Improvement in bodily pain and vitality on SF-36; improvement on general activity, mood, and work on Pain Interference Score	Not reported
Li, 2020 (31)	Retrospective cohort	40	Median MME 105 mg/d (range, 60-200) for median 96 months (range, 24-120)	Low AM cortisol, DHEAS, and/or ACTH (59%); peak cortisol <= 18 mcg/dL (496.6 nmol/L) after ACTH$^{1-24}$ stimulation (41%)	Hydrocortisone (95%) or prednisone (5%); dose not reported	Improvement in fatigue, musculoskeletal pain, and weight loss	Symptomatic improvement in 70% of patients with clinical follow-up, resolution of OAI in 70% of patients with biochemical follow-up
Gibb, 2016 (10)	Prospective, cross-sectional; reports on	2	39-year-old female on 185 mg/d MME	Peak cortisol <437 nmol/L (15.9 mcg/dL) after ACTH$^{1-24}$ stimulation	Hydrocortisone 10 mg AM/5 mg PM	Improvement in lethargy and fatigue	Not reported

(Continued)

TABLE 2 Continued

	Study Type	N	Opioid Use*	OIA Assessment	Intervention	Outcomes	Follow-up
	patients who did not pass ACTH$^{1-24}$ stimulation test		37-year-old female on 100 mg/d MME				
Kondo, 2022 (34)	Case report	1	46-year-old female on 90-120 mg/d MME for 48 months (transdermal fentanyl)	60-minute cortisol 15 mcg/dL (413.8 nmol/L) after ACTH$^{1-24}$ stimulation; AM ACTH 4.7 pg/ml (1 pmol/L), AM cortisol 2.1 mcg/dL (57.9 nmol/L), DHEAS 39 mcg/dL (135.2 nmol/L)	Hydrocortisone 15 mg/d	Partial improvement in fatigue, appetite, and vitality	Unable to taper off opioid; hydrocortisone continued
Debono 2011 (35)	Case report	1	21-year-old female on 100 mg tramadol 4x/d	30-min cortisol 307 nmol/l and 60-min cortisol 419 nmol/l after ACTH$^{1-24}$ stimulation; AM ACTH 9.7 ng/L			

*Patients with noncancer pain unless otherwise noted.
MEDD, morphine equivalent daily dose; MME, morphine milligram equivalent; OAI, opioid-induced adrenal insufficiency.

mineralocorticoid replacement is likely not required in patients with OAI.

Lastly, there is no protocol to suggest how to follow hydrocortisone-treated OAI patients. Case reports suggest cessation of opioids or even reduction of opioids doses can lead to reversal of OAI (33) (Table 2). However, the lowest opioid dose at which the HPA axis recovers and the time to recovery are both unknown. Therefore, per clinician discretion, periodic clinical and biochemical assessment of OAI symptoms during continuous treatment and dose tapering is suggested.

Conclusions

Opioid treatment is associated with the development of OAI in a small subset of patients, and those treated for a longer time and at higher doses might be at increased risk. Active monitoring for OAI in these patients is warranted. As no specific diagnostic criteria are available for OAI, at this time, treating clinician should rely on Endocrine Society guidelines for diagnosis of central adrenal insufficiency. It is preferable that patients be diagnosed, treated, and monitored by a skilled endocrinologist. Patients should be periodically monitored for new clinical symptoms and signs of adrenal insufficiency and, if suspicious, ACTH$^{1-24}$ stimulation testing should be considered to confirm the diagnosis. If stimulation testing is unavailable, a combination of morning cortisol, DHEAS, and ACTH measurement may be used as initial screening. All tests should be performed several hours after the last opioid dose to avoid the acute HPA axis suppression observed shortly after opioid treatment.

If both clinical and biochemical evaluation suggest a high probability of OAI, the clinician should consider treatment with daily hydrocortisone as recommended for patients with secondary adrenal insufficiency from other causes. Treatment should be temporary in most cases while continuously monitoring HPA axis recovery, and hydrocortisone dose should be tapered down as opioid dose is reduced and HPA axis function recovers.

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EDITED BY
Henrik Falhammar,
Karolinska Institutet (KI), Sweden

REVIEWED BY
Hassan Soleimanpour,
Tabriz University of Medical Sciences, Iran
Behzad Nazemroaya,
Isfahan University of Medical Sciences, Iran

*CORRESPONDENCE
Shibo Liao
liaoshibo@163.com
Yimin Yan
yanyimin180@163.com

†These authors have contributed equally to this work

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Etomidate-induced hypokalemia in electronic cigarette users: two case reports and literature review

Wenchang Wu[1,2†], Can Xia[2†], Lulu Gan[2], Shibo Liao[1*] and Yimin Yan[1,2*]

[1]Department of Endocrinology, Xiaogan Hospital Affiliated with Wuhan University of Science and Technology, The Central Hospital of Xiaogan, Xiaogan, Hubei, China, [2]Medical College, Wuhan University of Science and Technology, Wuhan, China

Hypokalemia is a common clinical condition that can lead to muscle weakness, difficulty breathing, malignant arrhythmias, and even death. This report describes two cases of severe hypokalemia resulting from the use of electronic cigarettes containing etomidate, both accompanied by varying degrees of adrenal hyperplasia. In both cases, the patients were admitted to the hospital with lower limb weakness and difficulty walking. Relevant examinations revealed low blood potassium, low cortisol, high adrenocorticotropic hormone, low renin, and low aldosterone levels in the patients, with Case 2 also having significant hypertension. In both cases, adrenal CT scans showed thickening of the adrenal glands. After the delivery of potassium supplementation in both cases, blood potassium levels gradually returned to normal and muscle strength gradually improved. The case reports are followed by a review of the literature on etomidate and its related mechanisms of action with discussion of its association with hypokalemia.

KEYWORDS

etomidate, hypokalemia, adrenal hyperplasia, 11-beta-hydroxylase, hypertension

Introduction

Etomidate is a non-barbiturate, sedative-hypnotic drug used to induce anesthesia. Its primary mechanism of action is positive allosteric modulation of the γ-aminobutyric acid type A receptor, which enhances the inhibitory effects of the neurotransmitter γ-aminobutyric acid (1). The advantages of etomidate include rapid onset of effects, short duration of effects, fast drug metabolism, no histamine release, and stable hemodynamics.

Numerous studies have shown that etomidate can cause adrenal insufficiency primarily by inhibiting 11β-hydroxylase, and this effect is dose-dependent. However, very few studies on the relationship between etomidate and hypokalemia have been reported. Recently, during our clinical work, we admitted two patients with hypokalemia caused by the use of

electronic cigarettes containing etomidate. Here we present these two cases and review the relevant literature to better understand the association between etomidate and hypokalemia.

Cases Report

Case 1

A 29-year-old Han male was admitted to the hospital presenting with "lower limb weakness for 2 days". The patient had experienced sudden onset of lower limb weakness 2 days previously that made him unable to walk and was accompanied by abnormal sensations such as pain and numbness in the lower limbs. His blood potassium level was measured to be 1.69 mmol/L in the outpatient department, leading to the diagnosis of hypokalemia and hospitalization.

Physical examination: body temperature, 36.3°C; pulse rate, 90 beats/min; respiratory rate, 19 breaths/min; blood pressure, 132/88 mmHg; and body mass index (BMI), 27.4 kg/m^2. No significant abnormalities were found on cardiovascular, pulmonary, or abdominal examinations. Muscle strength was grade 4 for all limbs, with moderate muscle tone. The patient stated he had no significant medical history. He reported intermittent use of electronic cigarettes containing etomidate for the previous 3 months. Each e-cigarette used by the patient contains 2.0 ml of e-liquid. The patient typically consumes one e-cigarette every two days. The patient's parents have no history of smoking and exhibit no symptoms indicative of hypokalemia.

Laboratory tests: blood potassium, 4.7 mmol/L; 24-h urinary potassium excretion, 123.31 mmol/24 h (reference range: 25–100 mmol/24 h); 24-h urine volume, 1900 mL; cortisol levels: 8:00 AM 8.34 μg/dL (reference range: 6.2–19.4 μg/dL), 4:00 PM 1.04 μg/dL (reference range: 2.3–11.9 μg/dL), 12:00 AM 9.04 μg/dL (Figure 1); adrenocorticotropic hormone (ACTH), 52 pg/mL (reference range: 6–48 pg/mL); aldosterone in supine position, 5.01 pg/mL (reference range: 60–360 pg/mL); renin activity in supine position, 0.56 ng/mL/h (reference range: 0.15–2.33 ng/mL/h); aldosterone in upright position, 5.000 pg/mL (reference range: 50–313 pg/mL); and renin activity in upright position, 0.64 ng/mL/h (reference range: 1.31–3.95 ng/mL/h). Endocrine examination revealed: prolactin, 16.60 ng/mL (reference range: 2.55–16.04 ng/mL); follicle-stimulating hormone, 1.75 mIU/mL (reference range: 1.5–11.8 mIU/mL); luteinizing hormone, 3.63 mIU/mL (reference range: 1.1–25 mIU/mL), estradiol, 56.40 pg/mL (reference range: 7.63–42.6 pg/mL); progesterone, 0.41 ng/mL (reference range: 0.05–0.149 ng/mL); testosterone, 0.89 ng/mL (reference range: 2.2–10.5 ng/mL); and parathyroid hormone, 63.94 pg/mL (reference range: 15–65 pg/mL). Adrenal CT scan showed bilateral adrenal gland thickening (Figure 2). No significant abnormalities were detected on routine blood tests, blood gas analysis, glucose tolerance test, thyroid function tests, catecholamine measurement, and antinuclear antibody spectrum analysis. Thyroid ultrasound, cardiac ultrasound, and chest CT were normal.

At admission, the patient's blood potassium level was 1.69 mmol/L, and he was given intravenous infusion of 10% potassium chloride solution and oral administration of 20 mL of 10% potassium chloride solution. His blood potassium level did not return to normal readily, and an urgent blood gas analysis showed a potassium ion level of 1.5 mmol/L and an actual bicarbonate level of 27.5 mmol/L. The patient was then treated with central venous infusion of 9 g potassium chloride at a rate of 0.5 g/h, and his blood potassium level had risen to 4.01 mmol/L when rechecked. His muscle weakness gradually disappeared, and he was prescribed oral sustained-release potassium chloride tablets (0.5 g three times a day). With this treatment, his blood potassium level remained within the normal range. Before discharge, his blood potassium level was measured to be 4.84 mmol/L, with an ACTH level of 5 pg/mL and 24-h urinary potassium excretion of 31.60 mmol. At that time, the following cortisol levels were measured: 8:00 AM 1.02 μg/dL (reference range: 6.2–19.4 μg/dL), 4:00 PM 2.23 μg/dL (reference range: 2.3–11.9 μg/dL), and 12:00 AM 14.01 μg/dL.

Case 2

A 32-year-old Han male patient was admitted to the hospital for "weakness in all limbs for half a day." The patient experienced sudden onset of weakness in all limbs without an obvious cause approximately 12 hours previously, accompanied by difficulties in standing up, turning over, and walking. His blood potassium level was measured to be 1.80 mmol/L in the outpatient department, leading to the diagnosis of hypokalemia and hospitalization.

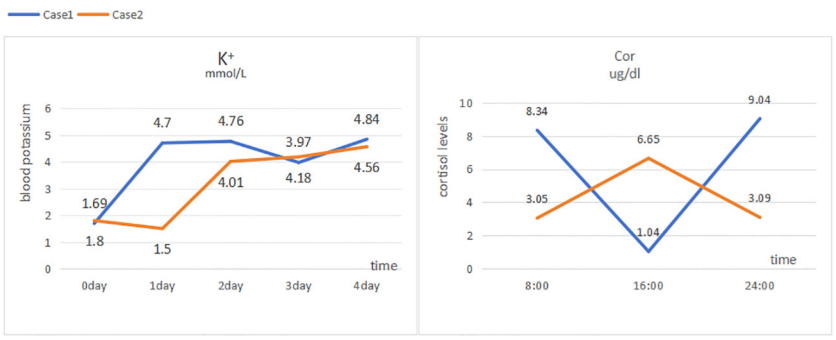

FIGURE 1
Fluctuations in blood potassium and cortisol levels in Case 1 and Case 2.

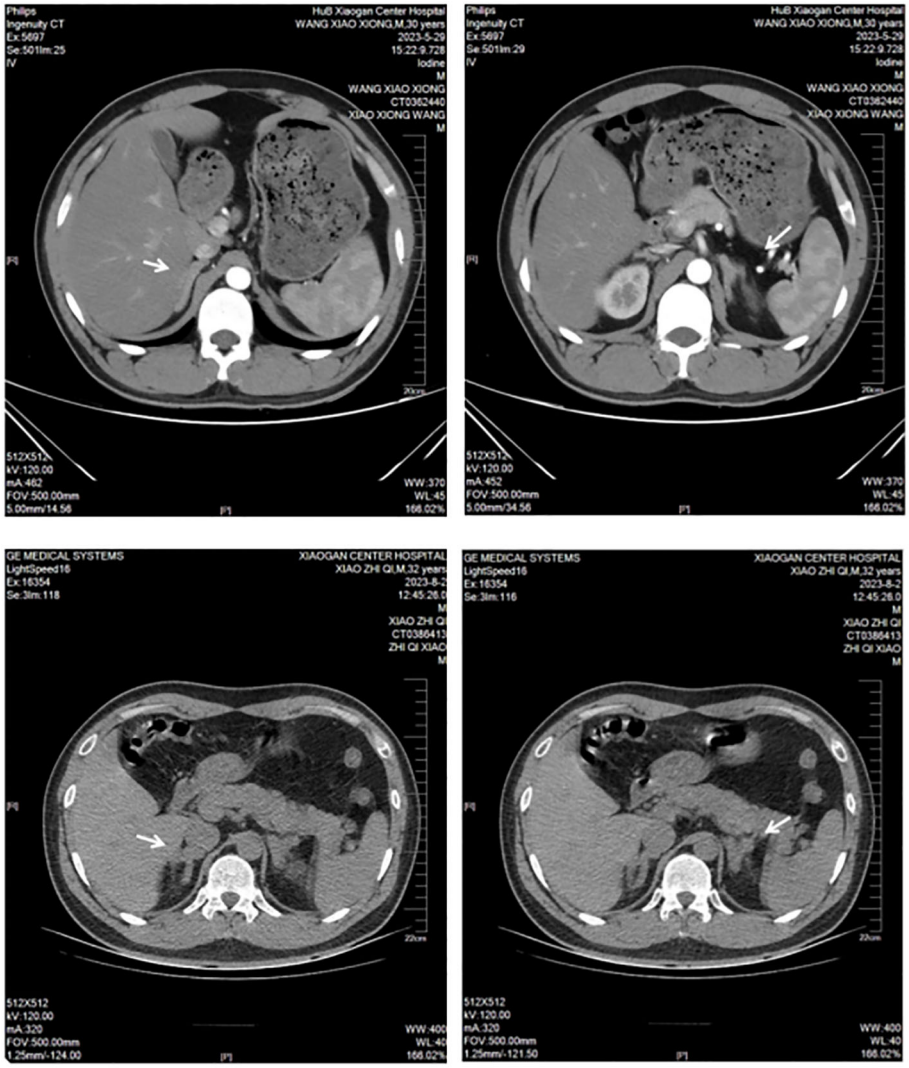

FIGURE 2
Adrenal CT images for Case 1 and Case 2.

Physical examination: body temperature, 36.5°C; pulse rate, 86 beats/min; respiratory rate, 19 breaths/min; blood pressure, 161/96 mmHg; and BMI, 24.45 kg/m^2. No significant abnormalities were found on cardiovascular, pulmonary, or abdominal examinations. Muscle strength was grade 2 for all limbs, with moderate muscle tone. The patient reported having no significant medical history and using electronic cigarettes containing etomidate for more than 2 months. Similarly, the patient's parents lack a history of smoking and display no symptoms indicative of hypokalemia.

Laboratory tests: blood potassium, 1.5 mmol/L (reference range: 3.5–5.3 mmol/L); 24-h urinary potassium excretion, 53.58 mmol/24 h (reference range: 25–100 mmol/24 h), 24-h urine volume, 2820 mL; cortisol levels: 8:00 AM 3.05 μg/dL (reference range: 6.2–19.4 μg/dL), 4:00 PM 6.65 μg/dL (reference range: 2.3–11.9 μg/dL), 12:00 AM 3.09 μg/dL (Figure 1); ACTH, 94 pg/mL (reference range: 6–48 pg/mL); aldosterone in supine position, 85.4 pg/mL (reference range: 60–360 pg/mL); renin activity in supine position, 0.38 ng/mL/h (reference range: 0.15–2.33 ng/mL/h); aldosterone in upright position, 63.39 pg/mL (reference range: 50–313 pg/mL); and renin activity in upright position, 0.38 ng/mL/h (reference range: 1.31–3.95 ng/mL/h). Endocrine examination revealed: estradiol, 48.60 pg/mL (reference range: 7.63–42.6 pg/mL); progesterone, 0.95 ng/mL (reference range: 0.05–0.149 ng/mL); and growth hormone, 138.40 pg/mL (reference range: 200–8000 pg/mL). Adrenal CT scan showed bilateral adrenal gland thickening (Figure 2). Routine blood tests, liver and kidney function tests, glucose tolerance test, thyroid function testing, and coagulation function testing showed no significant abnormalities. Thyroid ultrasound, cardiac ultrasound, and chest CT were normal.

At admission, his blood potassium level was 1.80 mmol/L. The patient immediately received intravenous infusion of 10% potassium chloride solution and oral administration of 20 mL of 10% potassium chloride solution. His blood potassium level did not return to normal readily, and urgent blood gas analysis showed a potassium ion level of 1.5 mmol/L and an actual bicarbonate level of 27.5 mmol/L. The patient was then treated with a central venous infusion of 9 g potassium chloride at a rate of 0.5 g/h. When rechecked, the patient's blood potassium level was 4.01 mmol/L, and muscle weakness gradually disappeared. The patient was later switched to

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oral sustained-release potassium chloride tablets (0.5 g), and his blood potassium level was maintained within the normal range. Before discharge, the patient had a blood potassium level of 4.56 mmol/L, an ACTH level of 53 pg/mL, and a 24-h urinary potassium excretion measurement of 53.58 mmol. His cortisol levels were as follows: 8:00 AM 10.47 μg/dL (reference range: 6.2–19.4 μg/dL), 4:00 PM 4.20 μg/dL (reference range: 2.3–11.9 μg/dL), and 12:00 AM 1.88 μg/dL.

In both reported cases, the patients presented with hypertension, hypokalemia, high ACTH, low cortisol, low renin, low aldosterone, and bilateral adrenal gland thickening (Table 1). Both patients also had a history of using electronic cigarettes containing etomidate. The patients were advised to quit smoking after discharge and to continue taking oral sustained-release potassium chloride tablets (0.5 g three times a day). Follow-up through telephone consultation confirmed no recurrence of hypokalemia symptoms after discharge.

Discussion

The use of etomidate as a γ-aminobutyric acid type A receptor agonist to induce anesthesia has the advantage of a minimal impact on the cardiovascular system. Etomidate typically does not induce significant hypotension during anesthesia induction at a dosage of 0.3 mg/kg. This stability is due to etomidate's minimal effect on sympathetic tone and its ability to preserve autonomic reflexes, including the baroreflex (2). The drug achieves this by acting as an agonist at α2-adrenoceptors, particularly the α2B subtype, which plays a key role in mediating peripheral vasoconstriction (3). Multiple studies have demonstrated that doses of etomidate used for anesthesia result in minimal changes in heart rate (less than 10%), while maintaining stable hemodynamic parameters such as central venous pressure, pulmonary artery pressure, cardiac index, and systemic vascular resistance (4). This favorable cardiovascular profile makes etomidate an optimal choice for induction of anesthesia in patients who are hemodynamically unstable or suffer from cardiac conditions. Significant adverse reactions to etomidate include postoperative nausea, vomiting, pain upon injection, muscle spasms, and involuntary muscle movements. It has been proposed that hemolysis could be a side effect associated with the presence of propylene glycol in etomidate formulations. In order to minimize these adverse effects, the World Health Organization (WHO) advises that the daily consumption of propylene glycol should not exceed 25 mg/kg (5).

