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001

Hydrocortisone treatment increases cholesterol availability in prolonged septic mice: effect on adrenal and muscle functioning

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Background
Sepsis is hallmarked by an immediate and sustained decrease in total-, HDL-, and LDL- cholesterol, associated with poor outcome and suggested to be a potential contributor to adrenal failure and muscle weakness in the critically ill. Although the underlying mechanisms are unclear, this sepsis-induced hypocholesterolemia has been postulated to be due to an increased conversion of cholesterol to cortisol. We hypothesized that hydrocortisone (HC) treatment, via reduced *de novo* adrenal corticosterone (CORT) synthesis, can improve the cholesterol availability and as such affect the adrenal gland and skeletal muscle. Our hypothesis was tested in a validated and clinically relevant mouse model of prolonged sepsis-induced critical illness.
Methods
In a catheterized, fluid-resuscitated and antibiotics-treated mouse model of prolonged sepsis (cecal-ligation and puncture), septic mice received either HC (1.2 mg/d) or placebo (PlasmaLyte), whereas healthy mice served as controls ($n=50$). After five days of sepsis, plasma CORT, HDL- and LDL- cholesterol were measured, and the presence of cholesterol esters (Oil Red 0), CD68+ cells and apoptosis was assessed in the adrenal cortex. Steroidogenic capacity of ACTH (100 nM) was evaluated in an *ex vivo* adrenal explantation study. Total body mass loss was measured to evaluate CORT-induced wasting, in addition to *ex vivo* muscle force, myofiber size and mRNA expression of atrogenes *(Trim63, Fbox32, Foxo3),* fibrogenes *(Ctgf, Tgf-B, MMP-9)* and markers of muscle regeneration *(Myostatin, Myf5, Myogenin).* Whole body composition was performed by EchoMRl to investigate loss of lean mass, fat mass and total and free water content.
Results
Plasma CORT was normalized in HC-treated septic mice, whereas the sepsis-induced reduction in plasma HDL- and LDL-cholesterol, and adrenocortical cholesterol ester content was attenuated as compared with placebo-treated septic mice ($P<0.05$), but without improving the blunted ACTH- induced CORT secretion. Increased presence of CD68+ and apoptotic cells was observed in the adrenal cortex of HC-treated septic mice, as compared with healthy controls and placebo-treated septic mice, respectively ($P<0.05$). Total body mass was median 13% further decreased in HC-treated septic mice, as compared with placebo-treated septic mice ($P<0.01$), with no additional effect on sepsis-induced loss of muscle mass, force and myofiber size. The sepsis-induced rise in atrogenes and fibrogenes was not further affected by HC treatment, whereas *Myostatin* and *Myf5lMyogenin* expression was respectively increased ($P<0.01$) and decreased ($P<0.05$), as compared with placebo-treated septic mice. An increased loss of lean mass and water content was observed in HC-treated septic mice as compared with placebo-treated septic mice ($P<0.05$), whereas fat mass was equally reduced.
Conclusion
Hydrocortisone treatment partly restored the sepsis-induced hypocholesterolemia in prolonged septic mice, but at a cost of impaired adrenal function, exacerbated wasting of lean body mass and suppressed muscle regeneration mechanisms.

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002

Protein tyrosine phosphatase receptor kappa regulates glycolysis, lipid metabolism and promotes hepatocyte reprogramming in obesity

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Fat accumulation, lipogenesis and glycolysis are key contributors to hepatocyte metabolic reprogramming and the pathogenesis of non-alcoholic liver fatty liver disease (NAFLD). The molecular mechanisms affected by steatosis and inflammation in the obese state remain unknown. Here we report that obesity leads to dysregulated expression of protein-tyrosine phosphatases in human livers. Protein Tyrosine Phosphatase Receptor Kappa (PTPRK) levels were increased in hepatocytes by steatosis and inflammation in humans and mice and positively correlates with PPARy-induced lipogenic PTPRK- PPARyupregulation is dependent upon Notch activation. PTPRK knockout male and female mice have reduced fat accumulation and liver steatosis after twelve weeks of obesogenic diet feeding. lmmunoblot and qPCR analyses of liver samples showed diminished levels of PPARy, nutrient-sensitive transcription factors (SREP1 and ChREBP), and lipogenic enzymes (FASN and ACC) in obese PTPRK knockout mice compared to controls. Phosphoproteomics analysis in isolated hepatocytes identified specific phosphotyrosine residues in fructose-1,6 bisphosphatase/FBP1 and glycolysis regulation as a target of PTPRK. Computational modeling and in vitro analysis confirmed FBP1-PTPRK interaction and PTPRK-mediated pFBP1 (Y265) dephosphorylation. Metabolomic analysis showed lower levels of dihydroxyacetone-phosphate and gIyceraIdehyde-3-phosphate, while pyruvate, a-ketoglutarate and ribose-5-phosphate were increased in livers from PTPRK knockout mice compared with controls. These metabolic changes in glycolysis/gluconeogenesis, TCA cycle and pentose phosphate pathway revealed metabolic reprogramming in hepatocytes mediated by PTPRK. Adenoviral-mediated overexpression of PTPRK increased glycolytic capacity in mouse hepatocytes. RNA-Seq analysis in human samples demonstrated positive correlations between PTPRK expression levels and glycolytic and lipogenic genes in NAFLD-associated hepatocellular carcinoma. Moreover, glycolytic-dependent hepatoma cell lines showed reduced colony-forming ability after PTPRK silencing in vitro and PTPRK knockout mice developed smaller tumours after diethylnitrosamine-induced hepatocarcinogenesis in vivo. Computational modelling identified potential PTPRK inhibitors, which selectively reduced PTPRK activity and decreased glycolytic rates in hepatoma cell lines and prevented PPARy overexpression in primary hepatocytes. Administration of these inhibitors to high-fat fed mice resulted in reductions in body weight, fat mass, glycemia, and liver fat content, underscoring their potential for managing obesity-associated liver and metabolic dysfunctions. In conclusion, our study defines an unprecedented mechanism for the development of NAFLD, revealing a key role of PTPRK on hepatic lipid metabolism, gluconeogenesis/glycolysis regulation and liver tumour development. We propose PTPRK as a potential target for metabolic liver dysfunction, and the identified inhibitors may represent promising candidates for therapy in obesity-associated liver diseases.

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003

Low-dose anti-thymocyte globulin in combination with verapamil reverses hyperglycaemia in newly diagnosed diabetic NOD mice

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Background
To arrest the immune-mediated destruction of the insulin-producing pancreatic beta cells in type 1 diabetes (T1D), interventional studies have predominantly focussed on two distinct approaches: arresting the ongoing immune response or protecting the pancreatic beta cells. Recently, both approaches using respectively low-dose anti-thymocyte globulin (ATG), an immune-depleting agent, and verapamil, a calcium channel blocker with beta cell protective and immune targeting properties, have demonstrated promising effects as single agents at T1D

onset. However, the primary challenge remains the achievement of a durable response, primarily attributable to the highly immunogenic response and the restricted availability of functional beta cells at symptom onset. Consequently, integrating general immunomodulatory with beta cell supportive therapy represents a promising approach to achieve superior and long-lasting outcomes, particularly when initiated at symptom onset.
Methods
We combined low-dose murine (m)ATG (250 µg per day on day 0 and 3; i.v.) with continuous administration of verapamil (1 mg/ml in drinking water) to study their efficacy in newly diagnosed diabetic non-obese diabetic (NOD) mice.
Results
Low-dose mATG reversed T1D in 39% of mice ($n=7/18$) 7 days after therapy start, but the effect waned to 22% of mice by 8-weeks follow-up. Verapamil stably reversed disease in 20% of mice ($n=3/15$). However, combining low-dose mATG with verapamil induced durable disease remission in 45% of mice ($n=9/20$; $P<0.0001$ vs. control). Especially in mice with mild hyperglycaemia (<350 mg/dl) at disease onset combination reversed 75% ($n=6/8$) of mice compared to 33% in either low-dose mATG ($n=3/9$ mice; $P<0.05$) or verapamil ($n=2/6$ mice; $P<0.05$). While combination therapy resulted in a more pronounced disease reversal, this was not mirrored in the levels of pancreatic insulin content. However, preservation of beta cell function, as indicated by C-peptide levels, was observed only in combination-treated mice. Regarding pancreas inflammation, only combination therapy reduced insulitis severity. Mechanistically, either low-dose mATG-treated group induced lymphocyte depletion (69% reduction vs. control) 3 days after therapy start, recovering by day 14. Flow cytometry analysis revealed a decreased percentage of $CD8^+$ T cells in the blood of either low-dose mATG-treated group at day 3, followed by a recovery of effector memory (EM) $CD44^{high}CD62L^-CD8^+$ T cells by day 14. Moreover, low-dose mATG was associated with an increased frequency of $FoxP3^+(CD25^+)$ regulatory T cells (Tregs) in the blood and pancreatic-draining lymph nodes (PLN) by day 14. Interestingly, only combination therapy had an increased ratio of Tregs-to-activated $CD44^{high}CD8^+$ T cells in PLN.
Conclusion
This is the first study to demonstrate that a short course of lowdose mATG in combination with verapamil protects the beta cells and mechanistically induces a transient imbalance in the frequency of immune cells favouring Tregs. This has to potential to establish enduring immune tolerance and confer sustained T1D remission in future clinical trials.

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004

Androgen deprivation modulates the adrenocortical stress response

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Background and aim
Androgens and glucocorticoids are regulated via the hypothalamus-pituitary-gonadal (HPG) and hypothalamus-pituitary-adrenal (HPA) axis, respectively. We aimed to check possible interference of androgen deprivation with the HPA axis and circulating glucocorticoid levels. Androgen deprivation is most widely used in the treatment of prostate cancer, but is also applied in gender-affirming care and males with hypersexual disorder. Current insights on the possible impact of androgen deprivation on the HPA axis are limited.
Material and methods
We chemically castrated 14-wk-old male mice using the GnRH-receptor antagonist degarelix (DGX) and supplemented them for 2 weeks with either vehicle (VEH) or the androgen dihydrotestosterone (DHT). The control group received no intervention (SHAM). We evaluated the HPA axis in basal condition as well as after stress, where we used the novelty-induced stress test and measured total serum corticosterone levels 10 and 120 minutes after stress induction.
Results
Androgen deprivation was confirmed by 5-fold reduction of seminal vesicle weight in DGX-VEH compared to SHAM, and supplementation of DHT treatment restored this parameter. Total serum corticosterone (CORT) levels at baseline were similar in the 3 different groups (SHAM, DGX, DGX-VEH). After 10 min of induced stress, DGX-VEH had higher total serum CORT compared to SHAM and DGX-DHT. Total serum CORT levels returned to baseline after 120 min in all groups. Furthermore, after 2 weeks of treatment no changes were observed in hypothalamic glucocorticoid receptor (GR) and androgen receptor (AR) mRNA expression. However, hypothalamic corticotropin releasing hormone (CRH) mRNA expression was increased by DGX, but was not affected by DHT treatment. Hepatic corticosteroid binding globulin (CBG) mRNA was 4-fold higher in DGX-VEH and restored to SHAM levels by DHT treatment. Protein levels of CBG in liver were unaffected, but serum levels of CBG were increased in the DGX group and restored to SHAM levels by supplementation of DHT.
Conclusion
Importantly, although androgen deprivation has no effect on basal total serum CORT levels, serum levels of CBG were increased. Even more, androgen deprivation leads to increased total serum CORT in circumstances of stress. We hypothesize that androgen deprivation might additionally sensitize the negative feedback loop of the HPA axis.

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005

Insulin resistance is associated with worse CGM-derived parameters in people with type 1 diabetes

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Background
An increasing number of people with type 1 diabetes (TlD) have co-existing insulin resistance (IR). Our goal was to investigate whether IR is associated with continuous glucose monitor (CGM)-derived parameters (glucometrics) such as time in range (TIR), time above range (TAR), time below range (TBR) and glycaemic variability (CV).
Methods
This is a retrospective analysis of two separate clinical studies (NCT04664036, NCT04623320). In the first database, IR was quantified according to the estimated glucose disposal rate (eGDR) formula: 21.158 + (-0.09 x waist circumference, cm)+ (-3.407 x hypertension)+ (-0.551 x HbAlc, %) and in the second, by performing a hyperinsulinaemic-euglycaemic clamp (HEC). All glucometrics were calculated over 28 days.
Results
A total of 287 subjects were included. The mean age was 46 ± 17 years, 55 % were male, median diabetes duration was 26 [14- 36] years, TIR equalled 57 ± 14 % and the median eGDR was 7.6 (5.6 - 9.3) mg/kg min. The cohort was divided in tertiles based on the eGDR. The tertile of people with the lowest eGDR (highest level of IR) had a higher TAR compared to the tertile with the highest eGDR (33 ± 14 vs 39 ± 15 %, $P<0.05$).). Using linear regression, eGDR was independently associated with TIR and TAR (= 0.016 and -0.021 respectively, $P<0.001$), and this adjusted for age, gender, diabetes duration, smoking status and alcohol intake. Logistic regression analyses showed that an increasing eGDR was independently associated with a higher TIR (OR 1.251, 95% Cl 1.120 - 1.399, $P<0.001$), lower TAR (OR 1.281, 95% Cl 1.146 - 1.443 $P<0.001$) and a higher TBR (OR 0.801, 95% Cl 0.801 - 0.994, $P<0.05$). In the 48 people undergoing a HEC, a higher TBR with increasing insulin sensitivity was present when analysing IR by the HEC-determined glucose disposal rate (M-value), but did not reach statistical significance.
Discussion and conclusion
Only a limited number of studies investigated the link between IR and glucometrics in TlD (1, 2). The strengths of our study are the detailed characterization of the patient population, the reasonably large number of HEC tests and the high-quality CGM data. To conclude, our study found that a lower IR, measured by eGDR, was independently associated with a higher TIR, lower TAR and a higher TBR. No significant association between glucometrics and the M-value was observed, probably due to a lack of power. These data suggest that measuring and targeting IR might benefit individuals with TlD in optimizing their glycaemic control.
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006

Effects of long-term testosterone treatment in transgender people without gender-affirming surgery: the elantes study

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Background
Since pelvic gender-affirming surgery (GAS) is no longer required for legal gender change, we expect a growing number of transgender men and gender nonbinary people who refrain from pelvic surgery. Little is known about the effects of long-term testosterone use on the reproductive organs. Polycystic ovaries and endometrial changes have been described in previous histology-based studies. Furthermore, cervical cancer screening (CCS) is still recommended in sexually active individuals with a cervix. Transgender men face several barriers to participate in classic CCS initiatives, which involve a pelvic examination. Different methods for CCS pose a promising alternative.
Methods
The ELANTES *(Effecten van Langdurig Testosteron)* study was a combined cross-sectional and retrospective study in adult transgender men or gender nonbinary people without previous GAS who started testosterone at least five years ago. A pelvic magnetic resonance imaging (MRI) was performed to map the anatomical changes in the reproductive organs. If pelvic MRI was already performed in the past, consent was obtained to assess the results. Additionally, human papillomavirus (HPV) detection in self-collected urine and vaginal samples as an alternative to the classic CCS was explored. Participants were invited to perform a Colli-Pee® urine sample and/or an Evalyn® vaginal brush. Quantitative polymerase chain reaction (qPCR) was used to detect HPV DNA. They also completed a short questionnaire.
Results
A total of 22 transgender men were included in the study. Nineteen of them participated in CCS. Mean age was 31.0 years. Mean duration of testosterone therapy was 70.0 months. Mean ovarian volume was 5.2 mL and mean follicle count was 12.7. Depending on the criteria used, 18.2-59.1% of participants fulfilled criteria for polycystic ovarian morphology (PCOM) diagnosis. Mean endometrial thickness (ET) was 3.3 mm. A thin ET (1-4 mm) was seen in 81.8% of participants. The remaining 18.2% had an ET of 5-7 mm. Intermediate-risk HPV prevalence was 11.7% for the Colli-Pee® samples and 17.6% for the Evalyn® brushes. No high-risk HPV was detected. The use of these self- collection devices was well-received and favoured over classic CCS by the participants.
Conclusion
No clinically relevant alterations in the reproductive organs were observed on MRI imaging in this small cohort of adult transgender men using long-term testosterone, who did not undergo pelvic GAS. Self-collected urine samples and vaginal brushes showed low prevalence for intermediate-risk HPV. No high-risk type HPV was detected. Self-sampling provides a unique opportunity to reach a population that is otherwise difficult to reach for CCS.

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007

Dose reduction of cyproterone acetate in trans women and the effect on patient- reported outcomes: results from the enigi study

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Context
Cyproterone acetate (CPA) is an androgen receptor blocker often used for testosterone suppression as a part of gender-affirming hormonal treatment (GAHT) in transgender women. In recent years, more concerns have been raised towards an increased risk of meningioma development, linked to CPA use in higher doses (25 mg or more per day).
Objectives
To determine if lower doses of CPA are equally effective in maintaining adequate testosterone suppression and feminization without increasing unwanted effects, measured by patient-reported outcomes.
Design
prospective cohort study
Methods
This longitudinal study was conducted at a specialized tertiary gender identity clinic in Ghent, Belgium. The participants were trans women ($n = 72$) taking a low (defined as 10 or 12.5 mg) or a high (defined as 25 or 50 mg) daily dose of CPA in combination with estrogens. Validated questionnaires assessing body image (Body Image Scale) and hormonal symptoms (Menopause Rating Scale) were used to measure patient-reported outcomes.
Results
After three months of GAHT, all but one participant had a suppressed serum testosterone, irrespective of CPA dose. Satisfaction with breast development was non-inferior in the low-dose CPA group compared to the high-dose CPA group ($P = 0.078$) after one year of GAHT. Hormonal symptoms evaluated through MRS after three ($P = 0.676$) or 12 months ($P = 0.684$) did not differ significantly between the low-dose and the high-dose CPA group. Sub-analysis for participants who decreased the dose of CPA from 25 to 12.5 mg during the first year of hormonal treatment showed an increase of hormonal complaints (evaluated by MRS), though not statistically significant.
Conclusion
Lower doses of CPA (10 or 12.5 mg per day) are equally effective in suppressing serum testosterone while maintaining an adequate feminization, measured by breast development satisfaction (Body Image Scale), in the absence of additional hormonal complaints after 3 or 12 months of GAHT.

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008

Post-hypoglycemic hyperglycemia are highly relevant markers for stratification of glycemic variability and remission status of pediatric patients with new-onset type 1 diabetes

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Aims
Recently, our team introduced the concept of post-hypoglycemic hyperglycemia (PHH) in the context that recovery of hypoglycemia impacts cardiovascular risk. GLUREDIA study aimed to evaluate whether PHH parameters correlated with glycemic homeostasis during the first year after type 1 diabetes onset and helped to distinguish pediatric patients undergoing partial remission or not.
Methods
In the GLUREDIA study, longitudinal values of clinical parameters, continuous glucose monitoring metrics and residual beta-cell secretion from children with new-onset type 1 diabetes were analyzed for one year. PHH is defined as any hypoglycemia followed within two hours by hyperglycemia. PHH parameters were calculated using an in-house built algorithm. Cross-sectional correlations between PHH parameters (i.e., PHH frequency, PHH duration, PHH$\subset$AUC$<$/SUB$>$) and glycemic homeostasis markers were performed using adjusted mixed-effects models.
Results
PHH parameters were strong markers to differentiate remitters from non-remitters (all $P < 0.001$), the most sensitive being PHH/Hyperglycemia duration ratio (cut-off < 0.02, sensibility: 86%, specificity: 68%). Among those, PHH$\subset$AUC$<$/SUB$>$ correlated with clinical parameters and continuous glucose monitoring metrics and inversely correlated with residual beta-cell secretion (all $R^2 > 0.22$, $P < 0.001$). Furthermore, combination of PHH parameters identified three groups of patients that might benefit from distinct therapeutic management. Finally, patient classification into four glucotypes, as previously described, independently validated PHH parameters as reliable markers of glycemic homeostasis and improved the segregation of patients with intermediate values of $IDAA_{1C}$ and $CPEP_{EST}$.
Conclusion
PHH parameters are new minimal-invasive and easily assessed markers of partial remission and glycemic homeostasis during the first year of type 1 diabetes that allow patient-specific therapeutic management.

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009

A retrospective study of people with familial hypercholesterolemia in a Belgian lipid clinic

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Background
Familial hypercholesterolemia (FH) is a genetic disease characterized by hypercholesterolemia and premature cardiovascular events. Early diagnosis and treatment can strongly reduce the cardiovascular burden.
Objective
We aim to describe the characteristics of patients with heterozygous FH followed in a tertiary hospital in Belgium.
Methods
We retrospectively studied a cohort of 321 patients with definite heterozygous FH who visited the UZ Leuven lipid clinic at least once between 1/1/2016 and 31/12/2020. Sociodemographic, clinical, and biochemical data were collected and analyzed. Data are represented as mean ±SD.
Results
The age at time of diagnosis of FH was 39 ±18 years. Patients with atherosclerotic disease (secondary prevention, 88/321) were older ($P<0.001$) and more often male ($P<0.001$). They had a higher body mass index ($P<0.001$), prevalence of (pre) diabetes ($P<0.001$) and hypertension ($P<0.001$) and had lower serum levels of low-density lipoprotein-cholesterol (LDL-C) ($P<0.001$) than individuals without atherosclerotic disease (primary prevention). A BMI >25 kg/m^{2}was associated with male gender ($P=0.007$), higher age ($P<0.001$) and a higher prevalence of (pre)diabetes ($P<0.001$), hypertension ($P<0.001$), liver steatosis ($P=0.001$), sleep apnea ($P=0.003$) and cardiovascular events ($P=0.002$). Moreover, significantly higher triglycerides ($P=0.002$) and lower HDL cholesterol levels ($P<0.001$) were seen in individuals with BMI >25kg/m^2 whereas the percentage of smokers ($P=0.107$), and LDL ($P=0.693$) and total cholesterol ($P=0.675$) were similar between the BMI categories. The average LDL-C in both primary (109 ± 53 mg/dl) and secondary (81 ±63 mg/dl) meet the targets of LDL-C as proposed by the 2019 ESC/EAS guidelines for the management of dyslipidemias. However, LDL-C levels in the subgroup of patients treated with PCSK9 inhibition therapy, and especially in the triple therapy group (combination of statin, ezetimibe and PCSK9 inhibitor), were markedly lower ($P<0.001$).
Conclusions
In this Belgian cohort, people with heterozygous FH remain undertreated and don't reach LDL-C targets as proposed by ESC. Reaching treatment targets in FH seems possible, although this requires combination treatment (with PCSK9-targeted therapy) in most patients. Earlier diagnosis of FH, more extensive lipid-lowering treatment and reimbursement options and a more holistic approach are needed to lower LDL-C and cardiovascular risk in patients with FH.