Additionally, the discovery of adrenal toxicity has limited the widespread clinical use of etomidate, and it is currently only used for induction of transient anesthesia in hemodynamically unstable patients. Because etomidate is not classified as a controlled psychotropic or anesthetic drug, a certain risk of abuse exists. Recently, electronic cigarettes containing etomidate have emerged, and adverse reactions to these products have gradually gained widespread attention. Here we report the cases of two patients who after smoking electronic cigarettes containing "etomidate," experienced severe hypokalemia with varying degrees of muscle weakness and even respiratory distress. Although the patients' symptoms eventually resolved with potassium supplementation, the impact of these events should be considered.

A significant association of etomidate with adrenal insufficiency has been established. Etomidate specifically and reversibly blocks 11α-hydroxylation and 11β-hydroxylation in adrenal steroid synthesis, leading to decreases in the synthesis of cortisol, corticosterone, and aldosterone (6) (Figure 3). Cortisol is the most abundant endogenous glucocorticoid (7). Recent research demonstrated that within a certain dosage range, single dosing and/or continuous infusion of etomidate can reduce plasma cortisol levels, although the levels remain within the normal physiological range (8). Pre-administration of vitamin C before etomidate injection mitigates adrenal suppression and diminishes the extent of serum cortisol reduction attributed to etomidate. Beyond its

TABLE 1 Laboratory test results for Case 1 and Case 2.

Laboratory test	Case 1		Case 2		Reference range
	Hospitalized	Discharge	Hospitalized	Discharge	
Potassium ion, mmol/L	1.69	4.84	1.80	4.56	3.5–5.3
Cortisol, μg/dL					
8 AM	8.34	1.02	3.05	10.47	6.2–19.4
4 PM	1.04	2.23	6.65	4.20	2.3–11.9
12 AM	9.04	14.01	3.09	1.88	
Corticotrophin, pg/mL	52	5	94	53	6–48
Aldosterone, pg/mL					
Standing	5.00		63.39		50–313
Supine	5.01		85.40		60–360
Plasma renin activity, ng/ml/h					
Standing	0.64		0.38		1.31–3.95
Supine	0.56		0.38		0.15–2.33

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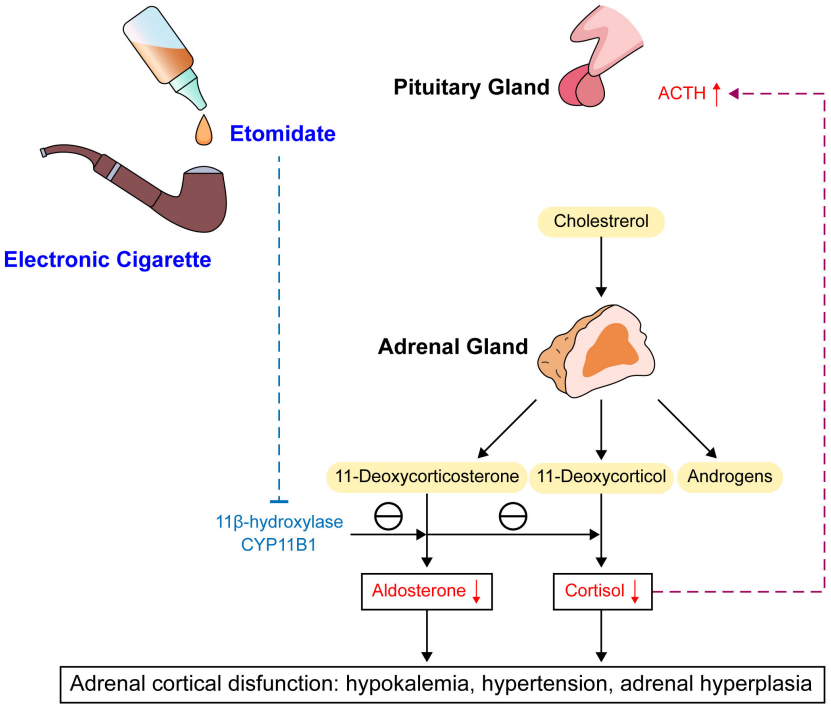

FIGURE 3
Mechanism and consequences of etomidate. ACTH, adrenocorticotrophic hormone; CYP11B1, Cytochrome p450 enzyme 11β-hydroxylas.

antioxidant properties, vitamin C additionally counteracts the suppressive impact of etomidate on adrenal gland functionality (9).

Additionally, adrenal cortex responsiveness to adrenocorticotropic hormone decreases. The reduction in cortisol level can provide feedback to stimulate the release of adrenocorticotropic hormone, causing adrenal hypertrophy and promoting synthesis of cortisol and corticosterone precursors. Through its effects on the activity of 11β-hydroxylase and 17α-hydroxylase, which result in decreased cortisol synthesis from 11-deoxycortisol and a decrease in aldosterone, etomidate can lead to temporary adrenal insufficiency. Notably, this effect of inhibiting adrenal cortex enzyme 11β-hydroxylase makes etomidate an effective acute treatment for severe Cushing's syndrome. In these patients, etomidate takes effect quickly and is the only steroidogenesis enzyme inhibitor that can be delivered parenterally, lowering the risk of liver damage compared to other drug treatments, such as ketoconazole (10).

Studies of the specific molecular mechanism of its effects indicate that etomidate, as an imidazole derivative, can inhibit cytochrome P450 enzyme 11β-hydroxylase (CYP11B1) in adrenal cortex cells, with the imidazole ring possibly being the main determinant of etomidate binding to CYP11B1. CYP11B1 is a key enzyme in glucocorticoid synthesis necessary for the conversion of 11-deoxycortisol to cortisol and 11-deoxycorticosterone to corticosterone (11). Animal experiments in crab-eating macaques showed that ketoconazole also lowers cortisol and corticosterone levels by inhibiting CYP11B1, with disproportionally greater increases in 11-deoxycortisol and 11-deoxycorticosterone compared with the decreases in cortisol and corticosterone. Long-term use of ketoconazole also was shown to increase pregnenolone and androstenedione production, possibly due to compensatory reactions to the decrease in cortisol (12). Etomidate and ketoconazole are both imidazole derivatives, which also explains the hormonal abnormalities observed in the present cases. Inhibition of CYP11B1 can increase the concentrations of precursor substances such as 11-deoxycorticosterone, 11-deoxycortisol, progesterone, and 17-hydroxyprogesterone (13). 11-Deoxycorticosterone is a precursor to aldosterone and has approximately 1/20 to 1/30 the mineralocorticoid activity, and an increase in 11-deoxycorticosterone can have effects such as sodium and water retention and increased potassium excretion. A study on adrenal functional tumors caused by non-aldosterone–dependent mineralocorticoid secretion found that such adrenal lesions can produce a large amount of 11-deoxycorticosterone, and the clinical manifestations are somewhat similar to those of primary aldosteronism, usually including hypertension and moderate to severe hypokalemia (14).

Regarding the timing of the effects of etomidate, a single dose of etomidate given before surgery usually results in a return to baseline levels of the inhibited enzymes by 24 hours after anesthesia induction, while continuous use leads to an inhibitory effect lasting longer than 24 hours, with possibly dose-dependency (15). Compared with propofol and dexmedetomidine, etomidate has a stronger adrenal suppression effect, and adrenal insufficiency can persist for a long time after discontinuation of continuous etomidate administration (16). Adrenal cortex dysfunction caused by inhibition of 11β-hydroxylase can manifest as low cortisol, high ACTH, low or normal aldosterone, high sex hormones, moderate to severe hypokalemia, hypertension, and compensatory adrenal

hyperplasia. This is consistent with the clinical characteristics observed in the two reported cases after smoking of electronic cigarettes containing "etomidate." Considering the known persistence of the inhibitory effect of etomidate with continuous use as well as the significant dose-dependency, long-term smoking of electronic cigarettes containing "etomidate" likely prolongs the inhibitory effect of the drug, allowing the development of severe hypokalemia, adrenal crisis, and a significantly increased risk of death. Thus, greater awareness of the risks of unregulated use of products containing etomidate is needed. For conditions involving cortisol excess though, the good therapeutic effects of etomidate warrant further investigation of its clinical value (17).

Conclusion

Our analysis of the two case reports revealed that both patients had been using electronic cigarettes containing etomidate for several months. Following this exposure, they presented with clinical and laboratory signs of hypokalemia, hypertension, elevated ACTH levels, and reduced renin and aldosterone levels. Imaging studies indicated adrenal gland thickening. Upon discharge and subsequent follow-up, it was noted that cessation of e-cigarette use led to the resolution of symptoms, including fatigue, abnormal sensations, and hypokalemia. This report describes the diagnosis and treatment processes for two cases of etomidate-related hypokalemia in adult male patients with a recent history of smoking electronic cigarettes containing "etomidate." The mechanisms of etomidate action were reviewed to better understand the development of etomidate-induced hypokalemia and adrenal insufficiency. Based on the presented cases and reviewed mechanistic information, healthcare providers should be aware of the possibility of that hypokalemia or symptoms of adrenal hyperplasia may be related to electronic cigarette use. Patients who present with these conditions and have a history of smoking should be asked about electronic cigarette use, and close monitoring of their blood potassium levels along with relevant examinations are needed to facilitate early treatment and improve their prognosis.

Author contributions

WW: Writing – review & editing. CX: Writing – review & editing. LG: Writing – review & editing. SL: Writing – review & editing. YY: Writing – review & editing.

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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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EDITED BY
Agnieszka Pazderska,
St. James's Hospital, Ireland

REVIEWED BY
Miles Douglas Thompson,
University Health Network (UHN), Canada
Kursad Unluhizarci,
Erciyes University, Türkiye

*CORRESPONDENCE
Thachanun Porntharukchareon
thachanun.por@cra.ac.th

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The existence of adrenal insufficiency in patients with COVID-19 pneumonia

Thachanun Porntharukchareon*, Bothamai Dechates, Supamas Sirisreetreerux, Phonthip Therawit and Kriangkrai Tawinprai

Department of Medicine, Chulabhorn Hospital, Chulabhorn Royal Academy, Bangkok, Thailand

Introduction: Infection with SARS-CoV-2 virus may result in long COVID, a syndrome characterized by symptoms such as dyspnea, cardiac abnormalities, cognitive impairment, and fatigue. One potential explanation for these symptoms is hypocortisolism.

Objective: To evaluate the prevalence of hypocortisolism in patients with a history of COVID-19 pneumonia.

Methods: Cross-sectional study of patients who were aged ≥18 years and had a 3-month history of radiography-confirmed COVID-19 pneumonia. Exclusion criteria included current or previous treatment with glucocorticoids and use of an oral contraceptive. Adrenal function was evaluated using a low dose (1ug) corticotropin stimulation test (CST). Serum cortisol levels were measured at 0, 30, and 60 minutes, and baseline plasma ACTH was also measured.

Results: Of the 41 patients enrolled, the median age was 62 years, 17 (42%) were female, and all 41 (100%) had severe pneumonia at baseline. Eleven patients (27%) had hypocortisolism, as evidenced by peak cortisol of less than 402.81 nmol/l after low dose (1 μg) CST. Of these 11 patients, 10 (91%) had secondary hypocortisolism (median ACTH 6.27 pmol/L, range 4.98–9.95 pmol/L) and one had primary hypocortisolism (mean ACTH 32.78 pmol/L). Six of the 11 patients with hypocortisolism (54.5%) reported symptoms of persistent fatigue and 5 (45.5%) required regular glucocorticoid replacement.

Conclusions: Our results suggest that hypocortisolism, predominantly caused by pituitary disruption, may emerge after SARS-CoV-2 infection and should be considered in patients with a history of COVID-19 pneumonia with or without clinical hypocortisolism.

KEYWORDS

adrenal insufficiency, long COVID syndrome, COVID-19 pneumonia, hypocortisolism, low dose synacthen test

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Highlights

We evaluated the low dose corticotropin stimulation test in patients with history of COVID-19 pneumonia. Our study suggested that participants with history of COVID-19 pneumonia may have adrenal insufficiency predominantly by pituitary disruption. Physicians should be aware of the hypocortisolism in these patients who present with clinical symptoms such as shock, nausea, vomiting, and fatigue.

Introduction

Coronavirus disease 2019 (COVID-19), caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), was first identified in Wuhan, China, on December 31, 2019. Since then, the disease has evolved into a global pandemic, fueled by the emergence of multiple SARS-CoV-2 variants. To date, more than 600 million confirmed cases of COVID-19 have been reported worldwide (1). The disease is primarily known for its respiratory symptoms, which range from mild cough to severe acute respiratory distress syndrome (ARDS). However, COVID-19 has also been linked to a variety of multisystemic manifestations, including cardiovascular complications, neurological sequelae, and endocrine dysfunction. In addition, many individuals with COVID-19 experience persistent symptoms, known as "long COVID," which includes fatigue, headache, dyspnea, and brain fog lasting more than 12 weeks after the initial onset of the illness. Long COVID can involve several organ systems, including the cardiovascular, neurological, gastrointestinal, and endocrine systems (2, 3).

Among the endocrine disorders potentially associated with COVID-19, adrenal insufficiency has gained increasing attention. Adrenal insufficiency can occur in various clinical contexts, including primary adrenal failure (Addison's disease) and secondary adrenal insufficiency due to hypothalamic-pituitary disorders. The possible link between COVID-19 and adrenal insufficiency is multifaceted. First, the direct cytopathic effects of SARS-CoV-2 on the adrenal glands are a consideration, given the presence of angiotensin-converting enzyme 2 (ACE2) receptors (4), which are the cellular entry points for SARS-CoV-2, in adrenal cortical cells. Second, the systemic inflammatory response triggered by COVID-19, characterized by a cytokine storm and immune dysregulation, may lead to adrenal gland dysfunction through various pathways, including direct tissue damage, disruption of the hypothalamic-pituitary-adrenal (HPA) axis feedback mechanisms, and impairment of adrenal steroidogenesis (5).

During the severe acute respiratory syndrome (SARS) pandemic in 2002–2004, Leow et al. (6) evaluated the function of the hypothalamic–pituitary axis in 61 patients with SARS infection and found evidence of hypocortisolism in 40% of the cohort. The proposed mechanisms included reversible hypophysitis or direct hypothalamic damage caused by the virus. However, data on the prevalence and causes of adrenal insufficiency following SARS-CoV-2 infection are currently limited. Consequently, this study aims to evaluate the prevalence of adrenal insufficiency in adult patients who were discharged from the hospital after being diagnosed with COVID-19 pneumonia and had a 3-month history of COVID-19.

Materials and methods

Study design

This was a cross-sectional study that enrolled consecutive patients with a history of COVID-19 pneumonia of 3 months duration at Chulabhorn Hospital, Chulabhorn Royal Academy, Thailand. The study was approved by the Human Research Ethics Committee Chulabhorn Research (Project code 140/2564) and was conducted in accordance with the Declaration of Helsinki and its amendments. All participants provided written informed consent prior to inclusion. The study was registered at the Thai Clinical Trials Registry (TCTR20220606002).

Participants

Participants were recruited from a COVID-19 cohort ward and acute respiratory unit clinic at Chulabhorn Hospital between March 1, 2022 and April 20, 2022. Eligible patients had a 3-month history of COVID-19 pneumonia and were aged ≥18 years. A diagnosis of COVID-19 was confirmed by real-time reverse-transcriptase polymerase chain reaction of SARS-CoV-2 RNA in nasopharyngeal swab specimens. The severity of COVID-19 was defined as critical, severe, and non-severe according to the World Health Organization (WHO) criteria (7). Critical COVID-19 was defined by as acute respiratory distress syndrome, sepsis shock, sepsis, or the need for life-sustaining therapies such as mechanical ventilation or vasopressor therapy. Severe COVID-19 was defined as one or more of (a) oxygen saturation <90% on room air, (b) signs of pneumonia, or (c) signs of severe respiratory distress. Non-severe COVID-19 was defined as the absence of any criteria for severe or critical COVID-19. Pneumonia was confirmed by imaging with either a chest radiograph or computed tomography (CT) of the chest. Among the exclusion criteria were comorbidity with pituitary disease or adrenal disease; unstable clinical condition; chronic kidney disease (creatinine >1.5 mg/dL); severe hepatitis (alanine aminotransferase >1.5× upper limit of normal); pregnancy or plans to become pregnant; and concurrent use of oral contraceptive pills, steroids (oral, inhaled, topical, or intra-articular), and other medications known to affect cortisol-binding globulin (including oral estrogens). Suitable subjects identified from a review of case notes were contacted in person or via the telephone.

Image analysis

COVID-19 pneumonia was confirmed by chest radiography or CT scan. CT scans were performed on the first and second day after COVID-19 diagnosis. The severity of pneumonia on CT was scored according to the COVID-19 Reporting And Data System (CO-RADS) classification using lobar-based assessment. In brief, each of the five lung lobes was subjectively scored from 0 to 5 (0, no involvement; 1, <5% involvement; 2, 6–25% involvement; 3, 26–50% involvement; 4, 51–75% involvement; 5, ≥76% involvement). The total score was the sum of the individual lobar scores and ranged from a minimum of 0 to a maximum of 25. Total scores of <7, 8–17,

and 18–25 were classified as mild, moderate, and severe pneumonia, respectively (8).

Study protocol

Blood samples were collected at baseline for measurement of plasma ACTH and serum cortisol, free thyroxine (FT4), thyroid-stimulating hormone (TSH), anti-thyroglobulin (anti-Tg), and anti-thyroid peroxidase (anti-TPO). Corticotropin stimulation test (CST) were performed as described below. Participants with hypocortisolism were evaluated for potential causes other than SARS-CoV-2 infection by magnetic resonance imaging (MRI) of the pituitary gland or an adrenal CT protocol as appropriate.

Corticotropin stimulation test

Low-dose (1 μg) CST were performed between 8 AM and 10 AM. An intravenous catheter was inserted into an antecubital vein and 0.4 mL (1 μg) of a synthetic ACTH 1–24 solution (Synacthen®, Novartis, Chippenham, UK) in 0.9% sodium chloride was administered through the catheter, followed by flushing with 15 mL of 0.9% sodium chloride. Blood samples were collected immediately before Synacthen injection to determine baseline ACTH and cortisol, and at 30 min and 60 min after Synacthen injection to determine the cortisol response to stimulation. A diagnosis of hypocortisolism was defined as a peak cortisol level of <402.81 nmol/L based on the new criteria for the Roche Elecsys Cortisol Generation II assay (Roche Diagnostic, Mannheim, Germany) (9, 10). Primary hypocortisolism was defined as a baseline plasma ACTH level >2× the upper limit of the reference range (11).

Laboratory investigations

Serum cortisol and plasma ACTH were measured using automated electrochemiluminescence immunoassays (Roche Elecsys Cortisol Generation II assay and Roche Elecsys ACTH assay; Roche Diagnostic, Mannheim, Germany). For plasma ACTH measurement, blood was collected in EDTA tubes and immediately transferred to the laboratory. FT4, TSH, anti-Tg, and anti-TPO were also measured using automated electrochemiluminescence immunoassays on the Roche Elecsys system. Reference ranges were 2.86–13.86 pmol/L for plasma ACTH, 11.97–21.88 pmol/L for FT4, and 0.27–4.2 mIU/L for TSH.

Statistical analysis

The sample size calculation was based on an infinite population proportion formula (12) and previous analysis of the prevalence of hypocortisolism in SARS-CoV-2 patients (6). Data analysis was conducted using STATA/SE version 16.1 (StataCorp LP, College Station, TX, USA). Continuous data are presented as mean ± standard deviation (SD) or the median with interquartile range (IQR) as appropriate. Normally distributed continuous data were compared using a Student's t-test for two groups or by one-way analysis of variance for three or more groups. Non-normally distributed continuous data were compared using the Mann–Whitney U test for two groups or the Kruskal–Wallis test with Dunn's *post hoc* test for three or more groups. Categorical data were compared using a Chi-squared test. An analysis of weight change was conducted using analysis of covariance (ANCOVA) to compare participants with hypocortisolism to those without hypocortisolism at both baseline and the three-month follow-up. The relationship between two continuous variables was determined using Pearson's correlation. The α level for statistical significance was set at 0.05.