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010

Cortical bone assessment and determinants in children and adolescents with Klinefelter syndrome

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Aims
Several studies show a deficit in cortical bone in adults with Klinefelter syndrome (KS). A correlation between the adult cortical bone deficit and impaired bone accumulation during childhood or adolescence remains unclear. Therefore, we evaluated the cortical bone by automated digital X-ray radiogrammetry in KS children and adolescents and evaluated potential associations with hormonal, radiographic, and anthropometric factors implicated in bone mineral accumulation in childhood and adolescence.
Methods
Automated bone age readings at the left hand and wrist by the BoneXpert method, performed between 2011 and 2021, together with anthropometric measurements were retrieved from 50 KS adolescents (aged between 3.6 and 19 years), none of them treated with testosterone substitution. Bone health index (BHI) by the BoneXpert method and digit 2 and digit 4 lengths by an inbuild measurement program were calculated from the left-hand X-ray. Results of lumbar spine bone mineral density (LS BMD) and routine hormonal measurements of LH, FSH testosterone, estradiol, 25 OH vitamin D and PTH were available in respectively 30/50 and 22/50 patients.
Results
Mean (SD) chronological age (CA) was 12.0 (3.79) and 52% were prepubertal. Mean (SD) height Z-score was 0.68 (1.20). Bone ages (BA) ranged between 3.3 and 19.0 years, showing a median advancement of + 1.2 years. While mean (SD) bone age Z-score (0.07 (1.14)) was not significantly advanced, mean BHI Z-score (-0.56 (0.99)) was significantly decreased ($P<$ 0001). Five patients (10%) had a BHI Z-score below -2. Mean (SD) LS BMD was 0.84 (0.19). All 30 studied patients had a normal BMD Z-score. Of the 14 patients having a Tanner G2 or more, 11 presented with an elevated FSH value, whereas only 4 showed a decreased testosterone value. Twelve (52%) of the 23 investigated patients had a serum 25-OH vitamin D level below 20µg/l. KS males with a BHI Z-score below – 1 ($n=13$) were significantly older (mean age 14.69 vs 11.07) and had significantly lower mean serum 25 OH vitamin D concentrations (11.70 vs 26.19 µg/l) in comparison with those of normal BHI scores ($P<0.001$). BHI Z-score did not correlate significantly with serum LH, FSH, testosterone, estradiol, and the Z-scores of bone age, LS BMD and D2/D4. In six patients BHI and LS BMD Z-scores differed with >2 SDS.
Conclusion
Cortical bone accumulation at the appendicular skeleton assessed by DXR is only slightly reduced in KS children and teenagers. Older age and lower circulating 25-OH vitamin D were clinical at-risk conditions for a lower BHI. BHI Z-scores, which were unrelated to the D2/D4 ratios and the LS BMD Z-scores, cannot be used in clinical practice to predict the LS BMD Z-score.

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011

Development of the KASAI score as a predictive tool for the uni- or bilateral form of primary hyperaldosteronism

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Adrenal venous sampling (AVS) is considered as the gold standard test for primary aldosteronism (PA) subtype diagnosis. However, it is also an invasive and technically challenging procedure. In order to reduce the use of AVS, we propose a new score predicting a unilateral (UPA) or bilateral (BPA) form of PA using biological and radiological parameters. The score was retrospectively developed on a cohort of 72 patients who underwent AVS at the Cliniques Universitaires St-Luc Bruxelles between 1993 and 2021. Based on the AVS, UPA was identified for 51 patients and BPA for 21 patients. Four predictive parameters of uni- or bilateral forms of PA were highlighted by logistic regression analysis and integrated into the KASAI score: minimal serum potassium (<3.0 — 3.0 to 3.4 - >3.4 mmol/l), supine aldosteronemia (<25 - 25 to 45 - >45 ng/dl), aldosteronemia at the end of the saline perfusion test (<20 - 20 to 25 - >25 ng/dl) and the results of the adrenal imaging (no abnormality, unilateral or bilateral abnormality). Depending of the results, 0,1 or 3 points were respectively assigned to each parameter and the sum corresponded to the KASAI score. A score greater than 9/12 identified a unilateral form, a score less than 4/12 a bilateral form, while performing AVS remained indicated for a score between 4 and 9 (grey zone). The KASAI score was calculated for 52 patients in the cohort (some data missing for the others). The score may have avoided the AVS in 40% of the patients (20/52 patients with a score < 4/12 or > 9/12) and correctly identified among them 11 UPA and 8 BPA. Only one UPA patient was falsely identified as BPA. In conclusion, we propose a new clinic-radiological score allowing to bypass AVS in 40 % of the patients with PA. An external validation of the score is however required.

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012

Endocrine manifestations in 22q11.2 deletion syndrome: a retrospective single center cohort study

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Introduction and objective
Patients with the 22q11.2 deletion syndrome (22q11DS) frequently display cardiological and psychiatric diseases, but are also at increased risk for endocrine manifestations. The aim of this study was to evaluate the screening, prevalence, and management of hypoparathyroidism and thyroid disease in patients with 22q11DS, to evaluate the metabolic profile and to compare these results with current literature and guidelines.
Design
We performed a retrospective study of patients with genetically confirmed 22q11DS, followed at the outpatient clinic of psychiatry of the University Hospitals Leuven between 1996 and 2022, resulting in a cohort of 75 patients. Medical history, medication, and laboratory results concerning hypoparathyroidism, thyroid dysfunction, and the metabolic profile were collected.
Results
Of the total cohort, 26 patients (35%) had at least one hypocalcaemic episode. Parathyroid hormone (PTH) was measured during hypocalcaemia in only 12 patients, with 11 having normal or low PTH, confirming a diagnosis of hypoparathyroidism. Seventeen patients (23%) had recurrent episodes of hypocalcaemia. Adherence to the guidelines was low, with 13% of patients having a yearly serum calcium evaluation, 12% receiving daily calcium supplements, and 20% receiving non-active vitamin D. Hypothyroidism was present in 31 patients (44%) and hyperthyroidism in 6 patients (8%). Information on body mass index (BMI) was available in 52 patients (69%), of which 38% had obesity (BMI $>$ 30 kg/m^2).
Conclusion
Hypoparathyroidism, hypothyroidism and obesity are common endocrine manifestations in patients with 22q11DS, but are probably underdiagnosed and undertreated. 22q11DS patients might benefit from multidisciplinary follow-up including an endocrinologist.

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013

Acquired idiopathic isolated adrenocorticotropic hormone deficiency: a descriptive systematic review of a heterogeneous and underreported disease

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Purpose
Deficiency of the adrenocortical axis is, except for glucocorticoid-induced adrenal deficiency, most commonly associated with failure of multiple pituitary axes and is less common isolated. Isolated adrenocorticotropic hormone deficiency (IAD) has been first described by Steinberg et al. in 1954. Acquired idiopathic IAD is defined by a secondary adrenal insufficiency with otherwise normal pituitary function, absence of structural pituitary defects, no history of prolonged glucocorticoid therapy and exclusion of other causes of failure of the HPA axis. Due to the nonspecific clinical presentation and consequently underreporting of IAD, precise data on the prevalence and incidence are lacking. Nevertheless, idiopathic acquired IAD is considered to be a rare disease. In this descriptive systematic review we aimed to analyse the clinical characteristics, association with auto-immune diseases and follow-up of previously published cases.
Methods
A structured search was conducted after developing a search strategy combining terms for acquired (idiopathic) IAD. Articles describing an adult patient presentation with diagnosis of ACTH deficiency using dynamic testing, no deficiency of other pituitary axes and MRI of the brain/pituitary protocolled as normal, were included. Exclusion criteria were cases describing congenital IAD, cases with another etiology for IAD and articles were full text was not available.
Results
In total 41 articles were included consisting of 84 cases of acquired idiopathic IAD. Cases were mostly female (55%) with mean age of diagnosis 53.5 $\pm$ 18 years. Lethargy was the most common presenting symptom (35%), followed by weight loss (25%), anorexia (23%) and myalgia/arthralgia (11%). 30% of cases had an autoimmune disease at diagnosis. The most frequent auto-immune disease was Hashimoto hypothyroidism (17%). Pituitary antibodies were positive in 27% of cases where measured. Most cases of IAD associated with malignancy could be attributed to glucocorticoid use.
Conclusion
Main findings of this descriptive systematic review, including the largest case series of acquired idiopathic IAD to date, suggest an association between acquired idiopathic IAD and underlying auto-immune etiology due to the high prevalence of auto-immune diseases in this population. The value of the presence of anti-pituitary antibodies has yet to be investigated. In this review we could not withhold acquired idiopathic IAD as a form of paraneoplastic syndrome as authors have recently suggested. Our systematic review also highlights the lack of a clear definition and diagnostic work-up. Based on the findings in this review a proposition is made for a flow chart to diagnose acquired idiopathic IAD.

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014

In otherwise healthy young men, Trabecular Bone Score (TBS) is significantly lower in case of insulin resistance: an analysis of the SIBLOS study

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Background
Over the last two decades, type 2 diabetes mellitus (T2DM) has increasingly been recognized as a fracture risk factor, despite the occurrence of normal to even increased bone mineral density (BMD) (1). The dual energy X-ray absorptiometry (DXA) derived trabecular bone score (TBS) is considered to be a proxy of bone quality and has been reported to be diminished in diabetic patients (2) and insulin resistant, non-diabetic postmenopausal women (3).
Objective
To assess whether insulin resistance already associates with TBS in young healthy men.
Methods
999 young healthy men were recruited in the region of Ghent and surrounding communities and were cross sectionally assessed for multiple bone, muscle and metabolic parameters as part of the SIBLOS study. Of 459 participants, aged 35 +/- 5.4 years, data on both TBS and fasting glucose and insulin were available. Insulin resistance was defined by a Homeostatic Model Assessment of Insulin Resistance (HOMA-IR, which is the product of fasting glucose with fasting insulin, divided by a constant) of greater than or equals to 2.17 (4). Statistical significance of DXA-derived parameters between the insulin resistant and insulin sensitive group was assessed using t-tests.
Results
Of the 459 participants, 89 (19.4%) were insulin resistant. Mean TBS +/- standard deviation in this group was 1.42 +/- 0.08, which is slightly lower than the insulin sensitive group: 1.46 +/- 0.08 (P= 0.002). Quantitatively, only total hip BMD tended to be slightly higher in the insulin resistant group (1.09 +/- 0.14 g/cm^2 vs 1.07 +/- 0.13 g/cm^2, P= 0.011), while at the level of the femoral neck and the lumbar spine, no statistical significant difference could be noted (0.89 +/- 0.13 g/cm^2 vs 0.88 +/- 0.13 g/cm^2, P= 0.220 and 1.06 +/- 0.12 g/cm^2 vs 1.05 +/- 0.13 g/cm^2, P= 0.282, respectively).
Conclusion
Despite being years ahead of established T2DM, insulin resistant young men tend to have a lower TBS. However, because of the small order of magnitude, no relevant statements about bone quality on the level of the individual patient can be made based upon TBS alone.
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015

The thiazide challenge test differentiates primary hyperparathyroidism from secondary hyperparathyroidism due to idiopathic hypercalciuria

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Background
Treatment of primary hyperparathyroidism (PHPT) and secondary hyperparathyroidism due to idiopathic hypercalciuria (SHPT-IH) is markedly different. Nevertheless, differentiating one from another remains a challenge and robust diagnostic tools are lacking. The thiazide challenge test (TCT) has been proposed to aid clinicians in their decision making. However, evidence supporting its use is non-existent.
Materials and methods
We performed a retrospective analysis of 25 patients who underwent a TCT at the Ghent University Hospital (Belgium). We assessed serum and urinary samples before and after testing, clinical and imaging outcomes as well as therapy and long-term follow-up to evaluate the efficacy of the TCT. Based on literature, other potentially useful parameters were calculated.
Results
The TCT was considered inconclusive in three cases (12%). PHPT was diagnosed in thirteen (52%), and SHPT in nine (36%) patients. Baseline serum albumin-adjusted calcium (AACa) and serum total calcium (TCa) were similar between patients with PHPT and SHPT-IH. During the TCT, albumin-adjusted calcium (AACa) rose 0,11mmol/l (±0,10) in patients with PHPT and 0,0071mmol/l (±0,10) in patients with SHPT-IH. The change in AACa is significantly different between both groups (one-sided $P=0{,}025$). A similar result was found for serum total calcium (TCa), which rose 0,14mmol/l (±0,12) in patients with PHPT compared to 0,012mmol/l (±0,15) in patients with SHPT-IH (one-sided $P=0{,}024$). The TCT has a calculated sensitivity of 81,8%, a specificity of 77,8% and a likelihood ratio of 3,68. We observed no differences in serum parathormone (PTH) levels and urinary calcium excretion (UCE) between patients with PHPT and SHPT-IH (101,7ng/l (±26,9) vs. 105,7ng/l (±53,8) and 10,9mmol/24 hours (±3,0) vs. 9,4mmol/24 hours (±3,2) respectively). The calcium-phosphorous ratio (Ca/P), the PTH-inhibition rate (PTH-IR) and the parathyroid function index (PF-index) did not differ significantly between patients with PHPT and SHPT-IH during the TCT. Mean serum potassium levels declined from 4,6mmol/l (±0,4) to 3,8mmol/l (±0,4) during the TCT ($P<0{,}001$). No severe hypokalemia (<3,0mmol/l) was observed. Creatinine values did not change significantly during the TCT.
Conclusion
The TCT can aid in discriminating patients with PHPT from those with SHPT-IH based on the rise in serum calcium. It can be easily used in all patients with nephrolithiasis or hypercalciuria, an elevated PTH, and a normal to slightly elevated serum calcium. Notwithstanding mild hypokalemia occurs frequently, we observed no severe side effects. Other variables such as serum PTH, UCE, Ca/P, PTH-IR and PF-index did not differentiate between both groups. Larger prospective trials are necessary to reassess the relevance of different biochemical parameters and the diagnostic potential of the TCT.

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016

SHBG assay performance and the effect of SHBG assay choice on calculated free testosterone

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Objective
To investigate performance of sex hormone binding globulin (SHBG) assays of different manufacturers and the effects of assay choice on calculated free testosterone (cFT).
Methods
Anonymized serum samples of 113 men and 106 women were randomly selected from excess material from the routine clinical lab at the University Hospital of South Manchester. SHBG levels were measured using three different immunoassays (IA): Roche (Cobas e801), Abbott (Alinity) and Siemens (Immulite). Total testosterone levels were determined at Ghent University Hospital using liquid chromatography followed by tandem mass-spectrometry (LC-MS/MS). Free testosterone (FT) levels were calculated using the Vermeulen formula for every SHBG IA. Free testosterone levels were also measured (mFT) using equilibrium dialysis followed by LC-MS/MS (ED LC-MS/MS). Results from the different assays were compared using Friedman test followed by Wilcoxon signed-rank tests with Benjamini-Hochberg correction. Method comparison for SHBG and FT was performed using Bland-Altman and Passing-Bablok analysis. The mean SHBG result and mFT were used as references for SHBG and FT respectively. FT analysis was performed separately for men and women.
Results
Mean SHBG values for all assays were 47.06 (+22.41), 45.86 (+22.22) and 44.57 (+21.04) nmol/l for Abbott, Roche, and Siemens respectively. Mean FT values for men were 7.23 (+2.66), 7.41 (+2.70), 7.51 (+2.73) and 5.23 (12.21) ng/dl for Abbott, Roche, Siemens, and ED LC-MS/MS respectively. Mean FT values for women were 0.30 (+0.15), 0.31 (+0.15), 0.31 (10.15) and 0.22 (+0.12) ng/dl. SHBG results differed significantly between the different lAs ($P<0.001$). Passing-Bablok analysis shows that the Abbott IA had a proportional difference of +3.3% in comparison to the average of the lAs while the Siemens IA had a proportional difference of -2.3%. The Roche IA did not show a significant proportional difference compared to the average. No significant constant differences were observed. cFT results similarly differed between the different lAs and compared to mFT ($P<0.001$) in both men and women. In both sexes a significant proportional and constant difference was observed between mFT and all calculated equivalents. In men, the proportional difference of cFT compared to mFT ranged from +22.3 to +25.2% with a constant difference of +0.82 to +0.9 ng/dl. In women, the proportional and constant differences ranged from +24.6 to +27.9% and +0.026 to +0.029 respectively.
Conclusions
The SHBG IAs showed good agreement with only minor differences between measurements. Subsequently, the effects on FT calculations were limited with minor variation in differences between cFT and mFT. While these differences were statistically significant, they are not expected to affect clinical decision making due to their limited effect.

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017

Unilateral mydriasis as a first presentation of pituitary macroadenoma

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Background
Pituitary adenomas are the most prevalent tumours in the sellar region and can either be functioning, with autonomous pituitary hormone secretion, or non-functioning. Clinically evident adenomas occur in 1 in 1,100 in the general population. Pituitary adenomas have traditionally been subdivided into microadenomas (< 10 mm) or macroadenomas (≥ 10 mm), with macroadenomas comprising 48% of clinically relevant adenomas. Non-functioning pituitary macroadenomas (NFPM) typically present with visual field defects, headache, and/or hypopituitarism secondary to local mass effect. The current recommended first-line therapy for NFPM is transsphenoidal surgery [1].
Case
A 62-year-old female consulted the neurosurgery clinic following a referral by her private practice ophthalmologist and neurologist with complaints of new-onset left unilateral mydriasis. Her medical history consisted of chronic tension headache, arterial hypertension, and diabetes mellitus type 2. She denied recent worsening of her headache, blurry vision, or visual field defects. Physical examination confirmed a unilateral left-sided mydriasis non-reactive to light (Figure 1, panel A). Additional ophthalmologic and neurologic examination was unremarkable. Magnetic resonance imaging (MRI) of the brain showed a large pituitary macroadenoma with invasion of the left cavernous sinus (Figure 1, panel B). Biochemical evaluation showed no signs of hormonal hyper- or hyposecretion. After obtaining informed consent, the patient underwent transsphenoidal endoscopic pituitary adenoma resection. Post-operative computed tomography (CT) scan showed a residual nodular component at the level of the left cavernous sinus, most compatible with tumour remnant. The anatomopathological examination was compatible with a pituitary adenoma. The mydriasis persisted post-operatively.
Discussion
To our knowledge, this is the first case report describing new-onset unilateral mydriasis as the first presentation of a NFPM. However, acute ophthalmoparesis as a presenting symptom of pituitary adenoma has been described before [2,3]. The oculomotor nerve is the most medial cranial nerve in the lateral wall of the

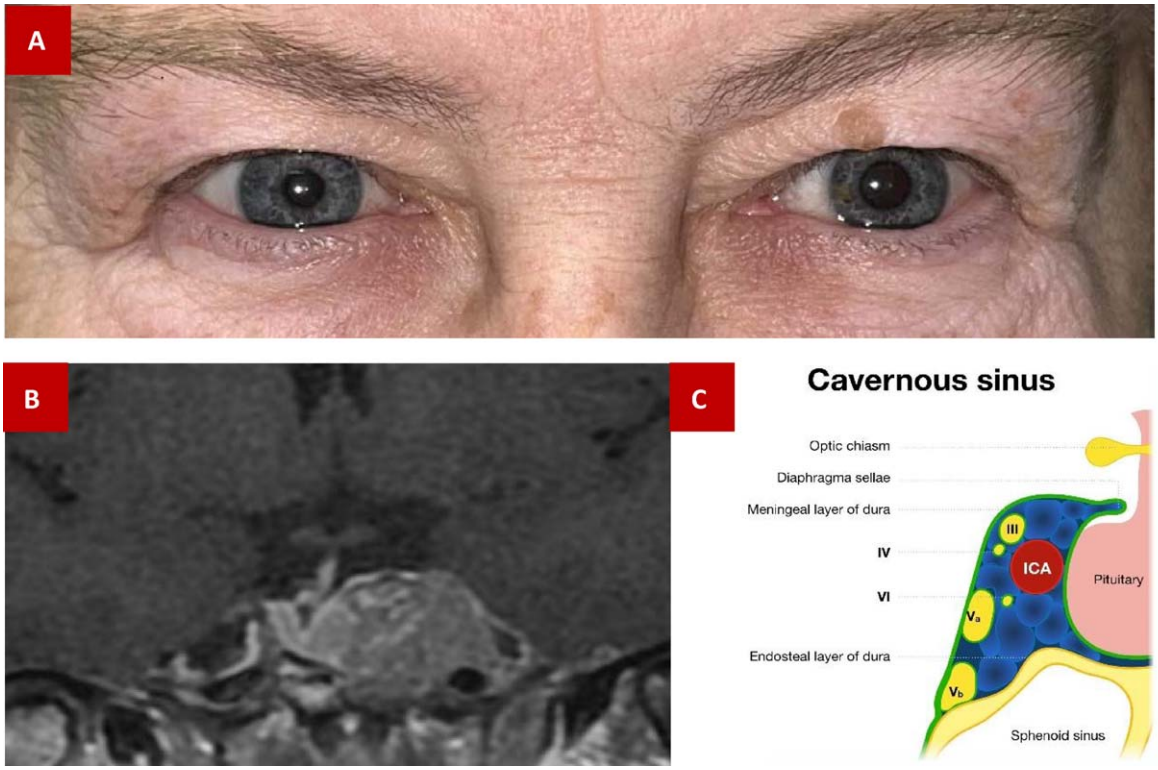

Figure 1. Left unilateral mydriasis (A). Pituitary macroadenoma invading the left cavernous sinus (B). Anatomy of the cavernous sinus showing the relation between the pituitary gland and the oculomotor nerve, courtesy of Frank Gaillard, Radiopaedia.org, rID: 54907 (C)

cavernous sinus (Figure 1, panel C) [2]. It is responsible for most eye movements and carries parasympathetic fibers that innervate the sphincter pupillae muscle. Contraction of this muscle causes miosis. Theoretically, compression of these fibres could result in the loss of papillary constriction, resulting in mydriasis. The anatomical proximity of the oculomotor nerve to the pituitary gland suggests that external pressure by the macroadenoma may have contributed to the observed mydriasis in this case.

Conclusion

Our case report highlights a peculiar presentation of a NFPM with new-onset unilateral mydriasis. While ophthalmoparesis involving the oculomotor nerve has been previously documented, this specific manifestation of oculomotor nerve dysfunction in relation to NFPM has not been reported before. This case emphasizes the importance of considering pituitary macroadenomas as a potential cause of unexplained ophthalmologic symptoms, even in the absence of hormonal abnormalities.

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018

Is this pancreatic NET responsible for an ectopic ACTH-dependent Cushing's syndrome?

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Introduction

Cushing's syndrome (CS) is a medical condition resulting from a chronic excessive exposure to glucocorticoids. ACTH-dependent CS are mostly caused by pituitary adenoma (about 80-85% of the cases) and, in a lower proportion, by ectopic ACTH secretion (about 10-15% of the cases). The exact origin of an ectopic secretion of ACTH is not always easy to identify. The most common locations are bronchus, lung and pancreas but it can also be located in thymus, thyroid and within a pheochromocytoma (1,2). We describe here the case of an ACTH-dependent hypercorticism due to astill unidentified tumor.