Results

Participants

Medical records of 2719 patients seen at our hospital between March 1, 2022 and April 20, 2022 were reviewed. A total of 2463 patients were excluded (182 patients <18 years of age, 2 patients were deceased, and 2279 patients had no documentation of pneumonia by radiography). The remaining 256 participants were contacted in person or by telephone and 215 declined to participate. Finally, a total of 41 patients were enrolled in the study (Figure 1).

The study cohort comprised 41 patients, with a mean (SD) age of 57.1 (13.7) years. Among them, 17 (41%) were female. All of the patients had severe COVID-19 pneumonia at baseline based on the WHO criteria (6). Thirty-seven patients underwent chest CT; based on the CO-RADS system of pneumonia classification (7), 36 of the 37 patients (87.8%) had mild pneumonia and 1 patient (2.4%) had moderate pneumonia. None of the patients developed acute respiratory failure. Most patients were obese (29/41; 70.7%) and the mean body mass index (BMI) of the full cohort was 28.85 kg/m^2. Fifteen patients (36.7%) received dexamethasone treatment for COVID-19. Of the fifteen COVID-19 patients who received glucocorticoid treatment, fourteen were administered dexamethasone at a dosage of 6 milligrams per day for a duration of 10 days. One patient received dexamethasone at a dosage of 18 milligrams per day for 5 days, followed by 6 milligrams per day for an additional 5 days. Other baseline characteristics of the study participants are shown in Table 1.

Eleven (27%) of the 41 participants showed evidence of hypocortisolism in the CST (Figure 2). The mean baseline cortisol level was 198.92 ± 83.87 nmol/L, and 3 patients had baseline cortisol levels <137.95 nmol/L. Of the 11 patients with hypocortisolism, 10 (90.9%) were diagnosed with central hypocortisolism based on low-to-normal ACTH levels (median ACTH 6.27 pmol/L, IQR 4.98–9.95). The remaining patient had a plasma ACTH level of 32.78 pmol/L and was diagnosed with primary hypocortisolism. Six of the 11 patients (54.50%) reported persistent fatigue after resolution of the acute infection. Participants with hypocortisolism had nonsignificantly greater reduction in body weight compared to those without hypocortisolism (-2.00 kg, 95% CI -4.42 to 0.42 vs.

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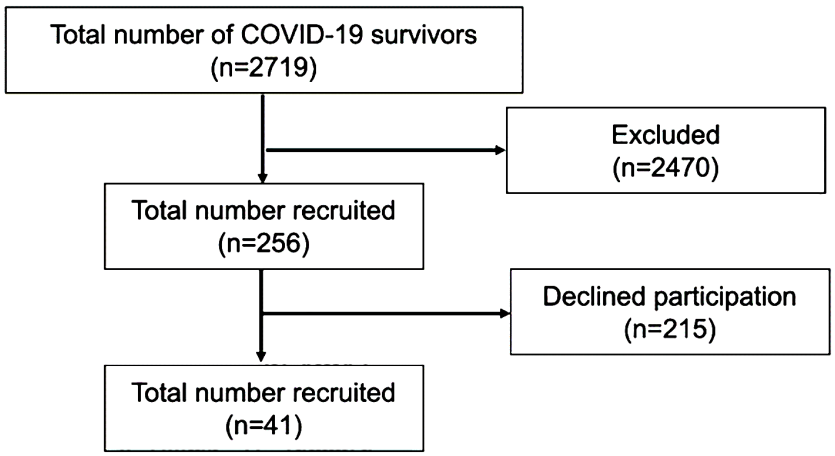

FIGURE 1
Study participation.

TABLE 1 Baseline characteristics of the 41 study participants.

Baseline characteristics	
Sex (female) (number, %)	17 (41.5%)
Age (years) (mean and SD)	57.1 (13.7)
BMI (kg/m^2) (mean and SD)	29.4 (6.4)
Obesity (number, %)	29 (70.7%)
Diabetes mellitus (number, %)	13 (31.7%)
Hypertension (number, %)	23 (56.1%)
Cardiovascular disease (number, %)	1 (2.4%)
COVID-19 severity (WHO category) (number, %)	
Critical	0
Severe	41 (100%)
Non-severe	0
Symptoms (number, %)	
Fever	14 (34.1%)
Upper respiratory tract involvement	34 (82.9%)
Diarrhea	7 (17.1%)
Dyspnea	3 (7.3%)
Fatigue	8 (19.5%)
Rash	2 (4.9%)
Treatment (number, %)	
Dexamethasone	15 (36.6%)
Favipiravir	38 (92.7%)
Sotrovimab	3 (7.3%)
Convalescent plasma	6 (14.6%)

BMI, body mass index; CRP, C-reactive protein; IQR, interquartile range; WHO, World Health Organization.

0.21 kg, 95% CI -0.37 to 0.78, p = 0.079). Only 5 of the 11 patients received corticosteroid therapy for the treatment of COVID-19 (Table 2). Six of the 10 patients with secondary hypocortisolism underwent MRI of the pituitary and no abnormal lesions were found. Four patients did not perform MRI imaging due to personal reason. The patient with primary hypocortisolism underwent an adrenal CT protocol and had normal bilateral adrenal glands.

In addition to the varied cut-off levels for hypocortisolism, we implemented a more stringent cut-off value of 345 nmol/L for the low-dose synacthen test to ensure robustness in our analysis. Despite this stricter criterion, three patients (7%) still exhibited hypocortisolism. Detailed characteristics and laboratory test results for patients numbered 2, 3, and 5 are provided in Table 2.

Two of the 41 patients (5%) had abnormal thyroid function tests; one had iatrogenic thyrotoxicosis, and positive autoimmune thyroid antibodies. She was being treated with levothyroxine suppressive therapy for papillary thyroid cancer, and the second had subclinical hypothyroidism (thyroid-stimulating hormone 5.4 μIU/mL). Five patients (12%) were positive for anti-thyroid peroxidase or anti-thyroglobulin antibodies with normal thyroid function test. Further clinicopathological details of the study participants is provided in Supplementary Table 1

There is no statistical significance, but a trend towards more fatigue symptoms in participants with adrenal insufficiency compared to those without hypocortisolism. The odds ratio for fatigue was 3.19 (95% CI, 0.70 to 14.56), with a p-value of 0.135 in participants with hypocortisolism (Table 3). Furthermore, none of the participants reported symptoms of brain fog, headache, memory problems, or muscle pain. We also performed logistic regression analysis to determine the risk factors associated with hypocortisolism in the subsets of patients with (n = 11) or without (n = 30) hypocortisolism. Increased BMI (adjusted P = 0.019) was the only significant risk factor for hypocortisolism. Age, sex, treatment modality, glucocorticoid usage, history of COVID-19 vaccination, and disease severity were not associated with hypocortisolism (Table 3).

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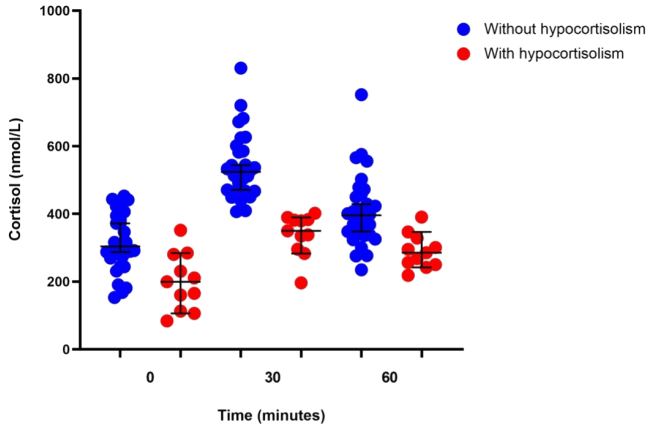

FIGURE 2
Corticotropin stimulation test results in patients with (n = 11) or without (n = 30) hypocortisolism.

Discussion

The results of our study suggest that hypocortisolism is a common complication in patients with COVID-19 pneumonia, with most cases in our cohort (10 of 11 patients) manifesting as central hypocortisolism. Additionally, a significant proportion of these patients in (55%) reported clinical symptoms of long COVID, including fatigue, insomnia, and dyspnea.

The underlying mechanism of hypocortisolism in COVID-19 patients is likely to involve the ACE2 receptor, the major functional receptor for infection by both SARS-CoV and SARS-CoV-2. Indeed, ACE2 has been detected in many human tissues, including endocrine glands such as the adrenal and pituitary glands (13–16). ACE2 receptors mediate viral entry in concert with S glycoprotein priming by the host cell transmembrane serine protease 2 (13, 14).

Many studies have reported cases of pituitary disruption after SARS-CoV-2 infection, including central hypocortisolism, central diabetes insipidus, hypothalamic hypogonadism, lymphocytic hypophysitis, and pituitary apoplexy. Primary hypocortisolism has also been reported in COVID-19 patients. Details of previously reported cases of pituitary dysfunction and primary hypocortisolism occurring more than 2 weeks after SARS-CoV-2 infection are shown in Table 4 (17–23, 25, 27, 28). The findings from those studies are consistent with our own results and support the conclusion that pituitary and adrenal function may be affected by SARS-CoV-2 infection.

TABLE 2 Clinical characteristics and laboratory values of patients with hypocortisolism (n=11).

Patient ID	Age (years)	Sex	CT score[a]	Treatment	Cumulative dose of Dex (mg)	Persistent fatigue	Cortisol level in CST (nmol/L)			ACTH (pmol/L)
							Baseline	30 min	60 min	
No. 1	73	M	2	Fa, C	None	Yes	199.5	383.5	256.6	12.80
No. 2[b]	29	M	2	Fa, C	None	No	210.8	295.8	218.8	19.07
No. 3[b]	33	M	NA	Fa	None	Yes	106.5	196.7	328.6	6.25
No. 4	63	M	4	S	None	No	284.5	389.8	268.2	32.78
No. 5[b]	51	M	NA	Fa	None	No	230.9	334.9	300.7	6.29
No. 6	46	F	1	Fa, Dex	60	Yes	351.8	381.6	295.2	6.05
No. 7	67	F	2	Fa, Dex	60	Yes	280.6	283.1	390.7	3.70
No. 8	21	M	2	Fa, C	None	Yes	84.1	402.0	250.2	5.41
No. 9	34	F	NA	Fa, Dex	60	No	165.3	379.9	285.3	9.00
No. 10	67	F	1	Fa, Dex	60	No	160.6	350.1	242.2	6.64
No. 11	71	F	6	Fa, Dex	60	Yes	112.8	338.3	346.8	2.82

ACTH, adrenocorticotropic hormone; C, convalescent plasma; CST, corticotropin stimulation test; CT, computer tomography; Dex, dexamethasone; F, female; Fa, favipiravir; ID, identification number; mg, milligrams; M, male; NA, not available; No, number; S, sotrovimab.
[a]CO-RADS classification.
[b]Patients who still had hypocortisolism when the cut-off for low dose corticotropin stimulation test was 345 nmol/L.

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TABLE 3 Evaluation of risk factors for hypocortisolism by logistic regression.

Characteristic	Patients with hypocortisolism (n=11)	Patients without hypocortisolism (n=30)	Crude OR (95% CI)	P value	Adjusted OR (95% CI)	P value
Sex, female	5 (45.5%)	12 (40.0%)	1.25 (0.31–5.04)	0.754		
Age, years (IQR)	51 (33–67)	62 (59–65)	0.95 (0.91–1.00)	0.067	1.04 (0.96–1.12)	0.380
BMI, kg/m^2 (IQR)	34.96 (29.86–37.52)	26.3 (24.61–30.0)	1.23 (1.05–1.43)	**0.009**	1.27 (1.04–1.54)	**0.019**
Comorbidities						
Obesity	10 (90.9%)	19 (63.3%)	5.79 (0.65 –51.50)	0.115		
Diabetes mellitus	5 (45.5%)	8 (26.7%)	2.29 (0.54–9.64)	0.258		
Hypertension	3 (27.3%)	20 (67.7%)	0.19 (0.04–0.86)	**0.032**	0.17 (0.02–1.23)	0.079
CT score (CO-RADS)						
<7	7 (72.7%)	28 (96.6%)	4.00 (0.22–72.18)	0.348		
8–17	1 (12.5%)	1 (3.4%)				
≥18	0	0				
Laboratory investigations						
SARS-CoV-2 antibody (mean ± SD BAU/mL) [a]	5475.57 ± 4646.10	17425.10 ± 7900.03	0.99 (0.99–1.00)	0.257		
Serum CRP (mg/L)	6.09 (3.33–12.83)	9.68 (4.60–12.76)	1.02 (0.98–1.05)	0.426		
Persistent fatigue	6 (60.0%)	8 (32.0%)	3.19 (0.70–14.56)	0.135		
Treatment						
Dexamethasone	5 (45.5%)	10 (33.3%)	1.67 (0.41–6.82)	0.477		
Favipiravir	10 (90.9%)	28 (93.3%)	0.71 (0.06–8.76)	0.792		
Sotrovimab	1 (9.1%)	2 (6.7%)	1.40 (0.11–17.17)	0.792		
Convalescent plasma	3 (27.3%)	3 (10.0%)	3.38 (0.57–20.10)	0.181		
COVID–19 vaccine[b]						
CoronaVac	2 (20.0%)	7 (23.3)	0.82 (0.14–4.80)	0.827		
BBIBP-CorV	2 (20.0%)	4 (13.3%)	1.63 (0.25–10.58)	0.611		
ChAdOx1-S	8 (80.0%)	24 (80.0%)	1.00 (0.17–5.98)	1.000		
BNT162b2	5 (50.0%)	22 (73.3%)	0.36 (0.08–1.60)	0.180		
mRNA-1273	2 (20.0%)	2 (6.7%)	3.50 (0.42–28.91	0.245		

BAU, binding antibody unit; BMI, body mass index; CI, confidence intervals; CRP, C-reactive protein; CO-RADS, COVID-19 Reporting And Data System; CT, computed tomography; IQR, interquartile range; OR, odds ratio; SD, standard deviation.
[a]Based on the WHO international standard for anti-SARS-CoV-2 Ig. One Elecsys-S unit = 0.972 × binding antibody unit.
[b]10 of the 11 patients with hypocortisolism had documentation of COVID-19 vaccination.
The values in bold denote statistical significance at $P < 0.05$.

In the present study, high BMI patients was significantly associated with an increased risk of hypocortisolism. This could be explained by high ACE2 expression in adipose tissue resulting in an increased viral burden, thereby increasing virus-associated damage to the endocrine glands (16). Other potential mechanisms are an increased proinflammatory phenotype associated with metabolic dysfunction and dysregulation of the renin–angiotensin pathway (29). Obesity is also an important risk factor for increased severity of COVID-19.

In our study, most of the patients with hypocortisolism exhibited non-critical COVID-19 disease severity. During the 6-month follow-up period, one patient received a daily dose of prednisolone at 5 mg, while the remaining patients received corticosteroids as needed, primarily during illness episodes.

Our study results differ from those of Clarke et al. (30) who previously found no evidence of hypocortisolism in patients with COVID-19 (30). However, the two studies differed in that we performed the CST using a low dose (1 μg) of CST whereas

TABLE 4 Literature cases of hypothalamic–pituitary dysfunction and primary adrenal insufficiency occurring more than 2 weeks after SARS-CoV-2 infection.

Study (ref. no.)	Patient age/sex	Time to onset after infection	Presentation	Results of investigations	Diagnosis
Secondary adrenal insufficiency					
Kenya et al. (17)	23/F	1 month	Fatigue, nausea, vomiting	• Basal cortisol 226.24 nmol/L; ACTH 1.08 pmol/L • hypocortisolism was confirmed with insulin tolerance test. • MRI pituitary: normal.	Secondary adrenal insufficiency
Central diabetes insipidus					
Sheikh et al. (18)	28/M	1 month	Polyuria, polydipsia, increased thirst	• 24-hr urine volume 7 L. • Serum sodium 153 mmol/L; paired serum and urine osmolality 300 and 93 mOsm/kg, respectively; urine sodium 16 mOsm/kg. • MRI brain: normal.	DI with concomitant myocarditis
Yavari et al. (19)	54/F	6 weeks	Thirst, polyuria, polydipsia	• 24-hr urine volume 13.3 L. • Serum sodium 144 mmol/L; paired serum and urine osmolality 298 and 164 mOsm/kg, respectively. • Urine osmolality 810 mOm/kg after intravenous desmopressin administration test. • MRI pituitary: normal.	CDI
Misgar et al. (20)	60/F	8 weeks	Polyuria	• 24-hr urinary volume 6 L. • Serum sodium 152 mmol/L; paired serum and urine osmolality 300 and 177 mOsm/kg, respectively. • MRI pituitary: enlarged pituitary with absent posterior pituitary bright spot on T1-weighted images; thickening of pituitary stalk.	CDI
Pituitary apoplexy					
Liew et al. (21)	75/M	1 month	Sudden onset severe frontal headache	• FT4 6.9 pmol/L (reference range: 10.5–24.5), TSH 0.1 mU/L (0.27–4.2), cortisol 57 nmol/L (133–537), testosterone <0.5 nmol/L (6.7–25.7), LH <1.0 U/L (1.7–8.6). • MRI: pituitary macroadenoma with recent hemorrhage.	Pituitary apoplexy with hypopituitarism
Hypothalamic hypogonadism					
Soejima et al. (22)	36/M	99 days	Insomnia, headache, dysgeusia, alopecia	• Free testosterone 19.09 pmol/L (22.56–61.42), FSH 4.2 IU/L (1.3–17), LH 3.0 IU/L (0.52–7.8). • MRI pituitary: partially empty sella.	Hypothalamic hypogonadism
Facondo et al. (17)	36/F	6 months	Secondary amenorrhea	• Estradiol <91.77 pmol/L (91.77–921.42), FSH 3.85 IU/L (3.0–8.0), LH 0.29 IU/L (1.8–11.78), TSH 1.71 mIU/L (0.27–4.2). • GnRH analog test: normal response. • TRH test: delayed response • MRI brain and pituitary: uncertain pituitary microadenoma 3 mm.	Hypothalamic amenorrhea
Lymphocytic hypophysitis					
Joshi et al. (23)	18/F	3 weeks	Acute onset headache	• Hormonal workup: within normal limits. • MRI brain: diffuse thickening and enlargement of the infundibulum with homogenous contrast enhancement.	Lymphocytic hypophysitis
Gorbova et al. (24)[a]	35/F	2 months	Symptoms of hypopituitarism	• Hormonal workup: hypothyroidism, hypocorticism, hypogonadism. • MRI: hypophysitis.	Hypophysitis and reversible hypopituitarism
Primary adrenal insufficiency					
Eskandari et al. (25)	18/M	2 weeks	Severe weakness, acute chest pain, hypotension	• Serum sodium 129 mmol/L; 8 AM cortisol 38.63 nmol/L; ACTH >396 pmol/L. • Cortisol levels at baseline and 60 min after 250 μg ACTH stimulation test were 49.67 and 281.42 nmol/L,	Autoimmune PAI and myocarditis

(Continued)

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TABLE 4 Continued

Study (ref. no.)	Patient age/sex	Time to onset after infection	Presentation	Results of investigations	Diagnosis
Primary adrenal insufficiency					
				respectively. • Anti-21-hydroxylase antibody: positive.	
Machado et al. (26)	46/F	3 weeks	Malaise, nausea, vomiting, hyperpigmentation, postural hypotension	• CT abdomen: adrenal infarction.	PAI with bilateral adrenal infarction
Sánchez et al. (27)	65/F	5 months	Abdominal pain nausea, vomiting, weight loss	• Serum sodium 117 mmol/L • Cortisol at baseline 71.73 nmol/L; ACTH at baseline 427.68 pmol/L • Cortisol at baseline, 30, and 60 min in 250 μg ACTH stimulation test were 63.46, 80.01, and 71.73 nmol/L, respectively • Anti-21-hydroxylase antibody: present. • CT abdomen: unremarkable.	Autoimmune PAI

ACTH, adrenocorticotropic hormone; CDI, central diabetes insipidus; CT, computer tomography; DI, diabetes insipidus; F, female; FSH, follicle-stimulating hormone; FT4, Free thyroxine; GnRH, gonadotropin-releasing hormone; hr, hour(s); LH, luteinizing hormone; M, male; MRI, magnetic resonance imaging; min, minutes; no., number; PAI, primary adrenal insufficiency; TRH, thyrotropin-stimulating hormone; ref, reference; TSH, thyroid-stimulating hormone.
[a]Only the abstract was available in English.

Clarke et al. used the standard CST (250 μg ACTH), which may have failed to detect patients with mild or early onset hypocortisolism (31). The results of our study are similar to those of Urhan et al. (32) except that the frequency of patients with hypocortisolism was higher in our study (27% vs 16.2%). This may be due to differences between the two studies in the severity and duration of COVID-19, especially because all of our study participants had severe COVID-19 pneumonia. Another difference between the two studies was that we evaluated adrenal function within 3 months of SARS-CoV-2 infection compared with the study of patients at 3–7 months post-infection by Urhan et al. (32).

A strength of our study lies in the utilization of a low cut-off value for serum cortisol (402.8 nmol/L) to define hypocortisolism, a measure that likely contributed to a decreased false-positive rate. Traditionally, the practical cut-off values for diagnosing adrenal insufficiency following standard and low-dose CST has been set at 500 nmol/L and has been widely accepted for an extended period (11, 33, 34). In our study, we adopted a similar approach by evaluating adrenal function using a low-dose CST, a strategy aimed at mitigating false-negative results. However, recent investigations, such as those conducted by Javorsky BR et al. utilizing the Elecsys® Cortisol II assay, have identified a lower cut-off value of 402.8 nmol/L for diagnosing hypocortisolism following a standard dose CST (9). Furthermore, we conducted imaging studies to explore other potential etiologies of adrenal insufficiency in the majority of the 11 study participants diagnosed with hypocortisolism.

However, it is important to acknowledge certain limitations in our study. A significant limitation is the absence of a widely accepted standard cut-off value for the low-dose CST specifically for the Roche Elecsys Cortisol Generation II assay. Although we used a lower cut-off value than previously established, the cut-off value for low-dose CST should ideally be lower than that for standard CST. Accurate diagnosis of hypocortisolism relies heavily on precise cortisol cut-off values, which are inherently assay and protocol-dependent. In our study, we set the peak cortisol level cut-off at 30 or 60 minutes of the low-dose CST at 402.8 nmol/L, based on recommendations from Javorsky BR et al. and Mongioi et al. (9, 10). However, it is worth noting that this approach may potentially lead to an overestimation of hypocortisolism diagnoses.

To address this concern, we explored alternative cut-off values suggested by Karaca Z et al. (35), which utilized different assays but employed low-dose CST. This study measured serum cortisol levels by radioimmunoassay (RIA) method and established a cut-off value of 345 nmol/L. Reassessment of our data using this cut-off revealed that three out of 41 participants (7%) remained diagnosed with hypercortisolism. Notably, all three of these participants did not receive corticosteroid treatment during COVID-19 management. As a result, eight participants exhibited cortisol levels falling within a grey zone for the diagnosis of hypocortisolism.

Due to the absence of a defined standard cut-off value for the low-dose CST by the Elecsys® Cortisol II assay, it is conceivable that some of these participants may indeed suffer from hypocortisolism. This result underscores the urgency of determining the appropriate cut-off value for the low-dose CST using the Elecsys® Cortisol II assay. A higher cut-off value provides more sensitivity but lower specificity, and vice versa. Therefore, further studies are urgently needed to establish an optimal cut-off value that balances sensitivity and specificity for accurate diagnosis of hypocortisolism. In addition, the sample size was small, and the study was conducted at a single center, both of which might impede the generalizability of our findings. Four participants with secondary adrenal insufficiency

did not undergo MRI of the pituitary gland, which could have identified potential pituitary abnormalities. This limitation was due to the participants' decision to decline the procedure when invited. Another limitation involves the potential for selection bias because participants may have been more inclined to join the study based on their concern about their post-COVID condition or the presence of symptoms resembling adrenal insufficiency. We made efforts to minimize this bias through formal consecutive invitations to all eligible patients, but it may still exist. Furthermore, there was no assessment of baseline pituitary (ACTH)-adrenal (cortisol) function in participants before their COVID-19 diagnosis or hospitalization. Determining whether participants had impaired function is challenging, especially during the critical phase of severe COVID-19 at presentation, which may lead to false-negative test outcomes. However, we addressed this limitation by excluding patients with a history of pituitary or adrenal disease and those concurrently using medications known to affect pituitary-adrenal function. Additionally, all study participants had severe COVID-19 pneumonia, and the results may not accurately represent patients with milder forms of the disease or critically ill patients.

Overall, the results of this study highlight the importance of monitoring for endocrine complications in patients with a history of SARS-CoV-2 infection. Further research will be needed to fully understand the underlying mechanisms of hypocortisolism following SARS-CoV-2 infection, and additional studies with larger sample sizes are needed to confirm the findings of this study and to better understand the prevalence and mechanisms of hypocortisolism in patients with COVID-19 pneumonia.

We conclude that patients with a history of COVID-19 pneumonia who present with clinical symptoms such as shock, nausea, vomiting, and fatigue should have hypocortisolism included in the differential diagnosis. These patients should also be followed over the long term to understand the persistence of adrenal insufficiency and its recovery rate. Finally, all patients who suffer from long COVID syndrome might benefit from an analysis of hypothalamic–pituitary axis function.

Data availability statement

The original contributions presented in the study are included in the article/Supplementary Materials, further inquiries can be directed to the corresponding author/s.

Ethics statement

The studies involving humans were approved by Human Research Ethics Committee Chulabhorn Research Institute. The studies were conducted in accordance with the local legislation and institutional requirements. The participants provided their written informed consent to participate in this study.

Author contributions

TP: Conceptualization, Investigation, Methodology, Writing – original draft, Writing – review & editing. BD: Investigation, Writing – original draft. SS: Investigation, Writing – review & editing. PT: Investigation, Writing – review & editing. KT: Conceptualization, Writing – review & editing.

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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.2024.1337652/full#supplementary-material

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EDITED BY
Henrik Falhammar,
Karolinska Institutet (KI), Sweden

REVIEWED BY
Sylvère Störmann,
LMU Munich University Hospital, Germany
Yuichi Yoshida,
Oita University, Japan

*CORRESPONDENCE
Joanna Sobolewska
joanna.sobolewska@wum.edu.pl

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Critical illness-related corticosteroid insufficiency (CIRCI) - an overview of pathogenesis, clinical presentation and management

Joanna Sobolewska*, Lukasz Dzialach, Pawel Kuca and Przemyslaw Witek

Department of Internal Medicine, Endocrinology and Diabetes, Medical University of Warsaw, Warsaw, Poland

According to the Society of Critical Care Medicine, critical illness-related corticosteroid insufficiency (CIRCI) characterizes hypothalamic-adrenal axis insufficiency following acute medical conditions of various causes, i.e., sepsis, septic shock, acute respiratory distress syndrome, community-acquired pneumonia, and status after major surgical procedures. Due to highly variable etiology, understanding the pathomechanism and management of CIRCI assumes relevance for all centers providing intensive care. During CIRCI, multiple peripheral adaptations develop, and cortisol distribution volume increases due to hypothalamic-adrenal axis dysregulation, alterations in cortisol metabolism, and tissue resistance to corticosteroids. The proper diagnosis and treatment of CIRCI may be challenging in many cases. Although we have been acquainted with CIRCI since 2008, it remains a difficult condition with widely variable approaches among clinicians due to inconsistent high-quality study results determining the effect of corticosteroids on mortality. Corticosteroids are widely used in acutely ill patients, highlighting the necessity for reliable knowledge to support crucial clinicians' decisions in daily medical practice. In this review, we provide an overview of the clinical management of patients with CIRCI based on current recommendations and selected studies.

KEYWORDS

critical illness-related corticosteroid insufficiency, CIRCI, intensive care, sepsis, septic shock

Introduction

Critical illness-related corticosteroid insufficiency (CIRCI) is a clinical condition with demanding diagnostics and individualized management. Since it may develop in the course of medical conditions of highly variable etiology, understanding the pathomechanism of CIRCI, as well as its manifestations, seems to be crucial for physicians of multiple specialties (1–3). Despite the numerous studies that have been presented, CIRCI remains a poorly understood clinical state, and the currently available knowledge still leaves many outstanding concerns unsolved. Our article aims to summarize current recommendations and selected research as on diagnostics and management of patients with CIRCI. The introduction of the term "CIRCI" is dated back to 2008, the year the Society of Critical Care Medicine (SCCM) used it to describe hypothalamus-pituitary-adrenal (HPA) axis insufficiency in the course of acute medical conditions (4). Previously, "relative adrenal insufficiency" was used (5). The significant focus of the CIRCI terminology has been to emphasize functional etiology, thus reflecting the concept that adrenal insufficiency (AI) in critical conditions may develop without structural defects in the HPA axis (1).

The pathogenesis of CIRCI

The pathogenesis of CIRCI involves dysregulation of the HPA axis, alteration of cortisol metabolism and tissue resistance to corticosteroids (2, 6). The HPA axis response to systemic inflammation during critical conditions may be reduced or dysregulated, and the reactions previously considered adaptive may be inadequate in the acute condition (2). However, a prompt increase in systemic corticosteroid availability is then essential to efficiently prevent an inadequate immune response but also to induce cardiovascular (such as fluid retention, vasoconstriction) or metabolic effects by enhancing catabolism and decreasing anabolism (5, 7, 8). In acute conditions, multiple peripheral adaptations develop after brief activation of the HPA axis to maintain increased systemic availability of cortisol without its increased production (7).

Cortisol is secreted in a pulsatile pattern following a circadian rhythm, with physiological peak concentrations in the morning and a drop in concentration during the subsequent hours (2, 6, 9, 10). The HPA axis is crucial in maintaining homeostasis, and its proper activity is based on the principle of feedback, but in acute conditions, this regulation is far more complicated (2, 6, 7, 9, 10). Critical conditions of various etiologies, through neuronal and inflammatory signals, induce an accelerated release of adrenocorticotrophic hormone (ACTH) mediated by corticotropin-releasing hormone (CRH) and arginine vasopressin (AVP), resulting in a disruption of the circadian rhythm of cortisol production (2, 5, 7, 8). In this group of patients, hypercortisolemia most likely develops secondary to decreased cortisol metabolism rather than an increase in adrenal sensitivity to ACTH (2). Initially, cortisol concentrations rise in response to a significant increase in ACTH concentrations, which declines to near basal levels if inflammation persists for a prolonged duration (9). The response of the HPA axis to critical conditions is divided into an acute phase (a few minutes after the initial damage), a subacute phase (a few hours to several days after the initial damage) and a chronic phase (more than a few weeks after the initial damage) (5). The acute phase is characterized by a rapid increase in cortisol levels in reaction to an increase in ACTH levels and numerous peripheral adaptations (5). This relationship has been demonstrated in patients hospitalized in the Intensive Care Unit (ICU), where elevated ACTH levels occurred only transiently, such as during surgical procedures (7). Similarly, a study by Raff et al., indicated that patients with sepsis admitted to the ICU, evaluated within 24 hours, had elevated serum total and free cortisol levels but without an accompanying sustained increase in ACTH concentrations (11). However, this study had its limitations, as it involved a relatively small number of patients with sepsis (22 patients) (11). In a prospective study involving 392 critically ill patients requiring ICU hospitalization for more than seven days, ACTH levels were reduced or normal until the 28th day of hospitalization, and free plasma cortisol levels were elevated (12).

Cortisol is principally metabolized in the liver and kidneys, and the essential enzymes responsible for the initial stages of metabolism are 5 α/β-reductase and 11β-hydroxysteroid dehydrogenase type 2 (11β-HSD2), respectively, whose activity decreases in response to inflammation (2, 5). This prolongs the half-life of cortisol, thereby maintaining sufficiently increased systemic corticosteroid concentrations (7). Similarly, the inactive 11β-HSD2-mediated cortisol metabolite- cortisone, formed in the kidneys, may be converted back to cortisol in extra-adrenal tissues such as the liver, adipose tissue and muscles due to increased 11β-hydroxysteroid dehydrogenase type 1 (11β-HSD1) activity, which is modulated by inflammatory cytokines (2, 5, 13). The produced mediators, including TNF-α and IL-1β, affect the expression of 11β-HSD, which alters the sensitivity of cells to endogenous corticosteroids (13–15). Elevated pro-inflammatory cytokines during acute conditions can also inhibit adrenal cortisol synthesis and induce tissue resistance to corticosteroids (1). In a study by Boonen et al. involving 158 ICU patients and 64 controls, plasma total and free cortisol levels were higher in the experimental group, while ACTH levels were lower than in the control group (16). Moreover, the experimental group had significantly higher cortisol production and decreased cortisol clearance, resulting in a 3.5-fold increase in cortisolemia compared to the control group (16). In critically ill patients, hypercortisolemia is not followed by high ACTH concentrations, which has been described as "ACTH-cortisol dissociation", emphasizing the role of the adrenal response in the pathomechanism of CIRCI (5, 11). In a study conducted by Boonen et al., it was shown that in the course of critical conditions, elevated cortisol levels were associated with suppressed nocturnal pulsatile ACTH secretion (17). This implies that hypercortisolemia in acute conditions develops by ACTH-independent mechanisms and may even result in negative feedback on the HPA axis (8, 11, 17). Hepatic cortisol metabolism may also be accelerated or prolonged by selected drugs used in acute conditions that modify the activity of cytochrome CYP3A4, such as amiodarone, macrolide group antibiotics (azithromycin,

clarithromycin) or azole antifungals (1, 18). It has also been described that decreased cortisol metabolism may also occur secondary to critical illness-associated cholestatic liver dysfunction (3, 5, 19, 20).

The α glucocorticoid receptor (GR α) plays a crucial role in maintaining homeostasis and the physiological stress response (2). Both endo- and exogenous corticosteroids act through the GR (21). There are available studies in patients with sepsis that have shown reduced GR α expression in peripheral blood, which has been interpreted as a manifestation of generalized resistance to corticosteroids and, thus, a rationale for the use of hydrocortisone in higher doses to overcome resistance in sepsis or septic shock (7, 22). Sepsis is also characterized by an increased expression of the GR isoform β in circulating cells, resulting in an imbalance between GRα and GRβ (23, 24). However this insight's limitation may be the differential effects of cortisol and synthetic corticosteroids depending on the target tissue (7). A study by Teblick et al. found that during critical illness, specific adaptations of GRα expression occur, and primarily neutrophils are responsible for the earlier observations of a decrease in GRα expression in peripheral blood cells of patients with sepsis (25). On the other hand, most further vital tissues and organs showed increased GRα action (25). This variation in GR expression could prevent immune- suppressive off-target effects of increased systemic cortisol availability (22). That observation also contradicts the generalized resistance to corticosteroids in acute conditions and the use of "stress" doses of hydrocortisone (25). However, some studies demonstrated that quantitatively adequate and prolonged glucocorticoid supplementation increased GRα number and function in both circulating and tissue cells, reversing critical illness-associated cellular glucocorticoid resistance (24, 26). When assessing the administration of glucocorticoid therapy, the current understanding of the role of activated GC-GRα in immunomodulation and the course of critical illness should be considered. Figures 1, 2 summarize the HPA axis's response to critical conditions and adaptations leading to increased systemic cortisol availability.

In acutely ill patients, it is also worth considering other factors that may lead to iatrogenic adrenal suppression, such as the use of the antifungal drug, ketoconazole for opportunistic infections, or the anaesthetic agent etomidate (1, 3, 18), a side effect of which is becoming increasingly prevalent in life-threatening cases of hypercortisolemia (27, 28). Indeed, ketoconazole, one of the imidazole derivatives, is among the historical drugs used in managing Cushing's disease because it inhibits cortisol synthesis by affecting 11β-hydroxylase activity (29, 30). Similar effects on the steroid synthesis pathway are also reported with etomidate, which additionally inhibits 17α-hydroxylase activity (29, 30). Further clinical conditions that may lead to iatrogenic adrenal suppression also include cases of adrenal hemorrhage, which can occur in septic patients with coagulopathies (1), assembling imaging methods such as ultrasound, computer tomography or magnetic resonance imaging valuable in such cases (3).

The clinical manifestation of CIRCI

CIRCI may develop in the course of sepsis, septic shock, acute respiratory distress syndrome (ARDS), community-acquired pneumonia, cardiac arrest, trauma, burns, and after extensive surgical procedures (2). Clinical manifestations range from nonspecific, such as fever and weakness, nausea and vomiting, to

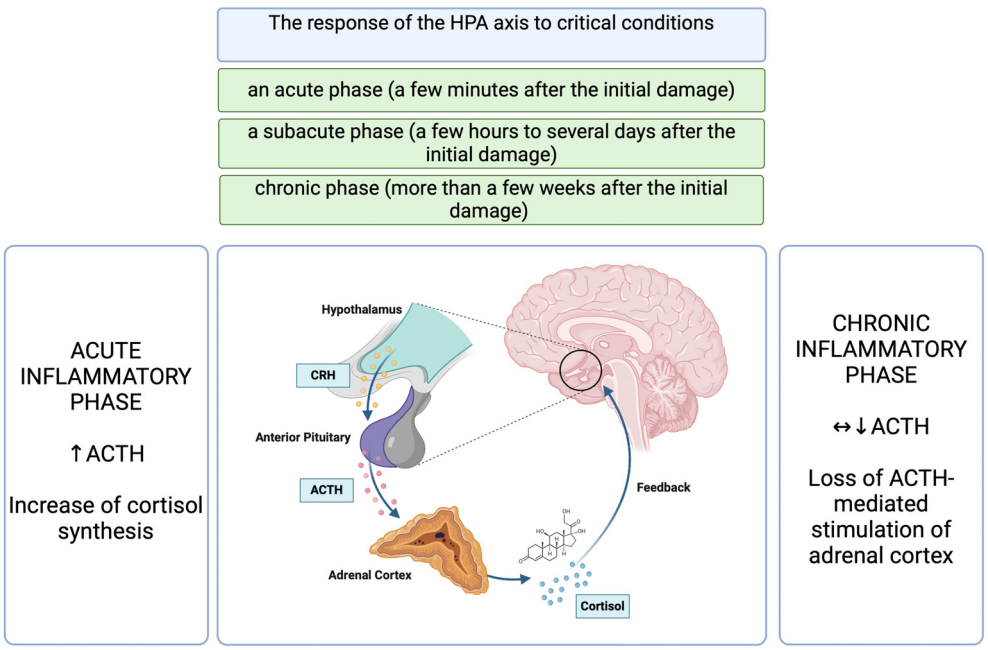

FIGURE 1
The response of the HPA axis to critical conditions (5, 7, 9, 11, 12). HPA, hypothalamic- pituitary- adrenal axis; CRH, corticotropin-releasing hormone; ACTH, adrenocorticotropic hormone. Figure created in BioRender.com.