Background

A Cushing's syndrome was diagnosed in a 57-year-old man with obesity, melanoderma, uncontrolled diabetes and amyotrophy. He had a medical history of metabolic syndrome with high blood pressure controlled with triple-combination anti-hypertensive drugs and type 2 diabetes treated with Metformin and Semaglutide. Despite the treatment with Semaglutide, he gained 10 kg during the last 5 months. Urinary free cortisol measurements were highly increased (6xULN). The overnight dexamethasone suppression test confirmed CS, plasma level of ACTH was in favor of ACTH- dependent CS and pituitary MRI showed a micronodule of 4mm. CRH and Desmopressine tests were both in favor of ACTH-dependent CS of ectopic origin. The 68Ga-DOTATATE PET/CT showed 2 lesions overexpressing somatostatin subtype 2 receptor (SSTR2): one in the pancreatic tail and one, with a lesser intensity, within a right pulmonary nodule. Our hypothesis was an ectopic ACTH secretion caused by this pancreatic tumor. Biopsy of the lesion demonstrated grade 2 neuroendocrine tumor. ACTH immunostaining was negative. As the general condition of the patient did not allow surgery, a radiofrequency ablation of the lesion was scheduled after a treatment with Etomidate to allow a rapid return to eucorticism. Post-procedure, ACTH and Cortisol didn't decrease as expected and a treatment with Ketoconazole was started. Given these results, a Petrosal sinus sampling was performed and was in favor of an ectopic secretion of ACTH. We therefore suspected that either the treatment had not been sufficiently effective or that there was another source of ectopic ACTH. The pulmonary nodule biopsy demonstrated aspecific inflammatory tissues.

Discussion

Ectopic ACTH secretion is a rare cause of CS (1). When the dynamic tests are in favor of an ectopic secretion, start a complex work to identify the source of the secretion. Medical imagings do not always reveal the origin of the ectopic secretion. Through our clinical case, we would like to discuss the difficulty of the clinical diagnosis, the exams needed and the risk of incidentaloma which can make the discovery of the origin of the secretion even more complex. According to the literature, 16% of the ectopic ACTH secretion remains unidentified. If the disease is located, surgery is the treatment of choice. Medical treatment should be reserved for cases where surgery cannot be offered. In some cases when hypercorticism is still uncontrolled with medication or surgery, the bilateral

adrenalectomy is an option but implies a lifetime hormonal substitution (3). Even when surgery is not possible, the prognosis of paraneoplasic secretion of unidentified tumor remains good (1,4).

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019

In search of the source; a challenging diagnostic work-up of ACTH dependent Cyclic Cushing syndrome

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Introduction

Cyclic Cushing Syndrome (CCS) is a rare entity of Cushing's syndrome (CS), characterized by a cyclic excretion of cortisol, resulting in periods of hypercortisolism intertwining with periods of hypo- or normocortisolism. Diagnosis of CS as such can be challenging, moreover the cyclicity of CCS may necessitate repeated testing. (1)

Case

A 68 year old woman consulted because of periodically appearing edema of the lower extremities. A blood test showed a hypokalemia of 2,04 mmol/l (reference 3,5-5,1mmol/l). At that time she was completely asymptomatic. Clinical examination was, except for obesity, unremarkable. CS was diagnosed based on an elevated 24-hour urinary free cortisol excretion (1117,2µg/24h - reference $<$90µg/24h), a high bedtime salivary cortisol (6,063 µg/dl - reference $<$ 0,107µg/dl) and lack of suppression of cortisol after an overnight 1 mg dexamethasone suppression test (1355nmol/l - reference 50nmol/l). A high ACTH (131 pg/mL - reference $<$46pg/mL), suggested Cushing disease (CD) or ectopic ACTH production. Pituitary MRI was unremarkable. During hospitalization, hypokalemia disappeared together with a normalizing morning cortisol and ACTH. Within a couple of days, a relapse occurred with immeasurably high serum cortisol levels, hyperglycemia and recurrent hypokalemia. FDG-PET-CT showed a hypermetabolic adenopathy at the right lung hilum, where a biopsy showed no abnormalities. Given the suspicion of CD, a sinus petrosus sampling with CRH stimulation was performed with the results being suggestive of a pituitary source of ACTH production. Despite a right hemiphysectomy, hypercortisolism persisted. A 68Ga-DOTATOC PET/CT was performed in search of an ectopic source of ACTH, showing increased uptake at the right lung hilum. A repeat biopsy showed atypical carcinoïd, which was resected. Unfortunately, hypercortisolism persisted. Lanreotide was commenced, but had to be stopped because of intolerance. Repeated episodes of hypercortisolism, intertwined with periods of hypocortisolism with the need for hydrocortisone therapy occurred. Ultimately, a bilateral adrenalectomy was performed. After surgery, hydrocortisone and fludrocortisone therapy was started, after which the patient no longer experienced episodes of leg oedema or electrolyte disorders.

Discussion

CCS is a rare entity of CS, characterized by fluctuating cortisol levels. Like CS, CCS can be associated with ACTH-dependent or ACTH-independent hypercortisolism. CD is the most frequent underlying disorder, followed by ectopic ACTH syndrome. (1) Symptoms do not differ from classical CS, but can fluctuate over time, coinciding with the cortisol levels. (2) In this case, the patient suffered from fluctuating leg oedema, hyperglycemia, hypokalemia in periods of hypercortisolism and hyponatremia in periods of hypocortisolism. In order to establish the diagnosis of CCS, late-night salivary cortisol, 24h urine free cortisol and low dose dexamethasone suppression test can be used. (2) Testing can be normal when tested in periods of normo- or hypocortisolism, advocating for repeat testing, ideally in a symptomatic period. (1) Diagnostic work-up of CCS is the same as in classical CS. ACTH dependent CS requires differentiation of a pituitary vs ectopic source. Pituitary MRI showing an adenoma or a positive sinus petrosus sampling is suggestive of a pituitary source. The latter test should be performed in an episode of hypercortisolism. (2) Imaging modalities for ectopic ACTH-producing tumors encompass CT-scanning, FDG-PET-CT or functional scanning (like 68-Ga DOTATATE PET-CT). (1) Treatment primarily involves resection of the primary tumor. In cases where no (residual) tumor can be found, pharmacotherapy is possible with somatostatin analogues, steroid synthesis inhibitors, dopamine agonists, and glucocorticoid receptor antagonists as possible treatment options. In case of inadequate control, bilateral adrenalectomy, combined with gluco- and mineralocorticoid replacement therapy, offers definitive treatment. (2)

Conclusion

This case highlights the complexity of diagnostic work-up in a patient with endogenous hypercortisolism, moreover complicated by a cyclic course of hypercortisolism. In patients with fluctuating symptoms, CCS should be kept in mind, with testing being performed during episodes of hypercortisolism.

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020

Hypercalcemia during pregnancy, how to manage it, discussion of a case study

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Introduction

Hyperparathyroidism (HPT) during pregnancy is rare but requires a specific approach for its diagnosis HPT is associated with a high incidence of associated maternal, fetal and neonatal complications, including miscarriage (3,5x-fold higher than expected), IUGR, prematurity and pre-eclampsia(1,2,3). The severity of complications is proportional to degree of maternal calcium level.

Case report

We report the case of moderate hypercalcaemia on primary hyperparathyroidism (PHPT) in a 43-year-old patient, 26 weeks pregnant, with a history of renal colic in 2015 and a caesarean delivery at 35 weeks for severe pre-eclampsia. The current pregnancy is marked by several episodes of lipothymia and hypertensive crises, therefore, given her history of severe pre-eclampsia, the patient is currently treated with Asaflow 160 mg and Aldomet 250 mg once daily. Hypercalcaemia was detected for the first time on 14 February 2023 at 14 weeks' gestation, with a blood calcium level of 3.38 mmol/l. Following a lapse in treatment, the first visit to an endocrinologist was on 11 May 2023 at 26 weeks' amenorrhoea. A biological workup was performed on 22/05/2023 and showed hypercalcemia corrected for hypoalbuminemia at 3.2mmol/l. In view of the risks inherent to hypercalcemia during pregnancy, it was decided to perform a parathyroidectomy (PTX) on the patient on 02/06/23. The preoperative localization work-up consisted of an ultrasound scan demonstrating the presence of a 14 x 9mm ovoid nodular lesion in the left lower pole, a localization confirmed by intraoperative palpation. Post-operative follow-up was marked by resolution of hypercalcemia and the absence of hungry bone syndrome. The 2-week post-operative foetal ultrasound follow-up was normal. Concomitantly we observed a postoperative resolution of the patient's hypertension.

Discussion

Current guidelines recommend that pregnant women with PHPT and an albumin-adjusted total calcium level consistenly $>$2.85 mmol/l should undergo PTX at the beginning of the second trimester(4). The purpose of this timing is to reduce the risk of the surgery and miscarriage, which occurs mainly in the second trimester(2). By contrast, there is still some controversy whether mild hypercalcemia is associated with adverse pregnancy outcomes. In te present case, the therapeutic decision was complicated by the fact that the first endocrinology referral took place during the 3rd trimester, a trimester in which operative complications are more common. On re-reading the case history, hypercalcemia was already present during the first pregnancy and may have favoured the occurrence of pre-eclampsia.

Conclusion

Hyperparathyroidism during pregnancy is under-recognized despite its high complication rate. We emphasize the importance of recognizing its presence ideally before pregnancy or as early as possible during pregnancy. If diagnosed, surgery should be proposed especially if the calcemia is above 2.85 mmol/l.

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021

A non-controlled Cushing disease treated with osilodrostat: A case report

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Introduction
Cushing disease (CD) is a rare pathology and associated with serious complications and an increased mortality (standardized mortality ratio 2.8) (1). The mortality risk is twice as high (standardized mortality ratio 5.7) if CD is biochemically not well controlled. Transsphenoidal tumor resection is the first line treatment. However, the need for second line treatment is high, as 5-50% of patients in remission after surgery relapse and consist of medical therapy to lower cortisol levels (2). Currently available medication in Europe and their effectiveness are: cabergoline (35%), pasireotide (44%), ketoconazole (44%), metyrapone (66%) and osilodrostat (81%) of which cabergoline and ketoconazole are available in Belgium (3, 4). Ketoconazole has, however, important side effects of which the most common are: hepatotoxicity (11-19%) and gastro-intestinal intolerance (4-19%) (5). We present you a case of a young women with treatment resistant CD well controlled with osilodrostat.

Case report
A 29-year-old woman was diagnosed with CD after developing spontaneous purpura, weight gain and oligomenorrhea. The laboratory findings showed a two-fold elevated prolactin (42,2 µg/l, <26.5µg/l) with a macro adenoma of 17mm on MRI pituitary. She was treated with cabergoline, resulting in a normalized prolactin level and shrinkage of the adenoma. Curiously, the 24h free cortisoluria (UFC) kept rising and the patient was diagnosed with CD for which she underwent twice a transsphenoidal adenectomy without achieving remission. In between the two surgeries the patient was treated with ketoconazole but experienced gastrointestinal side effects. Stereotactic radiosurgery (Gamma knife 60gy) was performed, but one year later UFC remained elevated (196 µg/24h, < 45). Therefore treatment with pasireotide was started in a dosage of 20mg per month and was complicated by developing diabetes mellitus without improvement of UFC (178µg/24h, <45). Ultimately a bilateral adrenalectomy was suggested, which the patient refused. Osilodrostat was started in compassionate use. After 5 months of systematically increasing the dose to 20 mg daily, eucortisolism was achieved for the first time since diagnosis (in combination with pasireotide) (Fig 1). Patient felt better and diabetes mellitus improved (HbA1c 8 to 5.4%). MRI pituitary 3 months after commencement of osilodrostat showed no remnant growth. However, after 7 months of treatment she complained of joint pains and tiredness and morning cortisol level was decreased to a level of 78.2 µg/dl (ACTH 473 ng/l) for which the dose of osilodrostat was gradually decreased. Currently, the patient is treated with a dose of 11 mg per day.
Discussion
The need for second line treatment to achieve remission in CD is high, but currently available medication in Belgium (cabergoline and ketoconazole) is insufficient and complicated by side effects. Osilodrostat is a new oral medication that inhibits 11beta-hydroxylase, better known as the final step in adrenal steroidogenesis. In 2022, the results of a phase III study were published (LINC4) with inclusion of 73 patients with CD (4). Twelve weeks after treatment 77% treated with osilodrostat showed normalization of UFC compared to 8% in the placebo group. After 36 weeks of treatment, normalization of UFC was achieved in 81% of the total study population. Furthermore, osilodrostat has a beneficial effect on the cardiovascular and metabolic parameters leading to a decrease in weight, fasting plasma glucose, systolic and diastolic blood pressure, and cholesterol. Osilodrostat is generally well tolerated. The most common adverse effects were related to hypocortisolism; anorexia (37.5%), arthralgia (35.4%), and

Figure 1. Evolution of the urinary free cortisol (UFC) level during dose titration of osilodrostat.

nausea (31.3%) and occurred mostly during the dose-titration phase. Osilodrostat is licensed in Europe for the use in CD but not reimbursed in Belgium. No head-to-head comparison between ketoconazole and osilodrostat exists but we conclude that in this particular patient case, osilodrostat had an added value in the treatment of CD.