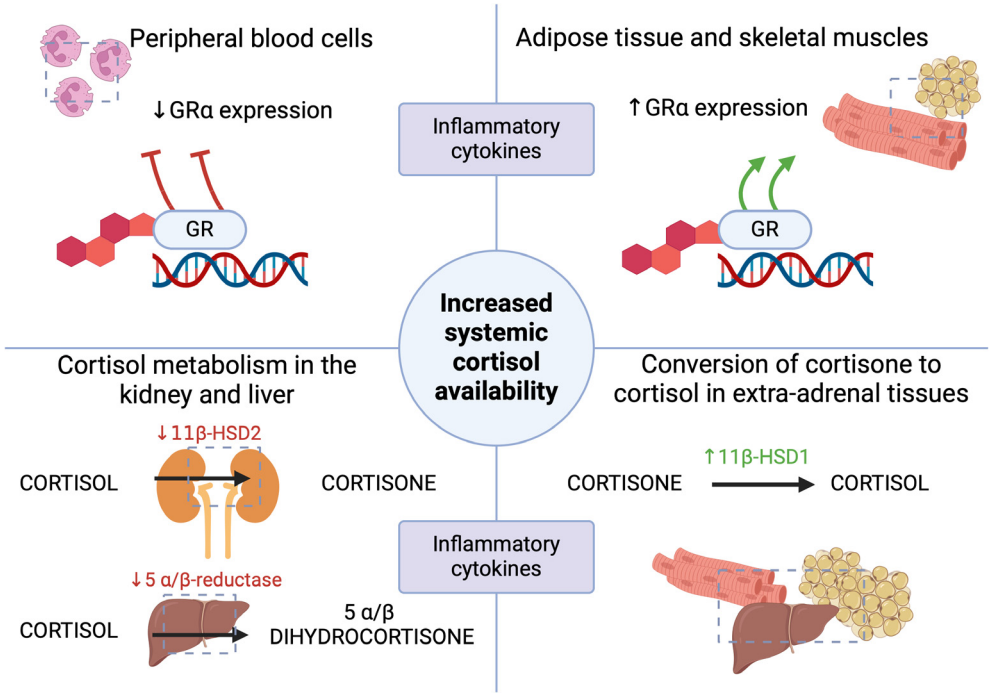

FIGURE 2
The mechanisms leading to the increased systemic availability of cortisol in acute conditions (1, 2, 5, 7, 9, 13–15, 22, 25). GR- glucocorticoid receptor; 11β-HSD1- 11β-hydroxysteroid dehydrogenase type 1; 11β-HSD2- 11β-hydroxysteroid dehydrogenase type 2. Figure created in BioRender.com.

symptoms involving multiple systems, such as confusion, delirium, coma; hypoxia, or hypotension refractory to fluid resuscitation (1–3). Possible clinical symptoms and laboratory and imaging abnormalities during CIRCI are summarized on Figure 3. Electrolyte abnormalities found in the course of CIRCI, such as hyponatremia and hyperkalemia, may be masked by intensive fluid therapy, but hypoglycemia or eosinophilia should suggest the suspicion of CIRCI, as these abnormalities are relatively rare in acute conditions (1).

Epidemiology of CIRCI

In a study by Hashemi-Madani et al. evaluating 99 patients admitted to the ICU, AI was found in 25.3% of patients, with no significant differences in the incidence of AI in patients with sepsis, severe sepsis or septic shock (31). Patients with positive blood cultures and greater C-reactive protein levels had a higher risk of developing AI; additionally, there was no significant difference in the average age of acutely ill patients with AI and those who did not develop hypocortisolism (31). In a retrospective single-center study assessing 145 patients with COVID-19 in critical conditions, 22.9% of these patients were likely to develop CIRCI (32). However, the results are slightly concerning, as in this group of patients, those who were treated with corticosteroids had a longer duration of mechanical ventilation, a higher risk of morbidity and mortality, and more severe organ dysfunction, emphasizing the unusual clinical manifestation of CIRCI in COVID-19 patients and appearing to be a factor for increased mortality in this group of patients (32). Similarly, in a study by Li et al., early (≤3 days after ICU admission) initiation of corticosteroid treatment (methylprednisolone was mainly administered) in patients with COVID-19 led to an increase in 90-day mortality (33).

Another important group are oncology patients with sepsis, who represent a high-risk subgroup for CIRCI, which may be overlapped by metastatic adrenal lesions or HPA axis dysfunction after radiation therapy (34). Existing data on the incidence of CIRCI in this group of patients appear to be limited, as diagnosed malignancy has formed an exclusion criterion in many trials (34). For instance, in a study by Sprung et al., oncological patients accounted for only 16.8% of the patients included (35). In a research by Bruno et al. evaluating 86 oncology patients with severe sepsis or septic shock, 59% of patients developed CIRCI, but the mortality rate in this study did not differ from the group that did not develop CIRCI (34).

CIRCI diagnostics

The 2008 SCCM guidelines already recommended that random plasma or serum total cortisol measurement <10μg/dL or change in baseline cortisol <9 μg/dL (cortisol) at 60 min in 250 μg cosyntropin test be used to diagnose CIRCI (4). The 2017 SCCM guidelines do not indicate the superiority of either test in this indication (36). Endocrine Society recommends that the 250 μg corticotropin stimulation test is the gold standard for diagnosing primary AI

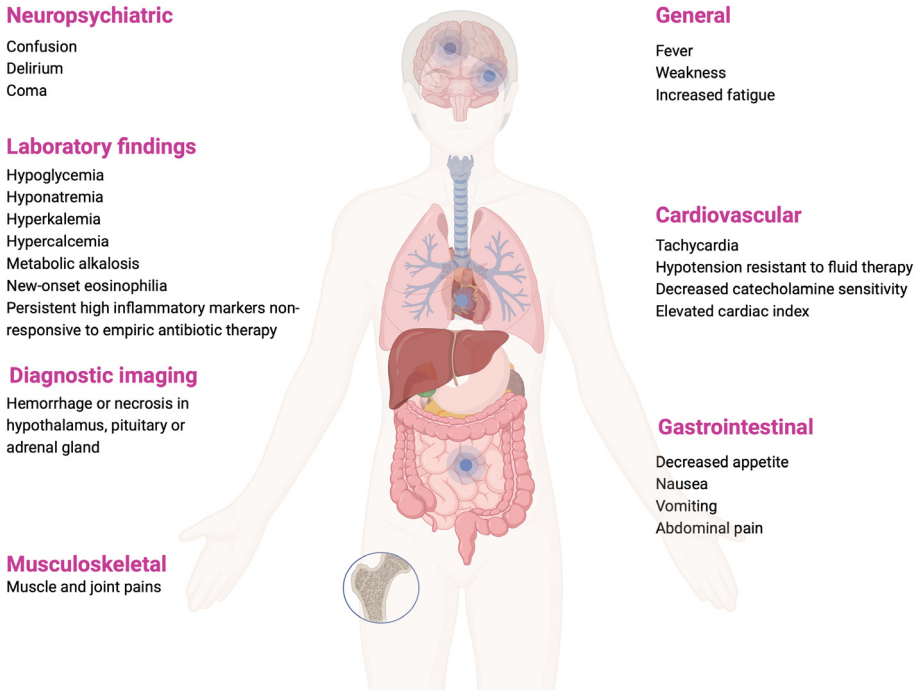

FIGURE 3
Clinical manifestations, laboratory and imaging findings that may occur in CIRCI (1, 3, 36). Figure created in BioRender.com.

(37). The term CIRCI does not include HPA axis abnormalities that were present before the onset of the acute condition (3). Even if the initial test results excluded CIRCI, it can develop in the subsequent days of infection, so it is essential to actively search for signs of AI and retest, especially if the patient's general condition worsens (1). In the reviewed literature, data about using a test with 1 µg cosyntropin, i.e. low-dose ACTH test for the diagnosis of CIRCI also appear (1, 38–41), however, current SCCM guidelines do not recommend this test (36). It is noteworthy to mention here the results of a single-center study conducted by Marik et al. involving 59 patients with septic shock, in which, depending on the type of test performed, a varying percentage of patients met diagnostic criteria for AI - 22% of patients in the test with 1 µg and 8% of patients in the test with 250 µg corticotropin (38). In another study by Widmer et al., moderate and major stress situations were associated with higher peak cortisol concentrations after stimulation with 250 µg than after 1 µg cosyntropin (42). In a survey involving 189 patients with septic shock, Annane et al. divided patients into three groups according to the response obtained in the 250 µg cosyntropin test and baseline cortisol levels, identifying patients with basal cortisol >34 µg/dL and response in the cosyntropin test (cortisol ≤9 µg/dL) as those with the highest risk of death and a median survival time of five days (43). This study underscores that a low increase in cortisol concentration in a test with 250 µg cosyntropin is predictive of mortality, independently of baseline serum cortisol concentration (43). The previously mentioned adaptive modifications to increase the volume of cortisol distribution significantly affect the perception of the stimulation test with cosyntropin in critical conditions, as the incremental response of total cortisol concentration is reduced and free cortisol concentration is normal (3, 7). In proportion to the severity of the general condition, the level of cortisol binding globulin (CBG) decreases, as we discuss below, the volume of corticosteroid distribution rises, and the incremental concentration of total cortisol in the test with cosyntropin is more inhibited (3, 7). This emphasizes not considering the cosyntropin stimulation test as a tool to identify patients who should be treated with exogenous glucocorticoids (7). The SCCM recommendations also do not suggest evaluating the hemodynamic response to hydrocortisone in the diagnosis of CIRCI, rather than performing a test with 250 µg corticotropin, as well as an isolated determination of ACTH levels, which, as we have discussed, can vary in the course of acute conditions (36).

In patients with suspected CIRCI, it is not recommended to measure free plasma cortisol concentrations instead of total serum cortisol levels (36). An estimated 80-90% of circulating cortisol is bound to CBG, 10-15% to albumin, and the remaining percentage occurs in unbound form (1, 2, 5, 44). Both CBG and albumin are among the negative acute-phase proteins (2), and decline in critical conditions, and thus changes in CBG concentrations significantly complicate reliable assessment of free cortisol levels (1, 2, 5, 36). Their concentrations also decrease secondary to bleeding, enteropathy or dilution secondary to fluid resuscitation (5).

It is also noteworthy to outline the variability of cortisol's binding capacity to CBG and albumin in relation to cortisol concentrations. CBG is a 50-60 kDa glycoprotein with a high affinity for cortisol (44, 45). An increase in cortisol secretion in response to psychological and physical stressors or tissue damage is

part of the "fight or flight" reaction, and similarly, in critically ill patients, the severity of surgery or infection positively correlates with the degree of cortisolemia (1, 5, 46). CBG binding capacity is saturated at cortisol concentrations in the range of 22-25 µg/dL, while when concentrations are higher, the proportion of albumin-bound cortisol and free cortisol rises, and the fraction bound to CBG remains unaltered (2). CBG concentration, total plasma cortisol concentration and albuminemia, may also be used to estimate the amount of free cortisol in plasma, using Coolens' formula in a modified version (5). However, this method should be interpreted cautiously in acutely ill patients (47). A study by Chan et al. highlighted the role of glycosylation as crucial in maintaining CBG function, both as a carrier of corticosteroids and as a modulator allowing differentiated release into tissues (44). Indeed, it was shown that the glycosylated form of CBG binds cortisol with significantly higher affinity than the non-glycosylated form and, interestingly, that an increase in body temperature by every two Celsius degrees doubles the concentration of free cortisol (44).

An interesting peripheral adaptation in acute conditions is also the increase in the distribution volume of cortisol due to decreased hepatic synthesis of cortisol-binding proteins and the altered affinity of cortisol for binding proteins in proportion to the severity of the disease (7). Recent findings suggest that the role of CBG during inflammation and sepsis is considerably increasing as a transporter protein delivering cortisol with immunomodulatory effects. Through a complex regulatory mechanism, in response to temperature and acidity, cortisol is directed to the highest-demand areas in sepsis conditions (45). The increase in free cortisol in acute conditions is also led by the effect of neutrophil elastase, which changes CBG to a form with low binding capacity (1, 5, 13, 45), as well as inflammatory cytokines that inhibit hepatic CBG synthesis (45). Decreased CBG levels affected one-third of patients with septic shock admitted to the ICU and were a risk factor for increased mortality, opening promising perspectives for rapid CBG assays as those with high prognostic value in sepsis (45). In a study by Dubey and Boujoukos, 39% of critically ill patients with concomitant decreased albumin levels had abnormal total serum cortisol levels despite preserved normal adrenal function (48). Although even if the free cortisol concentration could be measured reliably, it is difficult to estimate its recommended concentration, as it will differ depending on the severity of the patient's general condition (1, 7). Similarly, salivary cortisol determination is not recommended because of the possible changes in free cortisol concentrations described above but also due to the lack of cooperation enforced by the patient's critical condition, i.e., the inability of an unconscious or an intubated patient to use a salivette, and the limited availability of this type of assessment in many centers (36). Publications are available that evaluate free cortisol concentrations in saliva sampled in the morning (49), including after stimulation with synthetic ACTH (50)„ proving the usability of these determinations in diagnosing AI (49, 50); SCCM guidelines, however, do not recommend the use of this assay (36). According to the „Consensus on diagnosis and management of Cushing's disease", the determination of free salivary cortisol, however, performed in the evening (late night salivary cortisol) is used in the diagnosis of Cushing Syndrome and the detection of disruption of the circadian nadir of cortisol secretion in this group of patients (51).

Treatment with corticosteroids in selected clinical indications

Corticosteroids are likely among the most frequently used drugs in medicine (18). No conclusive data are available on the recommended dose, time of initiation and duration of therapy with corticosteroids in acute conditions, which, in clinical practice, have been used in patients with severe infections since the mid-20th century (21, 52). Synthetic corticosteroids bind glucocorticoid receptors with higher affinity, while mineralocorticoid receptors bind with lower affinity than endogenous corticosteroids (13). Furthermore, synthetic corticosteroids are not bound by CBG and are not inactivated by 11β-HSD, and thus exhibit stronger immunoregulatory potency than endogenous corticosteroids (13, 21). pa

Among clinicians, there are variable opinions about the usage of corticosteroids and their effect on survival in patients with sepsis (52). Sepsis and septic shock result in death from one in six to one in three patients (53). To properly expand on the issue, it is worth repeating the definition of sepsis and septic shock proposed by the International Consensus Sepsis- 3 (54). Sepsis is life-threatening organ dysfunction in the course of a dysregulated response to infection, as manifested as an increase of ≥2 points on the Sequential [Sepsis-related] Organ Failure Assessment (SOFA) scale (54). Septic shock refers to patients who require vasopressor therapy to ensure mean arterial pressure (MAP) ≥65 mmHg and serum lactate levels >2 mmol/L (>18 mg/dL) in the absence of hypovolemia (54). Septic shock is defined as a subgroup of sepsis presenting with more severe cellular, metabolic and hemodynamic abnormalities than in sepsis (54). Corticosteroids used in septic shock may accelerate the reversal of shock but may also have adverse effects on the adaptive capacity of the HPA axis (7). Sepsis is associated with an in-hospital mortality rate higher than 10%, while septic shock above 40% (54). The SCCM suggests that corticosteroids should not be used in adult patients with sepsis without associated septic shock (36). In the HYPRESS trial, with 380 adult patients with severe sepsis, evaluating whether hydrocortisone administration (200 mg in continuous intravenous infusion) prevents the development of septic shock, no significant differences were observed between the hydrocortisone-treated and placebo-treated groups (21.2% vs 22.9%) (55). In addition, in the hydrocortisone-treated group, patients were more likely to develop secondary infections and hyperglycemia (55).

The Surviving Sepsis Campaign's 2021 recommendations advise the usage of intravenous corticosteroids in adult patients with septic shock (weak recommendation; moderate quality of evidence), especially intravenous hydrocortisone at a dose of 200 mg/day in fractionated doses every six hours or as a continuous infusion (53). Considering the drug's pharmacokinetics, the use of instant-release hydrocortisone, when dosed two/three times a day, does not simulate the physiological circadian rhythm of cortisol secretion, including its natural, gradual decrease during the day

(18). Hence, intravenous infusion of hydrocortisone with the potential ability of flow rate modification has an advantage, improving the pharmacokinetic profile, which is possible in critically ill patients but impossible in patients chronically substituted with hydrocortisone on an outpatient basis (18).

The treatment of hydrocortisone at a dose of 50 mg every six hours, leads to supra-physiological cortisol concentrations, which may be important for the underlying tissue resistance to corticosteroids in CIRCI (1). The daily doses of hydrocortisone (200-300 mg) recommended for treating septic shock are equivalent to at least ten times the daily replacement dose for healthy individuals and about four times the average daily cortisol production in critically ill patients (16). It is suggested to initiate corticosteroid treatment when using doses of norepinephrine or epinephrine ≥ 0.25 μg/kg/min at least four hours after the start of treatment to maintain the recommended MAP (53). In clinical practice, hydrocortisone is also used in patients in septic shock when there is an increasing need for pressure amines in the absence of accompanying AI (3).

It is worth presenting three key randomized trials, which were the ones that significantly varied clinicians' opinions. In the ADRENAL trial, which enrolled patients treated with vasopressors and inotropic drugs for ≥4 hours to maintain MAP >60 mmHg, hydrocortisone treatment was given for a maximum of seven days or shorter until discharge from the ICU or death (56). The hydrocortisone treatment used in this study did not improve the 90-day survival of patients with septic shock compared to the placebo group (56). The hydrocortisone-treated group had a more rapid resolution of shock and less frequent requirement for blood transfusion compared to the placebo-treated group (56). In the multicenter, double-blind, randomized APROCCHSS trial, among 1,241 patients enrolled in the study, 90-day mortality from any cause was 6% lower in the group of patients treated with hydrocortisone along with fludrocortisone than in the placebo group (43% vs 49%, respectively) (52). Adding fludrocortisone (50 μg orally) to treatment was supposed to provide additional mineralocorticoid potency (52). Similarly, the number of days without the requirement for vasopressors and those without organ failure were higher in the hydrocortisone plus fludrocortisone group compared to the placebo group (52). In contrast, the number of days without mechanical ventilation was similar in both groups and importantly, hyperglycemia was more frequent in the group of patients treated with hydrocortisone plus fludrocortisone (52). The risk of secondary infections, gastrointestinal bleeding and neurological consequences was not significantly higher in the hydrocortisone plus fludrocortisone treatment group than in the placebo group (52). An interesting perspective on the role of hyperrenin hypoaldosteronism in critical conditions was presented by Nethathe et al., where the term critical illness-related mineralocorticoid insufficiency (CIRMI) was proposed for this type of dysfunction and indicated that hydrocortisone and fludrocortisone combination therapy should be considered in patients with septic shock (57). Another study that is also worth mentioning in the context of the role of mineralocorticosteroids is the FluDReSS trial, designed to evaluate hydrocortisone and fludrocortisone combination therapy in critically ill patients, which has completed recruitment and the results of which we are currently awaiting (58).

In the CORTICUS trial, which evaluated the efficacy and safety of low-dose hydrocortisone therapy among patients with septic shock, treatment did not improve survival (35). Hydrocortisone treatment did not significantly affect shock reversal, but shock resolved more promptly in the hydrocortisone-treated group than in the placebo group (35). This group also experienced more superinfections (35). The studies presented are summarized in Table 1. They appear to provide contrasting results, which may be explained by the fact that the patients in the CORTICUS and ADRENAL trials were less sick than those in the APROCCHSS trial, considering, for example, the higher vasopressors required. In addition, in both the ADRENAL and APROCCHSS studies, hydrocortisone was administered for seven days, but the pattern of drug administration differed-fractional doses versus continuous infusion. Controversy has arisen over the differing results of the indicated studies, enough that Annane, in his article "Why My Steroid Trials in Septic Shock Were 'Positive'?" accessibly summarized the five studies, indicating, among other reasons, that patients in worse general condition, i.e., with higher vasopressor requirements and greater severity of organ failure, benefited significantly from corticosteroid treatment (59). Results are also available from other studies that varied in the number of patients who experienced a reduction in the duration of septic shock during corticosteroid treatment (60, 61).

The SCCM recommends the use of hydrocortisone <400 mg intravenously or hydrocortisone equivalent in patients with severe forms of community-acquired pneumonia, indicating that

TABLE 1 ADRENAL (56), APROCCHSS (52) and CORTICUS (35) trials overview.

Study design	Trial		
	ADRENAL (56)	APROCCHSS (52)	CORTICUS (35)
Corticosteroid used	hydrocortisone	hydrocortisone combined with fludrocortisone	hydrocortisone
Route of drug administration	intravenous infusion	intravenous bolus (hydrocortisone); orally (fludrocortisone)	intravenous bolus
Dose per day	200 mg	200 mg (50mg every 6 hours) [hydrocortisone] 50 μg (fludrocortisone)	200 mg (50mg every 6 hours)
Treatment duration (days)	7	7	5
Number of patients	3658	1241	499
Mean age in the corticosteroid group (years)	62.3 ±14.9	66.0 ± 14	63.0 ± 14
Primary outcome corticosteroid vs placebo group* (%)	27.9 vs 28.8 P=0.50	43.0 vs 49.1 P=0.03	34.3 vs 31.5 P=0.51

*The primary endpoint in the ADRENAL study and APROCCHSS was 90-day mortality, in the CORTICUS study 28-day mortality (35, 52, 56).

corticosteroids shortened the length of hospitalization, reduced the possibility of the requirement for mechanical ventilation, the development of ARDS, but increased the risk of hyperglycemia, without causing other clinically significant complications (62).

Another important concern in a group of such interdisciplinary patients is cardiogenic shock, which has similar hemodynamic, inflammatory patterns and complications as septic shock and may involve CIRCI (63). In a single-center study conducted by Ducroq et al. involving 79 patients with cardiogenic shock, 42% of patients developed CIRCI, but in this research, this was not associated with an impact on 90-day mortality (63). Notably, exclusion criteria in this study included current ongoing corticosteroid therapy and use of etomidate (63).

Exogenous corticosteroid therapy has limitations, ranging from immunosuppressive effects to adrenal cortex inhibition (1, 13, 18). Corticosteroid-treated patients have been found to experience a significantly increased risk of gastrointestinal bleeding and perforation during hospital treatment, which was not found in outpatients (64). Through suppression of ACTH production, adrenal atrophy may occur, which, depending on the length of corticosteroid treatment, can persist even months after its termination (1). The possibility of developing the adrenal suppression described should be considered on an individualized basis for the patient, especially when corticosteroid therapy has lasted more than three weeks, and the patient has received more than 30 mg of hydrocortisone daily (or 7.5 mg prednisolone/day; or 0.75 mg dexamethasone/day) (1). Frequently used in medical nomenclature, "high" and "low" doses of corticosteroids vary depending on the indication for which they are applied (18). In rheumatological indications, the "very high dose" is referred to when the administration exceeds 100 mg of prednisone per day; in the indication of acute respiratory failure, the use of >30 mg of prednisone daily is similarly referred to as "high dose" (18). In a study by Min et al. evaluating the effect of short-term (1-2 weeks) use of high-dose corticosteroids (methylprednisolone at a daily dose of 48mg, equivalent to about 60 mg of prednisolone), in hospital but also outpatient pharmacotherapy, adverse effects were observed in 1/3 of patients (65). Once CIRCI is diagnosed and hydrocortisone treatment is initiated, therapy should be conducted with the minimum effective dose, and discontinuation should be attempted after recovery (3). The most recent SCCM guidelines do not specify how to conduct corticosteroid treatment discontinuation in CIRCI (36), but current Surviving Sepsis Campaign recommendations indicate that corticosteroids should be administered when vasopressor requirement persists (53). Interestingly, a retrospective cohort study by Carabetta et al. evaluated the impact of abrupt and gradual withdrawal of corticosteroids in patients in septic shock (66). Patients in the gradually reduced-dose group were more likely to experience hemodynamic instability than those in the abrupt dose reduction group (21.9% vs. 10.7%, respectively) (66). Hyperglycemia within 24 hours of the last dose of hydrocortisone was more frequent in the abrupt dose reduction group, while length of ICU hospitalization and in-hospital mortality were similar in both groups (66). In another retrospective study by Sobolewski et al., similarly, hemodynamic instability was more frequent in the gradual withdrawal group than in the abrupt discontinuation group (17.1% vs. 2.2%), while worsened glycemic control occurred in the gradual withdrawal group (67). Vanhorebeek et al., evaluated whether neuroendocrine disorders involving, among others, the corticotropic axis found during ICU hospitalization would resolve after discharge and how the function of this axis would fare in the evaluation made at five years following the acute condition (68). Total and free cortisol, as well as CBG levels, were similar in the study and control groups, while residual thyrotropic axis abnormalities persisted (68).

Conclusions

The data presented here demonstrate the lack of comprehensive research on CIRCI, which results in highly variable current approaches among clinicians. Diagnosing CIRCI in critically ill patients and managing corticosteroid therapy may be demanding due to limited data from high-quality clinical trials, particularly regarding the duration of corticosteroid treatment. Despite shedding new light on corticosteroid usage, numerous aspects of CIRCI still require further clarification and research. That underscores the importance for clinicians to expand their knowledge in this area of intensive care medicine and emphasizes the essential need for accurate patient assessment in critical conditions of various etiologies, particularly when the patient does not respond or worsens during ongoing therapy.

Author contributions

JS: Conceptualization, Formal analysis, Writing – original draft, Writing – review & editing. LD: Formal analysis, Writing – original draft, Writing – review & editing. PK: Supervision, Writing – review & editing. PW: Supervision, Writing – review & editing.

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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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EDITED BY
Henrik Falhammar,
Karolinska Institutet (KI), Sweden

REVIEWED BY
Chiara Lopez,
University of Turin, Italy
Lilah Morris-Wiseman,
Johns Hopkins Medicine, United States

*CORRESPONDENCE
Limin Zhu
zhulimin@rjh.com.cn

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Chronological outcomes of renal function after adrenalectomy in patients with primary aldosteronism across age groups

Yu Ma[1,2], Xiaofeng Tang[1,2], Qian Ge[1,2], Jianzhong Xu[1,2], Pingjin Gao[1,2], Jiguang Wang[1,2] and Limin Zhu[1,2]*

[1]Department of Cardiovascular Medicine, State Key Laboratory of Medical Genomics, Shanghai Key Laboratory of Hypertension, Shanghai Institute of Hypertension, Shanghai, China, [2]Department of Hypertension, Ruijin Hospital, Shanghai Jiao Tong University School of Medicine, Shanghai, China

Background: Patients with primary aldosteronism present with renal function decline after unilateral adrenalectomies. Our study aimed to assess the evolution of renal function after adrenalectomy in patients with primary aldosteronism across different age groups and to identify risk factors for postoperative renal function deterioration.

Methods: We included 210 patients with primary aldosteronism categorized into three age groups: <40, 40–60, and ≥60 years old. We followed up the patients for 1 month, 1 year, and 5 years after adrenalectomy to assess outcomes. Multivariate analyses were performed to identify predictors of renal function deterioration, and a univariate logistic regression analysis was used to assess the relationship between *KCNJ5* mutation status and the decline in renal function.

Results: Patients aged <40 years had a shorter duration of hypertension, higher preoperative diastolic blood pressure, and higher preoperative estimated glomerular filtration rate (eGFR) than did those in the other age groups. This group also exhibited the highest rate of complete clinical success, although there were no significant differences in complete biochemical success among age groups. Renal function declined in all three groups after adrenalectomy. However, changes in blood pressure and eGFR in the short- or long-term after adrenalectomy showed no significant differences among the three groups. Hypertension duration, preoperative systolic blood pressure (SBP), and plasma aldosterone concentration (PAC) were predictors of postoperative renal function deterioration. *KCNJ5* wild-type status was significantly correlated with the occurrence of chronic kidney disease after adrenalectomy.

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Conclusions: Unilateral adrenalectomy demonstrates favorable biochemical and clinical outcomes in patients with primary aldosteronism, irrespective of age. Long-term eGFR decline is similar among the different age groups. *KCNJ5* mutation exhibits a protective effect against the risk of chronic kidney disease after unilateral adrenalectomy.

KEYWORDS

primary aldosteronism, age, adrenalectomy, estimated glomerular filtration rate, *KCNJ5* mutation

Introduction

Primary aldosteronism (PA), characterized by inappropriately elevated aldosterone secretion, represents the most prevalent endocrine cause of secondary hypertension, affecting approximately 5–10% of patients with hypertension (1, 2). Excess aldosterone causes hypertension and hypokalemia, increasing the risk of cardiovascular and kidney diseases in PA (3–5).

Surgical adrenalectomy or mineralocorticoid receptor antagonists (MRAs) are the two recommended therapies, and the treatment choice is based on PA subtype and patient's preference (6, 7). Surgical treatment may resolve the excessive aldosterone production in patients with PA. A meta-analysis including 43 studies involving approximately 4000 patients with PA reported that the hypertension cure rate was approximately 50.6% for unilateral adrenalectomy (8).

Excess cardiovascular and cerebrovascular events and renal dysfunction in PA patients are ameliorated after adrenalectomy (4, 9–11). However, a significant proportion of patients develop renal dysfunction after adrenalectomy (12–15). For instance, Iwakura et al. (14) found that the prevalence of chronic kidney disease (CKD), defined as estimated glomerular filtration rate (eGFR) <60 mL/min/1.73 m^2, increased from 15.7% before adrenalectomy to 37.1% at 12 months post-surgery in the aldosterone-producing adenoma (APA) group. Our clinical observations also noted that certain older patients with PA experienced declining eGFR or developed CKD after adrenalectomy. Consequently, preserving renal function has become a critical consideration, in addition to blood pressure (BP) management, in patients with PA undergoing adrenalectomy.

Whether older patients benefit from adrenalectomy in terms of renal function needs further studies.

Recent studies have emphasized the vital role of somatic mutations in the *KCNJ5* gene in the pathogenesis of APAs. The prevalence of *KCNJ5* somatic mutations in APAs is notably higher in Chinese patients (70.7%–80.7%) (16–18) than in patients from Western countries (34%) (19). Arnesen et al. (20) demonstrated that the presence of *KCNJ5* mutations was related to better surgical outcomes in patients with APAs, including lower postoperative BP and a higher prevalence of clinical cure. Zhang et al. (18) further reported that *KCNJ5* mutation was a protective factor for achieving complete clinical success. However, studies on the relationship between *KCNJ5* mutation status and postoperative renal function changes are limited.

In this study, we aimed to investigate the chronological changes in renal function in patients with PA of different ages and explore the predictive factors of postoperative renal deterioration after adrenalectomy in the Chinese population. Additionally, we aimed to evaluate the potential benefits of unilateral adrenalectomy especially in elderly patients.

Methods

Patients

We recruited 218 patients diagnosed with PA who consecutively underwent unilateral adrenalectomy at the Department of Hypertension of Ruijin Hospital between April 2010 and June 2019. Finally, we included 210 patients with *KCNJ5* mutations and wild-type (WT) variants at postoperative follow-up.

PA was diagnosed according to the 2016 Endocrine Society PA management guideline (6). Briefly, before the diagnostic workup, angiotensin-converting enzyme inhibitors (ACEis), angiotensin II type 1 receptor blockers (ARBs) and β-blockers were withdrawn for at least 2 weeks, non-potassium-sparing diuretics for 4 weeks, and MRAs for 6 weeks. Non-dihydropyridine calcium channel blockers and α_1 blockers were prescribed for BP control, as necessary. Patients with a positive aldosterone-to-renin ratio (ARR) (ARR>24 [ng/dL]/[ng/mL/h]) were considered PA candidates. PA was confirmed using an intravenous saline loading infusion test (post-test aldosterone levels >100 pg/mL). The patients diagnosed PA underwent adrenal computed tomography scans. Adrenal venous sampling (AVS) was carried out in patients willing to undergo unilateral adrenalectomy. We conducted AVS by sequential procedure without ACTH stimulation and the selectivity and lateralization index is ≥2, respectively. Instead of

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the 1mg DST test, we performed the 24-hour urine cortisol levels dosage to exclude patients with cortisol co-secretion from undergoing AVS. Therefore, we did not measure other metabolites such as metanephrines for index correction beyond cortisol. APAs were verified via histopathology and immunohistochemical analysis (CYP11B2 staining positive). The adrenal tissues were screened for somatic mutations in the hot spot regions of KCNJ5, ATPase, CTNNB1, and CACNA1D. Clinical and biochemical success after adrenalectomy for unilateral PA was assessed using the Primary Aldosteronism Surgical Outcome (PASO) criteria (21). Complete clinical success was defined as achieving normalized BP (<140/90 mmHg) without use of antihypertensive medication after 6 months of follow-up. Normalized ARR and absence of hypokalemia were classified as complete biochemical success. All patients provided written informed consent, and the procedure received approval from the local ethics committee.

Patients follow up

We followed up the patients 1 month, 1 year, and 5 years after surgery to assess outcomes, including antihypertensive requirement, BP, eGFR, and levels of potassium, aldosterone, and renin. We calculated eGFR using the CKD-Epidemiology Collaboration equation (22). Renal function deterioration was defined as a decrease in eGFR at 1 month postoperatively ≥30% of the preoperative eGFR. CKD was defined as an eGFR of <60 mL/min/1.73 m^2.

Genotyping

Genomic DNA was extracted from APAs according to the standard methods. Sequences of KCNJ5, ATP1A1, ATP2B3, CTNNB1, and CACNA1D were amplified using primers described previously (17, 23–25). Sanger sequencing of the purified polymerase chain reaction products was analyzed using an Applied Biosystems 3730xl DNA Analyzer.

Statistical analyses

Continuous variables are expressed as mean ± standard deviations or medians with interquartile ranges, as appropriate. Categorical variables are presented as counts and percentages (*n* [%]). To compare continuous parameters, we used Student's t-test or one-way analysis of variance, depending on the data distribution. For categorical variables, comparisons were made using the χ^2 or Fisher exact test, as appropriate. Pearson's correlation analysis was performed to examine the relationship between changes in BP and eGFR. Univariate and multivariate analyses were conducted to identify the risk factors for postoperative renal function deterioration. Statistically significant was set a *P*-value of <0.05. We used SPSS Statistics 26.0 and GraphPad Prism 9.0 for all statistical analyses.

Results

Baseline characteristics of patients

Of 218 patients with PA, *KCNJ5* somatic mutations were observed in 164 patients. The *ATP1A1* (L104R) somatic mutation was detected in four APAs, and the *ATP2B3* (p. 422-426del) somatic mutation was detected in two APAs. Additionally, the *CACNA1D* (G403R) somatic mutation was identified in one APA, and 46 cases presented with the WT. We analyzed the clinical features of 164 patients with *KCNJ5* mutation and 46 patients with *KCNJ5*-WT mutation. The patients were divided into three groups according to their age at PA diagnosis. The baseline clinical features of study patients are presented in Table 1. In total, 74 patients were <40 years old (35.2%), 102 (48.6%) were 40–60 years old, and 34 (16.2%) were ≥60 years old. There were significant differences in hypertension duration, diastolic blood pressure (DBP), percentage of *KCNJ5* mutation, and eGFR among the three groups. Patients aged <40 years had a shorter duration of hypertension, higher DBP, and higher eGFR than patients in the other age groups. In our study, the prevalence of *KCNJ5* mutation was 75.2%. It was highest in patients aged <40 years (83.8%) and lowest in patients aged ≥ 60 years (58.8%). We observed no significant differences in the percentage of sex, body mass index (BMI), systolic blood pressure (SBP), plasma aldosterone concentration (PAC), urinary aldosterone level, plasma renin activity, serum sodium and serum potassium levels, or prevalence of hypokalemia among the three groups. Although not statistically significant, SBP was relatively higher in patients aged ≥60 years than in patients in the other age groups.

Outcomes after adrenalectomy

According to the PASO criteria, 43 (58.1%) patients aged <40 years, 24 (23.5%) patients aged 40–60 years, and 1 (2.9%) patient aged ≥60 years achieved complete clinical success (Supplementary Table 1). The analysis revealed significant differences in complete clinical success among the three groups (*P*<0.0001), with the <40 years old group having the highest success rate. Conversely, no significant differences in complete biochemical success were found among the groups (*P*=0.9169). All three groups exhibited a high prevalence of complete biochemical success. After adrenalectomy, none of the patients in the <40 years old group developed hyperkalemia. However, five of 102 (4.9%) patients aged 40–60 years and five of 34 (14.7%) patients aged ≥60 years presented with hyperkalemia (Supplementary Table 1).

Longitudinal changes in renal function and BP after adrenalectomy

Changes in BP and eGFR before and after adrenalectomy are shown in Figure 1. The SBP, DBP, and eGFR of all patients decreased 1 month after surgery (*P*<0.0001, respectively). SBP

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TABLE 1 Baseline clinical characteristics of patients.

	<40 (n=74)	40–60 (n=102)	≥60 (n=34)	*P*
Age (years)	34 (31–37)$^{\#,****}$	51 (47–55)$^{\$,****}$	64 (61–67)$^{\&,****}$	<0.0001
Female (n, %)	38 (51.4)	45 (44.1)	12 (35.3)	0.2829
HT duration (years)	1.5 (0.5–4)$^{\#,****}$	8 (4–13)$^{\$,****}$	15 (10–20)$^{\&,****}$	<0.0001
BMI (kg/m^2)	24.5 ± 4.1	24.0 ± 2.8	23.7 ± 2.7	0.4451
SBP (mmHg)	146.5 (137–156)	141.5 (133–157)	154 (140–160)	0.0583
DBP (mmHg)	90 (83–97)$^{\#,*}$	85 (80–94)	84 (79–90)$^{\&,***}$	0.0025
PAC (pg/mL)	314.6 (219.8–416.5)	296.9 (218.8–446.6)	345.8 (235.8–489.7)	0.6119
Urinary aldosterone (μg/24 h)	23 (17.3–72.5)	21.7 (16–67.6)	19.3 (13.6–30.6)	0.2835
PRA (ng/mL/h)	0.28 (0.14–0.58)	0.29 (0.15–0.67)	0.28 (0.15–0.66)	0.9436
Serum Na^+ (mmol/L)	141 (134.8–143)	141 (140–143)	142.5 (141–144)	0.0547
Serum K^+ (mmol/L)	3.16 ± 0.4	3.17 ± 0.4	3.2 ± 0.6	0.9445
Hypokalemia (n, %)	63 (85.1)	78 (76.5)	30 (88.2)	0.1853
KCNJ5 mutation n (%)	62 (83.8)	82 (74.5)	20 (58.8)$^{\&,**}$	0.0198
eGFR (mL/min/1.73 m^2)	115.8 (107.6–120.2)$^{\#,****}$	101 (86.8–107.7)$^{\#,****}$	86.8 (70.5–97.8)$^{\&,****}$	<0.0001

Values are expressed as means ± standard deviations, medians (interquartile ranges), or numbers (%). HT, hypertension; BMI, body mass index; SBP, systolic blood pressure; DBP, diastolic blood pressure; PAC, plasma aldosterone concentration; PRA, plasma renin activity; eGFR, estimated glomerular filtration rate. $^{\#}$<40 vs. 40–60, $^{\$}$40–60 vs. ≥60, $^{\&}$<40 vs. ≥60, ****P<0.0001, ***P<0.001, **P<0.01, *P<0.05.

and DBP were further reduced at 1-year post-surgery compared to that at 1 month after adrenalectomy, whereas no significant change was found in eGFR at 1 year versus at 1 month. SBP and DBP slightly increased, and eGFR continued to decline at 5 years postoperatively, whereas there were no significant differences compared with those at 1 year postoperatively.

In all patients, the change in SBP (preoperative SBP– 1-month postoperative SBP) was positively correlated with the change in eGFR

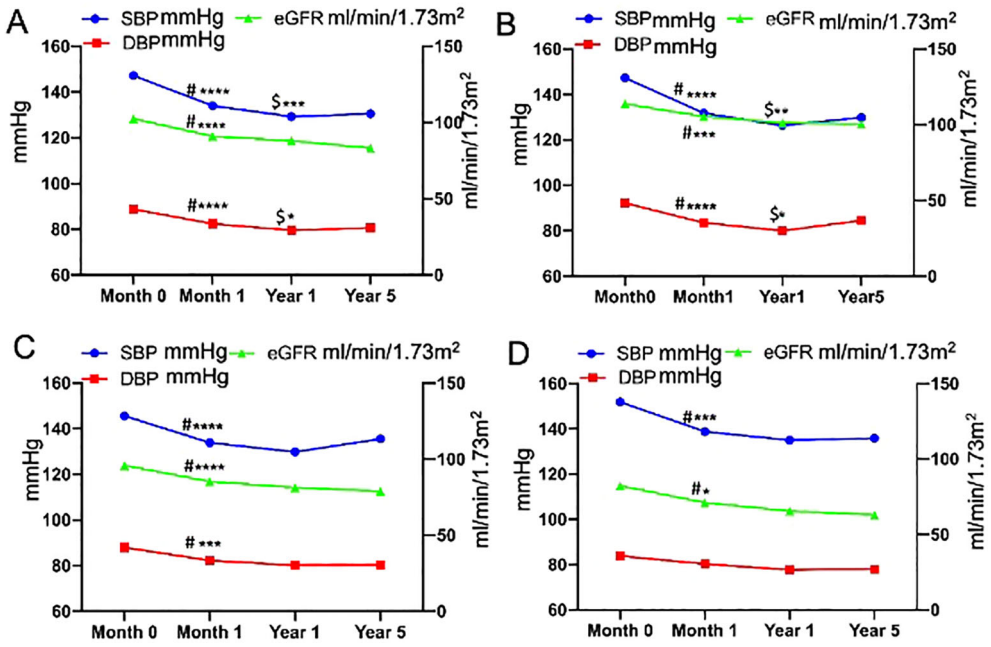

FIGURE 1
Longitudinal changes in blood pressure and estimated glomerular filtration rate before and after adrenalectomy [**(A)**, all patients; **(B)**, patients aged <40 years; **(C)**, patients aged 40–60 years; and **(D)**, patients aged ≥60 years]. $^{\#}$Month 0 vs. Month 1, $^{\$}$Year 1 vs. Month 1, ****P<0.0001, ***P<0.001, **P<0.01, *P<0.05.