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022

Primary infertility: a rare presentation of adrenocortical carcinoma

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Background

Adrenocortical carcinomas (ACC) are rare and aggressive endocrine tumours with an annual incidence of 1 to 2 per million.(1) Frequently, patients present with signs of hormonal excess. The majority are cortisol excreting carcinomas, followed by aldosterone or androgen excess.(2) Estrogen secreting ACC are extremely rare. We present a case of estradiol and progesterone excess in primary infertility caused by an ACC.

Case presentation

A 32-year-old woman was referred to the endocrinology department because of persistently high oestradiol and progesterone levels after hormonal stimulation in the context of ICSI (intracytoplasmic sperm injection) due to male infertility. She had no relevant history besides resection of a cystadenoma at the right ovary 3 months before presentation and used 4 mg folic acid daily because of child wish and a family history of cleft lip. Patient had no complaints besides the impression of more pronounced hair at the upper lip and amenorrhea since hormonal stimulation. Sequential biochemical analysis revealed rapidly increasing levels of estradiol and progesterone under hormonal stimulation (recombinant FSH (follitropin alfa) 150 IU daily in a gonadotropin releasing hormone (GnRH) antagonist protocol with cetrorelix 0.25mg daily from day 6 of the stimulation) with persistent elevation of progesterone (see table 1) after cessation of hormonal therapy. Additional testing showed elevated DHEAS (972 µg/dl, ref. 25.9-460 µg/dl), 17-OH progesterone (12.0 ng/ml, ref. 0.35-4.13 ng/ml) and androstene-dione (3.44 ng/ml, ref. 0.37-2.25 ng/ml) with mildly elevated testosterone (64.1 ng/dl, ref. 11-56 ng/dl). Estrone was extremely increased (2450 ng/l, ref. early follicular phase $<$150 ng/l). Inhibin B was low (36.7 ng/l, ref. $<$200 ng/l). Furthermore, morning cortisol was elevated at diagnosis (30.9 µg/dl). Low-dose dexamethasone (1 mg) did not suppress morning cortisol (31.3 µg/dl) and salivary cortisol at midnight was elevated (0.668 µg/dl). ACTH was suppressed in basal conditions ($<$1.51 pg/ml). Urinary normetanephrines and metanephrines were normal. CT scan without contrast enhancement showed an enormous mass of 30 HU, most likely situated at the left adrenal gland with an axial diameter of 12.9 cm (figure 1). Additional PET-CT demonstrated FDG-avidity and central necrosis in this lesion (figure 1) and no evidence of distant metastases. The tentative diagnosis of an estrogen and cortisol secreting ACC was made. After multidisciplinary evaluation the indication for maximal surgical resection was established and the patient underwent laparotomic left adrenalectomy in combination with left nephrectomy, splenectomy and resection of the pancreas tail. Solucortef treatment was started per-operatively. Anatomopathological evaluation confirmed the presence of ACC based on a Weiss score of 5 without vascular or lymph node invasion nor invasion in the adjacent structures. There was a low mitotic index of 5/50 HPF (high power field). After surgery, estrogen, estrone and progesterone levels soon dropped to baseline values and adrenal insufficiency occurred for which hydrocortisone substitution (20-10-10mg per day) was associated. Menstrual bleeding occurred 2 days postoperatively. Because of hypersecretion preoperatively and the tumour volume, treatment with mitotane was started 3 weeks postoperatively with acceptable tolerance. Patient was counselled that pregnancy is contraindicated until 1 year after cessation of treatment.(3) Re-evaluation with PET-CT is foreseen 3 months postoperatively. Discussion: Estrogen secreting ACC account for less than 2% of ACC. Feminizing adrenal tumours are a well-described but rare disease entity in males.(4) To our knowledge, this is the first case in literature of an estrogen and progesterone secreting ACC diagnosed because of primary infertility in a female patient.(5) Caution is needed in case of unexplained infertility or absence of hormonal variation during menstrual cycle in women.

Conclusion

Although extremely rare, estrogen secreting ACC can present in premenopausal women with loss of menstrual cycle. Clinicians should be aware of this rare but severe disease entity.

Table 1. Evolution of hormonal levels. NA: not available

	Start of stimulation	Day 13 of stimulation	Pre-operatively	10 days post-operatively	Reference
Estradiol	55.9	5400	220.5	$<$11.8	19-144 ng/l (follicular phase)
Progesterone	1.14	2.35	3.34	$<$0.21	$<$1.4 µg/l (follicular phase)
Cortisol before 9 am	NA	NA	30.9	3.3	5.3-22.4 µg/dl

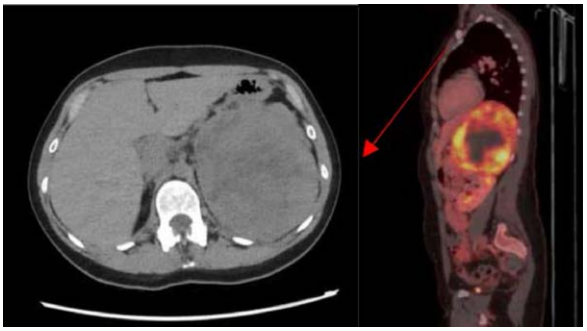

Figure 1. CT-scan without contrast enhancement (left): giant mass of 13 cm at the left hypochondrium. PET-CT(right): FDG-avidity with central necrosis.

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023

A unique cause of hypermetabolic adrenal incidentaloma

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Introduction/Background

Adrenal incidentaloma is defined as an asymptomatic adrenal mass greater than 1 cm, discovered during imaging performed for another purpose[1]. The main goal when discovering such a tumor is to rule out a hormonally active lesion or a malignant condition. Radiological characteristics on non-contrast CT or MRI can help to determine if a lesion is benign. If the lesion nature remains indeterminate after these exams, it is recommended to perform a $^{[18]}$FDG-PET/CT[1]. Here, we report a unique case of benign adrenal incidentaloma with abnormal radiological features and hypermetabolic pattern.

Case summary

A 53-year-old woman was referred to our Department of endocrinology in May 2019 for a bilateral adrenal incidentaloma. The lesions were discovered during an

MRI of the kidney. The radiologist described a left adrenal lesion of 16x15.5mm, without enhancement on T2 weighted images. There was no loss of signal intensity on out-phase imaging and the contrast media washout was delayed. The right adrenal lesion of 10x17mm had a characteristic pattern of adrenal adenoma. The left adrenal lesion was not present on a CT scanner performed in 2015, while the right lesion was stable. Clinical examination revealed weight loss and two centimetric lymphadenopathies : one in the right supra-clavicular area and the other in the left posterior cervical area. Considering the above and that the patient was an active smoker, an additional work-up was carried out. The aldosterone-to-renin ratio was a bit high due to beta-blockers, but aldosterone level was normal. Urinary metanephrines and normetanephrines, morning serum cortisol, 1-mg overnight dexamethasone suppression test, 17-hydroxyprogesterone, and DHEAS were normal. Abdominal non-contrast CT showed a growing left adrenal lesion (20x15mm) of 43 Hounsfield unit. Due to the rapid progression of the lesion and the atypical radiological features, a $^{[18]}$FDG-PET/CT was planned. The exam showed a hypermetabolic lesion (SUV max 7.2; adrenal to liver SUV ratio 1.95), without other suspect lesion. A laparoscopic left adrenalectomy was performed. Histopathologic analysis revealed an intra-adrenal lymphoid organ of 15 mm, localized in the medulla. There were numerous big germinal centers and no cellular atypia. Masson's trichrome stain suggested the presence of a capsule. On immunohistochemistry, B cells (CD20+/CD3-) and T cells (CD20-/CD3+) organization was normal, with a low CD68 staining. The surrounding adrenal tissue showed good corticomedullary differentiation, without any specific lesions. Early post-operative follow-up was unremarkable and the patient did not develop any problems over the following 2 years.

Discussion/Conclusion

Over 80% of the etiologies of resected adrenal incidentalomas are adrenocortical adenoma, adrenocortical carcinoma, pheochromocytoma and myelolipoma. Cystic lesions, ganglioneuroma and metastases account for less than 15% of the total, and the remainder are rare entities[1,2]. Elevated adrenal to liver SUV ratio and SUV max measures on $^{[18]}$FDG-PET/CT are useful tools to distinguish a benign from a malignant adrenal tumor, with a sensitivity ranging from 85% to 100%[1]. Therefore, the hypermetabolic status of the incidentaloma raised the question of a lymphoproliferative disorder. However, this hypothesis was quickly ruled out by the absence of cellular or architectural atypia and normal immunohistochemistry. The only publication reporting a benign intra-adrenal lymphoid structure described an isolated lymphoid follicle, also located in the medulla[3]. We report a bigger and more organized lymphoid structure which mimics a lymphoid organ. However, we were unable to classify it as secondary or tertiary lymphoid organ. Indeed, the adrenal medulla does not usually contain lymph nodes (secondary lymphoid organ) . Furthermore, we did not find surrounding tumor or chronic inflammation, characterizing tertiary lymphoid organs[4]. In conclusion, we describe a unique case of an adrenal incidentaloma suspected of malignancy, which turned out to be a benign ectopic lymphoid organ.

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024

A case of calcitriol-mediated hypercalcemia in a patient with solid tumor

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Case report

A 50-year-old man was referred by his family doctor to the emergency room in September 2020 for intermittent abdominal pain in the right upper quadrant for several months. He also mentioned weight loss and several episodes of fever with chills. The patient was an active smoker, consumed large amounts of alcohol, and had no known medical history, nor taking any medication. At physical examination, the patient appeared pale and cachectic, with tenderness on the right upper quadrant and slight defence, with no diminution of bowel sounds. Laboratory results showed microcytic anaemia (haemoglobin level of 7.6 g/dl) and severe - albumin adjusted - hypercalcemia (3.78 mmol/l). Protein electrophoresis showed no paraprotein (no spike/peak in the gamma zone) nor hypogammaglobulinemia. Biochemical workup (see Table 1) revealed suppressed PTH and PTHrP with elevated 1,25-dihydroxyvitamin. A contrast enhanced CT-scan of the abdomen revealed a heterogeneous mass of 12 cm of diameter in the liver with a possibility of kidney invasion accompanied by a dilatation of the intrahepatic bile ducts and a dilatation of the right kidney cavity. The same lesion was shown on the FDG PET-scan, without any other hypermetabolic lesion. On laparoscopic exploration, a tumoral mass invading the liver, the right kidney, the colon, the inferior vena cava and the duodenum with a walled retroperitoneum was seen. No resection was possible, and a biopsy revealed a sarcomatoid renal cell carcinoma. The patient was treated with hyperhydration and calcitonin. Due to remaining high calcium levels, zoledronic acid was administrated despite unfavourable dentist advice. Taking into account the possibility of a calcitriol-mediated hypercalcemia, 32 milligrams of methylprednisolone a day were initiated, with a certain degree of response. Denosumab was added given the new rise in calcium levels. Figure 1 shows the evolution of the calcium levels according to the treatment. The general condition of the patient continued to decline, and he sadly passed away. Staining for 1-alpha-hydroxylase was later performed on the biopsy, but remained negative.