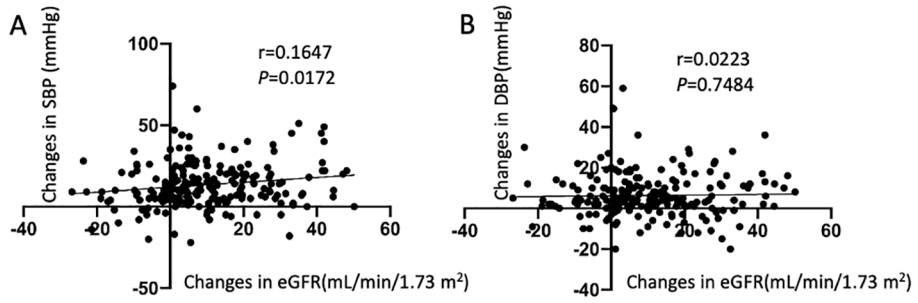

FIGURE 2
Correlation between the changes in estimated glomerular filtration rate (eGFR) and the changes in blood pressure. **(A)** Relationship between changes in systolic blood pressure and eGFR; **(B)** Relationship between changes in diastolic blood pressure and eGFR.

(preoperative eGFR– 1-month postoperative eGFR) (Figure 2), and the eGFR declined along with the decrease in BP. There were no significant differences in the changes in BP and eGFR among the three age groups, either in the short- or long-term after adrenalectomy (Table 2). Only the change in SBP in patients aged <40 years showed a significantly positive correlation with the change in eGFR (Supplementary Figure 1).

Univariate logistic regression analysis demonstrated that *KCNJ5* mutation status was significantly negatively correlated with the occurrence of CKD (odds ratio [OR], 2.825; 95% confidence interval, 1.123–7.110; *P*=0.027) (Table 3). However, it did not correlate with the changes in SBP, DBP, and eGFR, respectively. No significant differences were observed in CKD occurrence among the three age groups.

After adrenalectomy, the proportion of patients aged ≥60 years who experienced a drop in eGFR of >30% was higher than that in the younger groups, although this difference was not statistically significant (Supplementary Figure 2). The proportion of eGFR decreased by >30% or eGFR<60 mL/min/1.73 m^2 after adrenalectomy in patients with *KCNJ5*-WT was higher than that in patients with *KCNJ5* mutation. Additionally, the percentage of patients with postoperative hyperkalemia was also higher in patients with *KCNJ5*-WT than in patients with *KCNJ5* mutation (Table 4).

TABLE 2 Short- and long-term decrease in blood pressure and eGFR in different age groups.

	<40	40–60	≥60	*P*
SBP (mmHg)				
Short-term Long-term	3.8 ± 14.1 20.5 ± 22.3	3.1 ± 17.5 14.2 ± 19.7	0.9 ± 17.8 13 ± 18.2	0.8315 0.2906
DBP (mmHg)				
Short-term Long-term	0.7 ± 12.7 11.3 ± 17	2.8 ± 12.1 7.5 ± 12.6	2.4 ± 9.8 6.7 ± 7.3	0.9048 0.3567
eGFR (mL/min/1.73 m^2)				
Short-term Long-term	9.4 ± 15.7 14.8 ± 14.8	9.9 ± 16.5 15 ± 15.4	11.1 ± 15.9 17.6 ± 15.9	0.9207 0.7682

Short-term: pre-operation–1-month post-operation; long-term: pre-operation–5-years post-operation. eGFR, estimated glomerular filtration rate; SBP, systolic blood pressure; DBP, diastolic blood pressure.

Risk factors for postoperative renal function deterioration

Characteristics that were significant in the univariate analysis (*P*<0.1) were included in the multivariate analysis. Univariate logistic regression analysis showed that male sex, age, HT duration, preoperative SBP, PAC, and *KCNJ5*-WT were significantly associated with renal function deterioration (a decrease in eGFR at 1 month postoperatively ≥30% of the preoperative eGFR). Multivariate analysis revealed that HT duration, preoperative SBP, and PAC were predictors of renal function deterioration after adrenalectomy (Table 5).

Discussion

In this study, we presented the longitudinal changes in eGFR before and after adrenalectomy in patients with PA, focusing on different age groups. We also identified the risk factors for postoperative eGFR decline in these patients. Furthermore, our

TABLE 3 Relationship between the KCNJ5 mutation status and changes in blood pressure and renal function.

Variable	Univariate model	
	OR (95% CI)	*P* value
△ SBP (mmHg)	0.999 (0.976–1.023)	0.939
△ DBP (mmHg)	1.016 (0.983–1.050)	0.349
△ eGFR (mL/min/1.73 m^2)	1.000 (0.979–1.022)	0.992
CKD (eGFR<60 mL/min/1.73 m^2)	2.825 (1.123–7.110)	0.027

△ SBP, changes in systolic blood pressure between pre-op and 1-month post-op; △ DBP, changes in diastolic blood pressure between pre-op and 1-month post-op; △ eGFR, changes in diastolic blood pressure between pre-op and 1-month post-op; OR, odds ratio; CI, confidence interval; eGFR, estimated glomerular filtration rate; SBP, systolic blood pressure; DBP, diastolic blood pressure; CKD, chronic kidney disease.

TABLE 4 Changes in renal function in patients with KCNJ5-WT and KCNJ5 mutations.

Characteristic	KCNJ5 wild type (n=46)	KCNJ5 mutant (n=164)	*P*
eGFR decreased >30%, n (%)	11 (23.9)	19 (11.6)	0.035
eGFR<60 mL/min/1.73 m^2(%)	9 (19.6)	13 (7.9)	0.027
Hyperkalemia, n (%)	5 (10.8)	5 (3.0)	0.028

eGFR, estimated glomerular filtration rate.

findings suggest that somatic *KCNJ5* mutation may confer a protective effect against renal dysfunction after adrenalectomy.

Previous studies have reported that adrenalectomy reduced long-term mortality, major cardiovascular events, and incidence of congestive heart failure (26). Additionally, it also lowers incident atrial fibrillation in PA patients at long term (27) and improves metabolic outcomes (28). But studies on long-term postsurgical renal outcomes are scarce. Patients with PA have a higher risk of renal impairment and a greater prevalence of proteinuria than do patients with essential hypertension (EH) (29, 30). Sechi et al. (31) reported a decrease in eGFR and albuminuria during the initial 6-month treatment (surgical or medical treatment) in both the PA and EH patients, with a significantly greater change observed in patients with PA than in patients with EH. The subsequent rate of decrease in glomerular filtration was comparable between the two groups, whereas albuminuria did not progress further during the remainder of the follow-up period, indicating that renal dysfunction may be partially reversible with PA treatment. These findings underscore the importance of early PA identification for the effective prevention of long-term renal complications. Our findings indicate that eGFR decreases significantly during the initial 1-month period after adrenalectomy compared with that before surgery in all patients. In patients with PA, long-term aldosteronism leads to high blood volume, which increases BP and glomerular filtration. Excess aldosterone helps maintain eGFR and masks underlying structural renal damage. Accordingly, renal impairment can be revealed once aldosterone levels dramatically decrease after adrenalectomy (13, 32). We further found that a decrease in postoperative BP positively correlated with a decrease in eGFR, particularly in younger patients. However, no further significant reduction was noted in the long-term follow-up (1 and 5 years) after adrenalectomy across all age groups. We conclude that the underlying mechanisms may be associated with rapid aldosterone reduction, postoperative hypovolemia, an abrupt drop in BP, and renal hypoperfusion after adrenalectomy. Over time, all aforementioned factors achieve a new equilibrium. Considering these observations, monitoring postoperative BP changes is crucial. Considerations include discontinuing or reducing antihypertensive drugs 1–2 days pre-surgery, increasing postoperative fluid intake, and adopting a sodium-rich diet to prevent excessive BP drops, which may mitigate renal function decline.

TABLE 5 Risk factors for postoperative renal function decline 1 month after adrenalectomy.

Variable	Univariate model		Multivariate model	
	OR (95% CI)	*P* value	OR (95% CI)	*P* value
Male sex	2.135 (0.927–4.915)	0.075	1.611 (0.615–4.225)	0.332
Age	1.035 (1.001–1.070)	0.043	0.996 (0.947–1.049)	0.891
HT duration	1.081 (1.034–1.131)	0.001	1.085 (1.014–1.160)	0.018
BMI	1.111 (0.992–1.244)	0.07	1.158 (1.000–1.341)	0.050
SBP	1.033 (1.008–1.057)	0.008	1.037 (1.009–1.066)	0.009
DBP	1.016 (0.984–1.049)	0.339		
Serum K^+	0.775 (0.335–1.792)	0.551		
PAC	1.001 (1.000–1.003)	0.045	1.002 (1.000–1.003)	0.031
PRA	1.159 (0.732–1.824)	0.524		
eGFR	0.994 (0.974–1.013)	0.513		
KCNJ5	0.432 (0.189–0.988)	0.047	0.740 (0.277–1.973)	0.547

HT, hypertension; BMI, body mass index; SBP, systolic blood pressure; DBP, diastolic blood pressure; PAC, plasma aldosterone concentration; PRA, plasma renin activity; eGFR, estimated glomerular filtration rate; OR, odds ratio; CI, confidence interval.

Our study also revealed that the postoperative complete biochemical success rates were comparable among all three groups. In contrast, complete clinical success rates are lower among older patients than among younger and middle-aged patients, which is largely attributed to prolonged hypertension duration and existing vascular lesions in older patients. However, eGFR levels in older patients did not decline sharply after surgery compared with those in younger cohorts, suggesting the potential benefits of surgery in this demographic.

Additionally, we observed postoperative hyperkalemia in middle-aged (5/102) and older (5/34) patients, but not in younger patients. Although the incidence of postoperative hyperkalemia is relatively low in middle-aged and older patients with PA, it remains a concern. Close monitoring of renal function and serum potassium levels immediately after surgery is essential. We also discontinue all antihypertensive agents, including spironolactone and potassium-sparing antihypertensive agents after surgery and add calcium-channel blocker if needed, besides, we encourage patients to follow a low potassium diet for short-term. Long-term changes in BP may be influenced by age, lifestyle, and medication.

Recently, several studies have investigated the risk factors for postoperative renal dysfunction in patients undergoing adrenalectomy for PA, including preoperative eGFR, urinary albumin excretion, ARR, age, serum potassium level, BMI, and hypertension duration (14, 33–35). Kim et al. regarded a high preoperative eGFR is identified as a risk factor for postoperative renal dysfunction, whereas a low preoperative eGFR is associated with a likelihood of developing into CKD postoperatively (34, 36). Yoshioka et al. (37) reported that age was a critical predictor of kidney dysfunction. They found that adrenalectomy might result in a risk of renal impairment in patients with PA aged ≥50 years. However, our findings indicate that preoperative eGFR and age are not primary determinants of postoperative renal function decline.

Haze et al. (38) reported that higher SBP 6 months after PA treatment was associated with a higher risk of renal impairment over time, independent of the BP levels before treatment. Contrary to these findings, our data revealed that higher preoperative SBP was associated with lower postoperative eGFR. Our findings are partly consistent with those of previous studies demonstrating that a longer duration of hypertension, higher preoperative SBP, and elevated PAC levels are significant risk factors for kidney function deterioration after surgery. In addition, in our study, the prevalence of *KCNJ5* mutation was 75.2%, which is higher than that reported in Western countries. We also observed that the presence of somatic *KCNJ5* mutation was inversely correlated to the postoperative occurrence of CKD, suggesting a protective effect of *KCNJ5* mutation against CKD development after unilateral adrenalectomy. Yoshioka et al. (37) also reported that patients with APAs aged ≥50 years who progressed to CKD showed a higher incidence of *KCNJ5* mutation rates (75%). However, in that study, the deterioration of CKD was defined as progression to the CKD category, and the cutoff age was 50 years, which is different from the age classification and definition of postoperative renal function decrease used in our study. In addition, the sample size of their study was relatively small. KCNJ5 mutations are thought to be associated with florid PA phenotype. After unilateral adrenalectomy, the effects of KCNJ5 mutations are abolished, leading to the normalization of potassium levels and dramatically decreasing blood volume. These changes subsequently reduce renal workload and mitigates tubular damage, thereby protecting renal function in the postoperative period. In addition, patients with KCNJ5 mutations are younger, who have a shorter hypertension duration and less severe vascular remodeling. In contrast, KCNJ5-WT patients exhibit the opposite characteristics. Moreover, older patients with PA usually have concomitant EH, which negatively impacts renal function postoperatively. These may be the reasons why KCNJ5 mutations have a protective effect on postoperative kidney function.

Middle-aged and older patients can benefit from unilateral adrenalectomy, despite exhibiting slightly lower pre- and postoperative eGFR levels than younger patients. Therefore, surgical treatment remains advisable for older patients with PA if unilateral PA is confirmed using AVS. Considering the higher incidence of postoperative hyperkalemia in middle-aged and older patients, immediate postoperative follow-up is recommended.

Our study has some limitations. First, this was a long-term retrospective study in which other factors, such as lifestyle, the occurrence of diabetes, or other types of kidney disease, could potentially influence BP and eGFR during follow-up. Second, we assessed changes in renal function solely using eGFR and did not monitor albuminuria. Future studies should include monitoring the urinary albumin/creatinine ratio and 24-h urinary protein quantity during postoperative follow-up. Third, in our study, the follow-up time points were set at 1 month, 1 year, and 5 years after surgery, which may have caused us to miss the rebound in eGFR that occurs in the 1-3 months post-surgery.

In conclusion, our study provides evidence that unilateral adrenalectomy is beneficial for patients with PA, showing favorable outcomes in terms of both biochemical and clinical success, irrespective of age. Preoperative SBP, PAC, and hypertension duration were significant predictors of postoperative renal function impairment. Additionally, *KCNJ5* mutation appears to offer protection against CKD in patients with PA after unilateral adrenalectomy. Attention should be paid to the occurrence of postoperative renal function impairment and hyperkalemia, especially in older patients. These insights are expected to provide valuable guidance for the management of patients with PA.

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 humans were approved by The Ruijin Hospital Ethics Committee of Shanghai Jiao Tong University School of Medicine. The studies were conducted in accordance with the local legislation and institutional requirements. The participants provided their written informed consent to participate in this study.

Author contributions

YM: Data curation, Funding acquisition, Writing – original draft, Writing – review & editing. XT: Data curation, Writing – review & editing. QG: Investigation, Writing – review & editing. JX: Methodology, Writing – review & editing. PG: Formal Analysis, Writing – review & editing. JW: Formal Analysis, Writing – review & editing. LZ: Writing – original draft, Writing – review & editing.

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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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Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fendo.2024.1467742/full#supplementary-material

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EDITED BY
Rosario Ferrigno,
AORN Santobono-Pausilipon, Italy

REVIEWED BY
Salvatore Cannavo,
University of Messina, Italy
Christina Bothou,
Aarau Cantonal Hospital, Switzerland

*CORRESPONDENCE
Liam O'Murchadha
omurchal@tcd.ie

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Female fertility and pregnancy in autoimmune Addison's disease – a mini review

Liam O'Murchadha[1]* and Agnieszka Pazderska[1,2]

[1]Department of Endocrinology, St James's Hospital, Dublin, Ireland, [2]School of Medicine, Trinity College Dublin, Dublin, Ireland

Autoimmune Addison's Disease (AAD) is by far the most common cause of primary adrenal insufficiency in developed countries, occurring more commonly in women compared with men. The condition is associated with a spectrum of disorders affecting fertility and reproductive health. Premature ovarian insufficiency (POI) is a clinical condition defined by cessation of menstrual cycles and menopausal range gonadotrophins before the age of 40 years. This occurs with a prevalence of 1-2% in the general population, but has been estimated at 6-10% for women with AAD. One registry study demonstrated that one-third of those with AAD who develop POI, do so before the age of thirty. The onset of POI precedes or is contemporaneous with the diagnosis of AAD in the majority. It has also been demonstrated that women with AAD are more likely to use hormone replacement therapy. The pathophysiology of POI in this cohort is thought to be primarily through autoimmune mediated inflammation of the ovarian theca cells. In particular, cross-reacting autoantibodies to steroid-producing cells (StCA) have been identified which are present in AAD and POI. That said, when women with POI are excluded, fertility remains significantly reduced. Impaired adrenal androgenesis and resulting sex-hormone deficiency have also been implicated in subfertility in AAD. These lead to suboptimal follicular development. This, in turn, may also affect libido. Despite physiological glucocorticoid replacement therapy, patients with AAD consistently report reduced quality of life compared to matched controls. These factors may affect fecundity and likelihood of conception. Other autoimmune conditions such as hypothyroidism and type 1 diabetes occur with increased prevalence in those with AAD. These conditions have been shown to independently affect reproductive health. This review focuses on the current understanding of the factors and mechanisms impacting fertility in women with autoimmune Addison's disease.

KEYWORDS

Addison disease, fertility, STCA, premature ovarian insufficiency, autoimmune diseases, pregnancy

1 Introduction

Autoimmune Addison's Disease is a life-threatening condition caused by immunological activation and attack of the adrenal cortex, resulting in deficiencies in production of adrenal steroids & their metabolites (1). It is the most common aetiology of primary adrenal insufficiency in developed countries (2) and the condition can occur in isolation or as a component of autoimmune polyendocrine syndrome types 1 (APS-1) or 2 (APS-2), the former being a monogenic disorder caused by mutations in the AIRE gene (3). In most cases, this abnormal immune response is directed at the steroidogenic enzymes of the adrenal cortex, most specifically 21-hydroxylase, antibodies to which are readily measurable in serum of affected patients (4). There is a strong genetic basis, having particular associations with HLA susceptibility loci, such as DR3-DQ2 and DR4-DQ8 (5).

The management of AAD involves lifelong replacement of glucocorticoids and mineralocorticoids, at doses attempting to approximate physiological production of these hormones. However, current steroid replacement strategies fail to adequately match physiological cortisol production and lead to under- and overexposure to cortisol at various times of the day (6). Despite advances in knowledge of the management of this condition, people with AAD have reduced quality of life (QoL) scores and increased cardiovascular risk and premature mortality (7–9).

Adrenal insufficiency of all causes may be associated with adverse effects on fertility in women of reproductive age. This may be as a result of concomitant gonadotrophin deficiency in those with pituitary disorders (10) or due to the combination of a variety of factors such as anovulation and anatomical issues for those with congenital adrenal hyperplasia (CAH) (11). However, while it has been established for more than a decade that women with autoimmune Addison's disease are less likely to give birth (12) as indicated by lower Standardized Incidence Ratio (SIR) for birth after the diagnosis compared with before the diagnosis is made, there is a lack of robust evidence to elucidate the factors behind this. In this review, we summarize the current understanding of factors which may impact fertility & parity in women with AAD; namely premature ovarian insufficiency, the co-existence of other autoimmune disease, the potential role of diminished adrenal androgen production, psychosocial and factors related to libido and finally outcomes of pregnancy in this population group.

Below, we focus on 1) the factors pertaining to AAD which may affect female fertility and thereafter review 2) the implications of AAD for achieving a successful pregnancy.

1.1 Premature ovarian insufficiency

Premature ovarian insufficiency (POI) is defined as a clinical syndrome involving the cessation of menstrual cycles before the age of 40, elevation of gonadotrophins and low oestradiol (13). Most cases are idiopathic (14). POI is of clinical importance because of its effect on fertility and there is increasing evidence of its negative impact across a range of long-term health outcomes.