Discussion

Calcitriol-mediated hypercalcemia is a well-known entity in lymphomas and in granulomatous diseases (1,2). However, case reports of patients with solid tumors and calcitriol-mediated hypercalcemia remain scarce. Recently, Chukir and al. performed a retrospective study on 101 patients with solid tumors and hypercalcemia and showed that 45% of them had an elevated calcitriol, of which 76% also had an elevated PTHrP. This study also highlighted that those patients were rather poor responders to classical antiresorptive therapy, such as bisphosphonates or denosumab. (3) We observed similar findings in our patient. Two recent case reports of patients with calcitriol-mediated hypercalcemia showed an increased mRNAexpression of 1- h and vitamin-D receptors in cells from a gastrointestinal stromal tumor in one, and an expression of 1- h expression by the cells of a liposarcoma in another.(4,5) Our patient didn't have increased PTHrP levels (lab testing was the same and consisted in an immunoradiometric assay on two PTHrP epitopes, without cross reactivity with PTH 1-84, PTH C-term and mid-term), but showed elevated calcitriol levels, suppressed PTH, and low phosphorus levels. No bone metastases were diagnosed, ruling out hypercalcemia due to osteolytic lesions. Staining for 1- h on the biopsy did not show any activity n the patient's tumor. An argument in favour of this theory is the moderate response to high dosage of corticosteroids.

Conclusion

Physiopathology of hypercalcemia of malignancy is complex and might engage several mechanisms. Calcitriol-mediated hypercalcemia should be considered in case of solid tumors and complete biochemical workup remains crucial in order to better understand the underlying mechanisms and to predict poor responders to conventional antiresorptive therapy.

Table 1. Biochemical workup

Parameters	Lab value
Total calcium	3.78 (2.20-2.55 nmol/l)
Albumin	4.1 (3.4-4.8 g/dl)
Phosphate	0.62 (0.75-1.39 mmol/l)
Creatinine	1.12 (0.70-1.20 mg/dl)
Intact parathyroid hormone (PTH)	< 10 (< 49 ng/l)
Parathyroid hormone-related peptide (PTHrP)	< 20 (< 20 pg/l)
Thyroid-stimulating hormone (TSH)	1.99 (0.27-4.20 mU/l)
25-hydroxyvitamin D	19.9 (30-80 µg/l)
1,25-dihydroxyvitamin D	87.2 (29-83.6 ng/l)

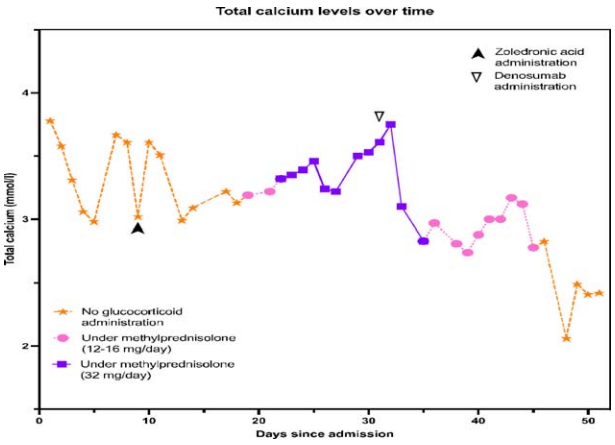
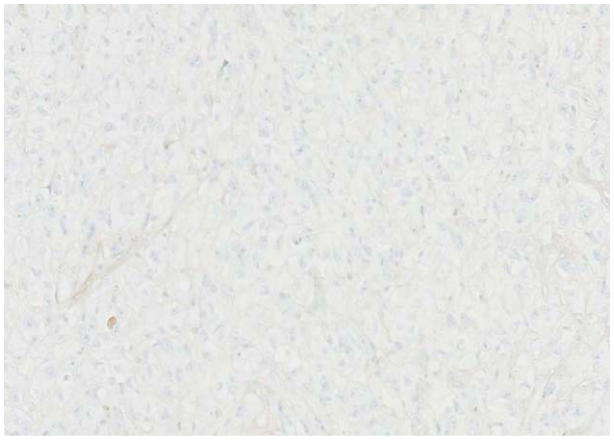


Figure 1: Total calcium levels over time

Anti-CYP27B1 immunostaining (1-alpha-hydroxylase) on the primary tumor of the patient. Negative tumor cells ; note the positive staining in a normal macrophage, shown in the lower left image.



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025

An extremely rare cause of psychosis, hypokalemia, and metabolic alkalosis

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Cushing's syndrome (CS) remains a diagnostic challenge due to an overlap with other clinical conditions such as pseudo-Cushing, which can be triggered by major depression, alcoholism, and metabolic syndrome. The most prevalent cause of CS is iatrogenic. When Cushing's syndrome is caused by endogenous cortisol secretion, most often an ACTH-secreting pituitary adenoma is the culprit and the syndrome is hence referred to as Cushing's disease. In very rare cases CS is caused by a cortisol-producing ACC. The prognosis of this extremely rare disease is generally poor but depends on the stage at diagnosis. Here, we present the case of a 40-year- old female who, as a result of an acute psychotic episode in the presence of severe hypokalemia and metabolic alkalosis, was diagnosed with liver-metastasized ACC. Despite treatment with ketoconazole, followed by adrenalectomy, and adjuvant therapy with mitotane, the patient showed rapid clinical deterioration and eventually palliative care was initiated. The patient died several weeks after her discharge. We address the current literature on the epidemiology, underlying causes, clinical presentation, diagnosis, treatment, and prognosis of CS. In the presence of (therapy-resistant) arterial hypertension, impaired glucose tolerance or diabetes, and psychiatric symptoms, screening for CS should be readily considered. Concomitant hypokalemia and metabolic alkalosis, which are caused by stimulation of the aldosterone receptor, should further increase clinical suspicion for CS.

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026

Solving the puzzle of MEN2B syndrome in an adolescent girl

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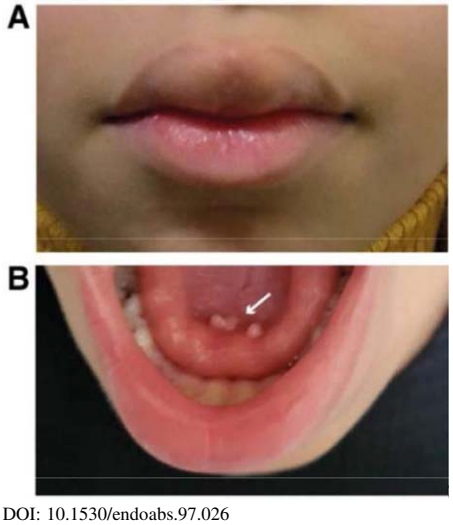
Introduction
MEN2B is a rare genetic tumor syndrome that causes medullary thyroid cancer at a young age and may lead to pheochromocytoma later in life. Early diagnosis is crucial for thyroidectomy before metastasis. This case report aims to increase awareness of MEN2B signs and symptoms and the need for early referral and treatment.
Case
A 10-year-old girl was referred for ultrasound imaging of a swollen submandibular gland that had been present for several months. In the past, she had been seen by an ENT specialist for a thickened upper lip present since birth, undergone surgery for clubfoot, and consulted several times for chronic constipation and poor weight gain. Ultrasound showed calcifications within the swollen submandibular gland and a nodule with calcifications in the right thyroid lobe, strongly suggestive of a thyroid neoplasm. In addition to the swollen left cervical gland, the patient's examination revealed thickened lips (Figure 1A) and mucosal neuromas on the tongue (Figure 1B). Her thyroid gland appeared normal on palpation. She had an elongated face and thin body habitus. Thyroid hormone and serum thyroglobulin levels were normal, but serum calcitonin was markedly elevated at 11290 ng/l (ref. $<$7.2ng/l). Urinary catecholamines were within normal limits. Ultrasound-guided thyroid biopsies confirmed medullary thyroid cancer. A computed tomography scan of the lungs revealed two small nodules ($<$ 1 cm) in the right lung. A positron emission tomography (PET) scan with Ga-DOTANOC showed heterogeneous uptake in the thyroid and some cervical lymph nodes but not in the pulmonary lesions. The patient underwent a total thyroidectomy with cervical lymph node dissection. The tumor had invaded several adjacent structures. Genetic analysis of lymphocytes and thyroid tissue revealed a pathogenic missense variant in the RET proto-oncogene in exon 16 (M918T), found in $>$95% of MEN2B patients. Calcitonin levels decreased after surgery but remained elevated at 5981 ng/l.
Conclusion
Medullary thyroid cancer in MEN2B is a highly aggressive disease. Clinical guidelines consistently recommend prophylactic thyroidectomy within the first few months of life. In this case, several diagnostic opportunities were missed despite the classic presentation of the disease. Early diagnosis and treatment could have resulted in a better prognosis, but this can only be achieved through increased awareness of this rare syndrome.
Figure 1. Phenotypical features of MEN2B syndrome. (A) thickened lips and (B) mucosal neurinomas on the tongue.



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027

Two family members with partial hypopituitarism and gingival fibromatosis caused by a missense mutation in KCNQ1

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Background
Childhood growth hormone deficiency (GHD) is a clinically heterogeneous condition and may have a genetic basis. Association with abnormalities of eyes, ears, palate and other parts of the forebrain or familial occurrence of GHD suggest a genetic cause (1). The occurrence of childhood GHD in association with gingival fibromatosis led to the identification of two autosomal dominant KCNQ1 missense variants (Arg116Leu and Pro369Leu). KCNQ1 gene encodes the alpha subunit of the voltage gated-channels, well known in the context of cardiac arrhythmia syndromes (long QT syndrome) (2). We present the case of a Belgian family, of which two family members were diagnosed with a heterozygous Pro369Leu mutation in KCN1Q1.
Case report
We report the clinical picture of a mother (45 years, subject 1) and a son (11 years, subject 2), both affected by the above-mentioned Pro369Leu mutation. The mother had been diagnosed with a GHD since childhood based on growth retardation. Evaluation of the other pituitary axis showed a thyrotrophic and gonadotropic deficiency. Dynamic testing of the HPA axis with revealed no adrenal insufficiency. Prolactin levels were fluctuating mildly elevated. MRI of the pituitary was normal (2021). She is being treated with growth hormone, thyroid hormone and female hormone (estradiol gel and Progebel). Cardiologic evaluation revealed no prolonged QT interval. A dilatation of the ascending aorta up to 40 mm was seen, for which further investigations are planned. She has no intellectual impairment and no obvious gingival hyperplasia. The current clinical picture is dominated by complaints of exercise intolerance and muscle pain, further deteriorated after a complicated covid-19 infection. The patient had two successful, assisted pregnancies through IVF, which resulted in the birth of two sons (°2011 and °2014). The firstborn child presented himself at the age of 7 years at the pediatric department due to short stature. Hormonal examination revealed a deficiency of the somatotropic and thyrotrophic axis for which substitution was started. Since he is in prepuberty, the gonadal axis cannot yet be assessed. He has a history of gingival hyperplasia for which surgical intervention was needed, adenotonsillectomy and a hydrocele. On physical examination facial dimorphism with coarse features and bilateral epicanthus was reported. He is diagnosed with severe learning disabilities (ADD, dyslexia, dyscalculia). Cardiac evaluation is scheduled. The son and his mother were referred to the geneticist. Genetic testing showed a heterozygous missense mutation c.1106C>T p.(Pro369Leu) in the KCNQ1 gene and a heterozygous variant c.1159G>A p.(Ala387Thr), presumably not pathogenic, in the ARID1B gene.
Conclusion
KCN1Q variants are well described in the context of cardiac arrhythmias (2). Recently, two autosomal KCNQ1 missense variants (Arg116Leu and Pro369Leu) have been shown to cause childhood GHD and gingival fibromatosis (3-5). To our knowledge, these are the seventh and eighth published case reports with a heterozygous Pro369Leu mutation to date. An overview of the clinical picture in individuals previously published with this pathogenic KCNQ1 variant are shown in Table 1. KCNQ1 are expressed in hypothalamic and pituitary cells and pathogenic variants may lead to reduced pituitary hormone secretion. The endocrine phenotypic spectrum is heterozygous ranging from a mild GHD to multiple pituitary hormone deficiencies. KCNQ1 are also expressed in gingival fibroblasts where it may have a proliferative effect (3). Gingival hypertrophy seems to be maternally inherited. This case report illustrates the diversity within and between affected families. With this abstract we wish to increase the awareness of this rare etiology of childhood GHD to learn more about the clinical spectrum and pathomechanism of KCNQ1 mutations.
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028

A diagnostic conundrum in Bardet-Biedl syndrome: when genetics precede clinical diagnosis

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Introduction
Bardet-Biedl syndrome (BBS) is an autosomal recessive, monogenic syndrome of obesity, with an estimated prevalence of 1:160.000. BBS is caused by mutations in one of the twenty-six genes that play a role in the function of primary cilia (1).