A recent individual patient meta-analysis of 15 observational studies (mostly prospective cohorts) reported that the risk of cardiovascular diseases was higher in women who had premature menopause compared with those whose menopause was at the age of 50-51 years (15). Women with POI are more likely than the general population to experience sexual dysfunction and depression (16). POI has been shown to be associated with lower bone mineral density scores compared with women who have menopause >40 years (17) and there is some evidence of increased rates of cognitive decline in women with POI (18).

The pooled prevalence of POI in the general population has been estimated in a recent meta-analysis of 31 studies (19) at as 3.7% (95% confidence interval: 3.1-4.3), with higher prevalence in low to medium Human Development Index countries. It has been reported that autoimmunity is implicated in 5 to 30% of cases of POI.

Premature ovarian insufficiency and early menopause occur more frequently in patients with primary adrenal insufficiency with estimates of 6-20% in this population (20–22). An observational population-based cohort study of the Norwegian National Addison Registry examined the records of 461 women with autoimmune Addison disease (23) and reported a prevalence of POI of 10.2%. One-third of these women developed POI before 30 years of age. Interestingly, in this cohort women with POI were more likely to have a longer duration of AAD than those without POI (26.8 vs 20.1 years, $P = .003$). There was a strong positive correlation between age at diagnosis of AAD and age at menopause ($P < .001$). It had previously been reported that even for AAD patients without POI, the median age of menopause was almost four years younger than for the general population (24).

Women with AAD because of autoimmune polyendocrine syndrome type 1 (APS-1) are reported to be at highest risk of developing POI, in excess of 40% (20).

The underlying mechanism to explain the high prevalence of POI in women with AAD is due to autoimmune oophoritis, itself due to the presence of autoantibodies against steroid producing cells (StCA) (25, 26). Steroidogenic enzymes are expressed in both the ovary and adrenal cortex. StCA antibodies are found in the majority of women with both conditions and the most important include steroid 17α-hydroxylase (17α-OH) and cytochrome P450 side-chain cleavage enzyme (P450scc) (27). Falorni (25) studied 57 patients with POI without adrenal insufficiency and 24 women with POI in association with AAD. In that study, 87% of women with POI and AAD had measurable steroid-cells antibodies, compared to none in the POI without associated adrenal insufficiency cohort.

The potential for using measurements of StCA to predict later developed of POI could be of clinical utility. De Ballis (28) followed 33 women under the age of 40 years with AAD, but without clinically overt POI, annually for a ten-year period to investigate the predictive capacity of StCA for the development of overt ovarian dysfunction. All women in the group with StCA at high titer at the start of the study (>1:32) developed ovarian failure in the follow up period, whereas none of the women without StCA at baseline either developed these antibodies or developed menstrual dysfunction

over the duration of the study period. Such evidence raises the promise of measuring StCA in women with AAD to help clinically guide those patients who might most benefit from fertility preservation treatment.

Erichsen et al. (22) used Standardized Incidence Ratio (SIR) for birth to assess changes in fertility & parity before and after a diagnosis of AAD. The SIR for birth before the women were diagnosed was 0.97 (CI, 0.845–1.095). After AAD diagnosis, this SIR fell to 0.69 and even when the subgroup with POI was excluded the number of observed births remained well below the expected rate, with an SIR of 0.72. Clearly, such results would point to other factors in play in reduced fertility in women with AAD, beyond POI.

It must be pointed out that, to date, there is no reliably effective way of restoring ovarian function for these women. POI likely presents as a spectrum of disease, with some women achieving a successful pregnancy without any specific treatment. Van Kasteren (29) reported in 1999, that women with POI still have a 5-10% chance of conceiving after the diagnosis is established.

There has, though, been increasing interest in evaluating interventions which might preserve ovarian function for longer. One randomized controlled trial (30) assessed DHEA supplementation versus placebo across several parameters. At the end of the treatment period, there was no significant difference in AMH concentrations, FSH levels or return of menses between the two groups. There was a higher antral follicle count (AFC) observed in the DHEA group, but the clinical significance of this in such a small study group (n=22) is unclear. Intraovarian injection of autologous platelet rich plasma (PRP) has been investigated in two observational studies (31, 32). In both studies, transvaginal PRP lead to a statistically significant increase in AMH and antral follicle count. In the former, 7% of patients achieved spontaneous pregnancy after PRP treatment. Randomized controlled trials to evaluate this intervention are clearly required, before one can comment on its effectiveness.

Finally, there are isolated reports of restoration of ovarian function in those being treated with immunomodulatory interventions for other indications. One such notable example (33) concerns a woman with AAD who had a 13-year history of POI, demonstrated by amenorrhea, hypergonadotrophic hypogonadism and evidence of StCA in the serum. Ovarian biopsy confirmed autoimmune oophoritis. Following eight months of treatment with azathioprine for ulcerative colitis, the woman had spontaneous return of menses and became pregnant shortly thereafter. Such cases, though rare, demonstrate the potential of immunomodulatory medications to alter the course of POI, even for women with longstanding amenorrhea.

1.2 Adrenal androgen production

Autoimmune Addison's Disease also results in deficiencies of adrenal androgens and their precursors, particularly DHEAS from the zona reticularis (34). However, unlike glucocorticoids and mineralocorticoids, androgens are not routinely replaced in clinical practice. There is a progressive age-related decline in DHEA concentrations in the healthy female population (35). In both primary and secondary adrenal insufficiency, it has been well established that (36–38) serum testosterone and related precursors are significantly reduced compared to those without the condition. Study of this topic in women is complicated by the fact that commercially available testosterone assays show divergent results when analyzing the low androgen levels typically seen in women compared with men (39).

Androgens are thought to play key roles in the regulation of ovarian follicular development. Follicles are the basic physiological unit, enabling reproduction. This has been revealed in animal models (40, 41) where knockout mice lacking the androgen receptor had reduced fertility, impaired folliculogenesis, reduced ovulation and increased rates of POI. Sen et al. (42) have demonstrated, again in mice, that androgens are promotors of follicular development by regulating the balance between follicular growth and apoptosis. This is achieved by suppressing follicular atresia by inducing the expression of miR-125b, a microRNA which suppresses proapoptotic protein synthesis and by enhancing follicular growth by upregulating FSH expression.

Lower than normal serum androgen concentrations observed in adrenal insufficiency might well suggest hypoandrogenism as a significant factor in the observed subfertility after the diagnosis of AAD, however no studies have examined this to date.

1.3 Psychosocial factors & libido

A number of studies have established the effects of circulating androgens on sexual motivation and responsiveness in women (43–45). Jacobsen et al. demonstrated, in a cohort of 560 healthy women aged 19-65 years, both free testosterone and androstenedione correlated well with measures of sexual desire. In a subgroup of women aged 25-44, total testosterone and DHEAS also correlated with the measures of sexual desire.

Knowing that women with AAD have lower androgen concentrations, it is plausible that sexual motivation and function would be adversely impacted in AAD, but clearly libido is a complex and subjective concept. It may be thought of as the culmination of a multiple hormonal, non-hormonal and environmental inputs. The degree to which AAD effects sexual function in women is not well established, with conflicting results reported by studies.

The most complete picture, to date, is provided by Erichsen et al. (22), who specifically examined sexual function in women with autoimmune adrenal insufficiency using the Sexual Activity Questionnaire (SAQ) (46), which looks at three areas; relationship status, reasons for sexual inactivity, and sexual functioning. In the study, 148 women with AAD did not report a reduction in sexual activity compared with 740 age-matched controls. In fact, women with AAD reported that they were comparatively more sexually active, had higher SAQ scores for sexual pleasure and reduced sexual discomfort; the precise reasons for these findings are not clear.

A smaller study (47) aimed to examine the prevalence of female sexual dysfunction in primary adrenal insufficiency using the

Female Sexual Function Index-6 (FSFI-6), which unlike SAQ includes female sexual desire among other parameters. The other parameters are arousal, lubrication, orgasm, satisfaction and dyspareunia. They identified that women with AAD report significantly increased sexual issues compared with the control group (68.2% versus 8.7%). Specifically, adverse differences in AAD for arousal, desire and sexual satisfaction were reported.

Most recently, the DREAM (48) group investigating dual release versus conventional hydrocortisone treatment reported data on sexual function in adrenal insufficiency (primary and secondary aetiologies), showing that 94% of the patients exhibited diminished sexual desire, however no associations were observed between sexual function and sex steroid levels. At the end of the intervention period, there was no significant difference in Female Sexual Function Inventory (FSFI) scores in either treatment group. For pre-menopausal women, rates of sexual activity seen were similar to that previously reported for healthy populations, but rates were significantly reduced in the postmenopausal group; the authors postulate their findings imply that for postmenopausal women specifically, there is a comparatively greater physiological role for adrenal androgens in sexual motivation relative to ovarian androgens.

Reduced quality of life (QoL) scores are consistently reported by women with AAD, despite adequate glucocorticoid and mineralocorticoid replacement (7). The effect of reduced QoL on libido and sexual function in this specific population has not been studied.

In summary, questions relating to sexual function in AAD have not been satisfactorily answered to date. Despite low androgens being associated with reduced sexual desire in the general population, such correlations in adrenal insufficiency have not been consistently demonstrated.

1.4 Co-existence of other conditions

Women with AAD are at much higher risk of developing other autoimmune endocrinopathies (3, 49). Addison's disease is a major component of the autoimmune polyendocrine syndromes APS-1 and APS-2. While AAD can occur in isolation, it is associated with the presence of other autoimmune conditions in 50–80% of cases. Current estimates are that of all people with autoimmune Addison's disease, about two-thirds have an autoimmune polyendocrine syndrome with APS Type 1 accounting for 15% of people with AAD (50). These co-existing autoimmune diseases include autoimmune thyroid disease, type 1 diabetes, gastritis, POI, vitiligo and hepatitis. Thyroid disease (51) and type 1 diabetes (52) are both independently associated with reduced fertility. Thyroid autoimmunity appears to confer an increased risk of miscarriage even with biochemical euthyroidism. One study has identified adverse effects of type 1 diabetes on fertility even before the woman is diagnosed with diabetes (53). Lin et al. have additionally reported in their study of Taiwanese women that type 1 diabetes in association with thyroid disease had a further negative impact on live birth rate.

It seems plausible therefore that the high prevalence of additional diseases seen concurrently with AAD has a contribution to the reduction seen in some aspects of fertility. That said, Bjornsdottir (12) showed in a subgroup analysis that women with isolated AAD also did have a lower parity.

2 Pregnancy

During pregnancy a number of physiological changes in the HPA axis and mineralocorticoid status occur in women without adrenal insufficiency (Figure 1). Firstly, alterations in glucocorticoid metabolism have been demonstrated in a study of plasma and urinary cortisol in pregnancy and the peripartum period (54). It has previously been reported (55) that increases in oestrogen during pregnancy drive elevations in cortisol-binding globulin and therefore elevations in total cortisol. However, Jung et al, in this longitudinal study (54), also demonstrated progressive elevations in plasma free cortisol concentrations and urinary free cortisol measurements through the course of the pregnancy, compared with non-pregnant controls. They found that mean serum free cortisol concentrations were 1.2-, 1.4-, and 1.6-fold elevated during the first, second, and third trimesters, respectively, compared with the control group and that urinary free cortisol concentrations also became progressively elevated, peaking in the third trimester with levels 3.1 times that seen in the non-pregnant group. These results suggest upregulation of the hypothalamic-pituitary-adrenal axis during pregnancy. The placenta was identified as a source of ACTH and CRH in the 1980s (56) and it has recently been discovered (57) that the human placenta releases CRH mRNA packaged within extracellular vesicles into the maternal blood. During pregnancy, CRH appears to play a role in regulation of pregnancy duration with elevated levels occurring in women who deliver preterm, while a lower concentration is associated with an extended length of pregnancy (58).

Mineralocorticoid metabolism during pregnancy is also altered in those with normal HPA axis activity. The entire renin-angiotension-aldosterone pathway is upregulated leading to elevations in aldosterone concentrations (59, 60). One study reported serum aldosterone rise from 8.0 ng/dl in the first trimester to 25.3 ng/dl during the third trimester of pregnancy (P = 0.001) and also demonstrated increased responsiveness of aldosterone production to ACTH (61). In addition, progesterone-induced inhibition of the enzyme 11β-hydroxysteroid dehydrogenase type 2 in the kidney results in decreased renal inactivation of cortisol to cortisone, and subsequently an increased activation of the mineralocorticoid receptor by excess cortisol (62).

The changes in gluco- and mineralocorticoid metabolism have important clinical consequences for women with AAD during pregnancy. Prior to the availability of reliable steroid replacement preparations, maternal mortality was reported to be as high as 35% with 1/3 of infants dying at time of delivery (63). Pregnant women with AAD may be at higher risk of developing adrenal crisis due to for example; hyperemesis during the first trimester or insufficient parenteral glucocorticoid in the puerperium (64). Current guidance emphasizes the need to take account of the increased physiological cortisol production during pregnancy described above, and that an increase in hydrocortisone dose of 20-40% should be implemented,

Summary of physiological changes in adrenal hormones during pregnancy	
Factors affecting glucocorticoid metabolism	• Oestrogen-driven elevations in cortisol-binding globulin and therefore elevations in total cortisol. • Progressive increases in serum and urine free cortisol concentrations throughout pregnancy, likely through overall upregulation of the HPA axis. • Placental derived production of CRH and ACTH. • Progesterone-induced inhibition of the enzyme 11b-hydroxysteroid dehydrogenase type 2, resulting in decreased renal inactivation of cortisol to cortisone.
Factors affecting mineralocorticoid metabolism	• Upregulation of entire Renin-Angiotensin-Aldosterone (RAAS) system, leading to increased aldosterone synthesis. • Increased responsiveness of aldosterone production to ACTH stimulation during pregnancy. • Progesterone-induced antagonism of mineralocorticoids through mineralocorticoid-receptor binding. • Progesterone-induced inhibition of the enzyme 11b-hydroxysteroid dehydrogenase type 2, resulting in decreased renal conversion of cortisol to cortisone. This conversion should ordinarily protect the mineralocorticoid receptor from overstimulation by cortisol.

FIGURE 1
Summary of physiological changes in pregnancy relevant to Addison's Disease.

especially in the final trimester (65). An increased dose of fludrocortisone during the latter stages of pregnancy is also frequently recommended, though robust evidence is lacking.

The outcome of pregnancies in women diagnosed and appropriately treated for AAD has typically been considered as favorable (66). However, a Swedish Registry Study from 2010 (12), found that the diagnosis of AAD was still a risk factor for adverse pregnancy events. This study compared 1118 women aged 15–47 during the period 1973 to 2006, who had a diagnosis exclusively of AAD with age matched women without AAD. Compared with controls, the odds of having childbirth were not significantly reduced in women before the AAD diagnosis but were significantly reduced after the diagnosis was made. The odds of having three or more infants were three times lower among women after their AAD diagnosis compared with controls. No women were diagnosed with AAD while pregnant. Infants delivered 3 years or less before the date of the mother's diagnosis of AAD were more often born preterm (adjusted OR, 2.40; 95% CI, 1.27–4.53). The closer to the AAD diagnosis that the mother delivered, the higher was the risk of preterm delivery, with a five times increased risk 1 yr or less before the mother's diagnosis of AAD. Risk of low birth weight was more than three times higher among infants delivered 3 yr or less before the mother's diagnosis of AAD (adjusted OR, 3.50; 95% CI, 1.83–6.67). Compared with the control group, the risks of caesarean (adjusted OR, 2.35; 95% CI, 1.68–3.27) and preterm delivery (adjusted OR, 2.61; 95% CI, 1.69–4.05) were more than doubled among mothers diagnosed with AAD. Birth weight did not differ between the two groups. There was no statistically significant increased risk of maternal complications observed and the authors stressed that the majority of both diagnosed and undiagnosed AAD ultimately had "normal" pregnancies with infants delivered vaginally, at full term without complications. They also highlighted that physician preference may have been a potential explanation for the increased caesarean section rate.

More recently, Bothou et al. (67), retrospectively analyzed pregnancy outcomes in 113 women with adrenal insufficiency, 44% with AAD. 15 of 55 women with AAD had at least one miscarriage previously, which was significantly lower than for

women with CAH. 8.9% of women had an adrenal crisis during the course of their pregnancy. 16% had a pre-term delivery, which was lower than for both CAH and secondary adrenal insufficiency (32% and 21% respectively).

Schneiderman et al. (68) performed a retrospective cohort study of data pertaining to the pregnancies of almost 8 million women in the USA between 2003 and 2011, 552 of which had adrenal insufficiency. It is important to point out that as this study utilized ICD codes to capture relevant patient data, it has included women with other forms of primary adrenal insufficiency (and potentially secondary AI) besides AAD. There was an increased rate of caesarean section and preterm delivery in the adrenal insufficiency group, though these women were also significantly older that the control population. Contrary to the data from earlier studies above, women with adrenal insufficiency were found to be at higher risk of a variety of adverse maternal outcomes, including premature rupture of the membranes and post-partum infections. Adrenal insufficiency was associated with a statistically significant increase in overall mortality risk with three deaths among 552 patients (OR 22.30, 95% CI 6.82–72.96). This represents a mortality rate of 0.5% for women with AAD in this study. Impaired fetal growth and increase congenital abnormalities were also identified.

These few studies offer some conflicting information on pregnancy and fetal outcomes for women with AAD. The increased rate of caesarean section does seem to be a somewhat consistent finding, though the reasons for this are likely to be complex and multifactorial. There is also evidence of increased risk of premature birth, which would fit well with the postulated role of CRH as one regulator of pregnancy duration. However, in another smaller retrospective questionnaire study of 54 women (69), the investigators did not find any increase in preterm delivery for women with AAD. These conflicting findings reinforce the need for further large, well designed prospective studies of pregnancy outcomes with AAD.

3 Conclusions

In summary, there are many knowledge gaps in our understanding of the extent to which AAD affects female sexual function and reproduction. It is well established that women with AAD are at higher risk of POI, but the literature would suggest that fertility remains significantly reduced, beyond the obvious effects of POI.

While observational studies have reported increased risk of preterm birth and caesarean section, it is still the case one can reassure pregnant women with AAD that the vast majority with well controlled disease will have uneventful pregnancies with no major maternal complications.

There are many potential factors that contribute to the observed reductions in parity after women are diagnosed with AAD. These include the role of androgen deficiency, the increased prevalence of other conditions which may independently have an effect and the impact observed on overall quality of life. However, these theories have not been consistently borne out by published literature to date and there remains many conflicts and contradictions to resolve so that we can provide comprehensive reproductive healthcare to our patients with autoimmune Addison's disease.

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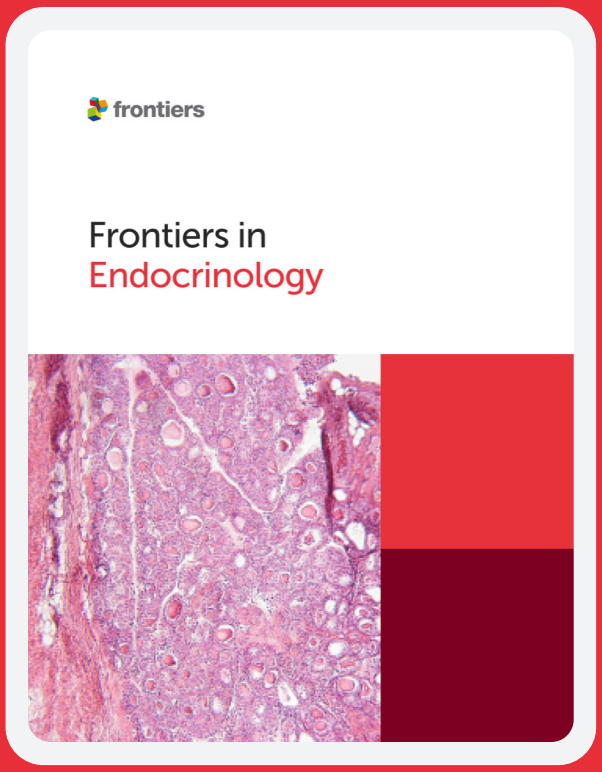

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