Table 1: Clinical picture in individuals with the pathogenic Pro369Leu KCNQ1 mutation

	INDIVIDUAL 1	INDIVIDUAL 2 (SON OF INDIVIDUAL 1)	INDIVIDUAL 3 (3)	INDIVIDUAL 4 (3) (SON OF INDIVIDUAL 3)	INDIVIUAL 5 (3) (SON OF INDIVIDUAL 3)	INDIVIDUAL 6 (3)	INDIVIDUAL 7 (5)	INDIVIDUAL 8 (4)
SEX	F	M	F	M	M	F	F	M
GROWTH RETARDATION AS CHILD	Yes, before 2 yo	Yes, before 2 yo	From 2 yo	ND	no	From 11-12 yo	yes	Yes; from 9 yo
MRI PITUITARY	Normal	ND	normal	Small hypophysis	ND	normal	normal	ND
GH DEFICIENCY	yes	yes	yes	yes	yes	ND	yes	yes
OTHER PITUATARY HORMONE DEFICIENCIES	TSH, LH/FSH	TSH	Gonadotropin, ACTH, thyrotropin	no	no	ND	Thyrotropin	no
PROLACTINEMIA	1.5 - 2 x ULN	ND	ND	1 - 1.5 x ULN	ND	ND	ND	ND
MUTATION INHERITED	NA	Yes/maternally	ND	Yes/maternally	Yes/maternally	no	no	no
GINGIVAL FIBROMATOSIS	no	yes	no	yes	yes	yes	yes	yes
LEARNING DIFFICULTIES	yes	yes	ND	ND	ND	ND	ND	no
CRANIOFACIAL PHENOTYPE								
-COARSE FACE	yes	yes					Yes	yes
-LONG PHILTRUM				Yes	yes			
-PALPEBRAL FISSURES		yes			downward		upward	
-BROAD NOSE	yes	yes		yes	yes		yes	
-THICK EARLOBULES	yes	yes		Yes	yes		yes	

YO: years old; ND: not defined; MS: milliseconds

Early-onset obesity, post-axial polydactyly, retinitis pigmentosa, renal or genitourinary abnormalities, learning disabilities and hypogonadism are considered primary features. Diabetes, speech deficit, hearing loss, anosmia, cardiomyopathy, and hepatic fibrosis are described as secondary features. Diagnosis is clinical and modified diagnostic criteria: four primary features or three primary plus two secondary features are required (2). The role of genetic testing remains controversial.

Case presentation

Two 17 -year-old female monozygotic twins of Palestinian origin were referred for obesity. They were born in a consanguineous marriage. Both had normal birth weight, showed rapid weight gain during childhood, but no food-seeking behaviour. Issue A had a history of strabismus surgery, and removal of two supernumerary teeth. Issue B had previously consulted the emergency department for abdominal pain, due to cystitis and urinary retention. Initial contact revealed obesity class 1 (BMI 31.8 and 32.45kg/m^2), an increased waist circumference, and no overt dysmorphic features. Because hormonal and metabolic screening came back negative, it was concluded as exogeneous obesity and a conservative weight-loss program was initiated. Issue A consulted the ophthalmology department 6 months later because of progressive deterioration of sight and photophobia. Comprehensive ophthalmological examination was consistent with retinitis pigmentosa. An exome-based RetNet panel, designed to screen for inherited retinal diseases, was analysed. A homozygous missense variant in exon 2/17 of the BBS2 gene on chromosome 16 (c.224T > G (p.Val75Gly)) was found, indicating BBS (likely pathogenic). After genetic testing, the patients were re-evaluated on the clinical diagnostic criteria of BBS (table 1a). Additional primary and secondary BBS features were identified, but they did not meet the full clinical criteria. However, given the results of genetic testing, diagnosis of BBS was considered confirmed. A trial with Dulaglutide was initiated, but no weight loss was observed and therapy was ceased after three months. The conservative treatment program was intensified and frequent follow up at the endocrine department was scheduled.

Discussion

This case highlights two limitations of the diagnostic model of BBS. The first is inherently due to the high variability and progressive onset of the phenotype, leading to low sensitivity of the criteria at young age, and a significant delay in diagnosis. Although this was described for all primary features in literature, in this case it was most evident for obesity and retinitis pigmentosa (1). After the genetic diagnosis of BBS, the patients were re-evaluated, but a strict clinical diagnosis could not be made, highlighting the second limitation to Beales' diagnostic model: the unclear role of genetic testing. Recent publications suggest that genetic testing can be used to confirm the clinical diagnosis (1, 3). In this case however, the genetic diagnosis of BBS preceded clinical diagnosis. To date, the identical homozygous single nucleotide variant c.224T > G (p.Val75Gly) in exon 2/17 of the BBS2-gene on chromosome 16 has been described in four families on ClinVar. Two of them have been described in literature (4,5) (table 1b). The confirmation of the diagnosis in our patients implies a fifth family identified, suggesting an upgrade in classification from 'likely pathogenic' to 'pathogenic' This may lead to earlier diagnosis and intervention.

Table 1 Beales' modified diagnostic criteria (2), applied to Issue A and B (a) and applied to Family 1 (4) and 2 (5), described in literature *

	3a		3b	
	Issue A	**Issue B**	**Family 1**	Family 2
Primary features				
Retinitis pigmentosa	+	+	+	+
Polydactyly	-	-	+	°
Obesity	+	+	+	°
Learning disabilities	-	-	+	°
Hypogonadism	-	-	°	°
Renal abnormalities	-	+	°	+
Secondary features				
Speech disorder/delay	+	-	°	°
Strabismus/cataract/astigmatism	+	-	°	°
Brachydactyly/-syndactyly	-	-	°	°
Developmental delay	-	-	°	°
Polyuria/polydipsia	-	-	°	°
Ataxia/poor coordination/imbalance	-	-	°	°
Mild spasticity	-	-	°	°
Diabetes mellitus	-	-	°	°
Dental crowding/hypodontia/small roots/high arched palate	+	+	°	°
Left ventricular hypertrophy/-congenital heart disease	-	-	°	°
Hepatic fibrosis	-	-	°	°

***when a characteristic was present in more than half of the family members, it was considered as positive ° information could not be retrieved from the original article**

Conclusion

The current standard for the diagnosis of BBS needs to be updated to clarify the role of genetic testing: due to evolution of the phenotype and the increasing availability of genetic testing, genetic testing may precede clinical diagnosis, leading to earlier diagnosis and intervention.

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029

Challenges in Pregnancy Diagnosis: A Case Report of Persistently Elevated hCG Levels

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Serum or plasma human chorionic gonadotropin (hCG) immunoassays are a cornerstone in diagnosis and monitoring of pregnancy [1]. Although these assays show very satisfying performance, pathophysiological (e.g., menopause induced pituitary secretion of hCG, familial hCG syndrome, exogenous hCG injections) and analytical (e.g., interference of heterophilic antibodies or anti-hCG antibodies, lack of specificity of older assays) determinants may rarely cause false positive results and lead to wrong diagnosis of pregnancy or associated trophoblastic disease [2]. We present the case of a 40-year-old female who was admitted to the emergency department (ED) for abdominal pain for a week in a context of treated respiratory infection. The patient blood test at admission notably showed high c-reactive protein (CRP) (52.7 mg/l [URL: 5]), hyperleukocytosis (10.84 103/pL [RR: 4.00 — 10.00]), high neutrophil count (8.56 10^3/qL [RR: 1.6 — 7.00]), normal urinalysis and positive total hCG (37.6 UI/l [URL: 5]). An ultrasound was performed and showed an absence of pregnancy in the uterus. The patient was released with a diagnosis of ectopic pregnancy of unknown location to be monitored. Two days later, hCG was still above the URL (39 UI/l), CRP was stable (54 mg/l), neutrophil count was still high (7.28 10^3/qL) and urinalysis was normal. As controls of hCG levels at days 5 and 7 showed a slow decrease in hCG but still suggested ectopic pregnancy, a single 50 mg dose of methotrexate was administered. The following days, she presented with persistent fever, thoracic and abdominal pain and persistent increased hCG values (40.8 UI/l). The second ultrasound was still negative for pregnancy. Given the severity of the presentation, a workup was conducted, and blood cultures were found positive for Streptococcus sanguinis. Cardiac ultrasound showed a 13 mm vegetation on the mitral valve signing endocarditis and the patient underwent emergency surgery for valvuloplasty. The patient then recovered progressively and the hCG has been decreasing while still above

threshold until 44 days post-surgery, when hCG was ultimately found negative. Laboratory investigations were performed alongside the clinical investigations to explain the inconsistence between hCG levels and the apparent absence of pregnancy. Routinely, hCG was measured using the Elecsys HCG+§ assay (Roche Diagnostics, Switzerland). Given the age of the patient, FSH levels were measured to exclude post-menopausal increased hCG. Free b- subunit and chromogranin were also measured to evaluate the risk of trophoblastic and neuro-endocrine malignancy, respectively. Additional investigations, based on recently published literature [3], were regularly performed on samples with positive hCG to exclude an analytical interference: testing samples using another immunoassay method, testing urinary hCG, treatment using heterophilic blocking tubes, serial dilutions, and treatment with polyethylene glycol. Given the absence of any detectable analytical interference, two hypotheses prevailed for the persistently high hCG values observed. The first was ectopic pregnancy in the peritoneum which led to infection by a digestive germ and consequent blood dissemination and endocarditis. The second is quiescent gestational trophoblastic disease (Q-GTD), which corresponds to the persistence of fully differentiated syncytio-trophoblasts [4]. It is a benign or inactive form of GTD. A persistent low serum hCG elevation is observed (usually between 50 and 200 mIU/mL), with no clinical or radiological evidence of pregnancy or tumour and often resolves spontaneously within 12 months but sometimes persists or progresses to malignant disease. Assessment of hyperglycosylated hCG (hCG-H) may help differentiate patients with Q-GTD from other causes of hCG elevation [5] but this measurement was not available for a clinical use in Belgium yet. A comprehensive algorithm would be most relevant to help both clinicians and laboratory specialists to handle suspicion of false positive hCG results and prevent harmful consequences for patients.

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