ORIGINAL RESEARCH—TRANSGENDER AND GENDER NONCONFORMANCE

Cross-Sex Hormone Therapy in Trans Persons Is Safe and Effective at Short-Time Follow-Up: Results from the European Network for the Investigation of Gender Incongruence

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ABSTRACT

Introduction. Data on the effects of cross-sex hormone therapy (CHT) are limited due to the low prevalence of gender dysphoria, small number of subjects treated at each center, lack of prospective studies, and wide variations in treatment modalities.

Aim. The aim of this study is to report the short-term effects of CHT on hormonal and clinical changes, side effects, and adverse events in trans men (female-to-male gender dysphoric persons) and trans women (male-to-female gender dysphoric persons).

Methods. This was a multicenter 1-year prospective study in 53 trans men and 53 trans women. Trans men received injections of testosterone undecanoate every 3 months. Trans women younger than 45 years received 50 mg cyproterone acetate (CA) and 4 mg estradiol valerate daily, whereas those older than 45 years received 50 mg CA daily together with 100 μg/24 hours transdermal 17-β estradiol.

Main Outcome Measures. Sex steroids, prolactin, liver enzymes, lipids, hematocrit, blood pressure, anthropometrics, Ferriman and Gallwey score, and global acne grading scale were measured. Side effects, adverse events, and desired clinical changes were examined.

Results. No deaths or severe adverse events were observed. Two trans men developed erythrocytosis, and two had transient elevation of the liver enzymes. Trans men reported an increase in sexual desire, voice instability, and clitoral pain (all $P \leq 0.01$). Testosterone therapy increased acne scores, facial and body hair, and prevalence of androgenetic alopecia. Waist–hip ratio, muscle mass, triglycerides, total cholesterol (C), and LDL-C increased, whereas total body fat mass and HDL-C decreased. Three trans women experienced transient elevation of liver enzymes. A significant increase in breast tenderness, hot flashes, emotionality, and low sex drive was observed (all $P \leq 0.02$). Fasting insulin, total body fat mass, and prolactin levels increased, and waist–hip ratio, lean mass, total C, and LDL-C decreased.

Conclusions. Current treatment modalities were effective and carried a low risk for side effects and adverse events at short-time follow-up. **Wierckx K, Van Caenegem E, Schreiner T, Haraldsen I, Fisher A, Toye K, Kaufman JM, and T'Sjoen G. Cross-sex hormone therapy in trans persons is safe and effective at short-time follow-up: Results from the European Network for the Investigation of Gender Incongruence. J Sex Med 2014;11:1999–2011.**

Key Words. ENIGI; Gender Dysphoria; Transsexualism; Hormone Treatment; Safety

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Introduction

Trans persons undergo cross-sex hormone therapy (CHT) to induce the secondary sex characteristics of the desired sex while reducing those of the natal one [1]. The act of using cross-sex hormones is also an affirmation of gender identity in many trans persons. The choice of type and dosage of hormones have not yet been established as randomized controlled trials, and comparative studies are lacking [2], and so, a variety of hormone preparations are currently used [3]. Female-to-male gender dysphoric persons, referred to as trans men, are treated with testosterone (T) preparations to induce virilization, sometimes preceded with progestagens to suppress menstruation [1]. To promote feminization, trans women (male-to-female gender dysphoric persons) usually receive estrogens in combination with antiandrogen and/or gonadal axis suppression medication to lower T levels and/or action [1].

Many centers in Europe use cyproterone acetate (CA), a progestational agent with androgen receptor-blocking properties [4–6], whereas spironolactone, a diuretic with antiandrogen action, is mostly used in the United States [7,8]. Other centers also use gonadotropin-releasing hormone analogues [9,10], nonsteroidal androgen receptor blockers, or 5-alpha reductase inhibitors. In addition, type of formulation, hormone dosage, and route of administration (oral, transdermal, or intramuscular) may differ between centers. These wide variations in treatment modalities and the low prevalence of transsexuality, and therefore small number of subjects treated in each center, hamper our knowledge on the effects and side effects of CHT. Furthermore, hormonal therapies have changed considerably in the past years, and most current evidence is based on older treatment regimens. The use of long-acting T preparations has significantly increased in trans men, and the use of ethinyl estradiol (EE) has decreased in trans women due to the increased risk for cardiovascular disease [11].

Aims

The aim of this study was to investigate the physical and physiological effects, side effects, and adverse events of commonly used CHTs. We present the first multicenter prospective study in a well-described cohort of trans persons treated according to a standardized treatment protocol.

Methods

Study Population and Sex Hormone Therapy

This research is part of the European Network for the Investigation of Gender Incongruence (ENIGI), a collaboration of four major West European gender identity clinics (Amsterdam, Ghent, Hamburg, and Oslo) [12] created to study the diagnostics and treatment of gender dysphoria. We present current data from the Department of Endocrinology at the Ghent University Hospital and the University Hospital in Oslo. All patients diagnosed with gender dysphoria and referred to our departments between February 2010 and August 2012 were invited to participate in this prospective study (N = 152). We included only hormone-naive trans persons. After screening, by thorough medical history and determination of serum sex steroids, 44 individuals were excluded. A total of 53 trans men and 53 trans women participated in our study (Figure 1). Patients were followed every 3 months during the first treatment year.

Trans men received injections of 1,000 mg intramuscular T undecanoate (Nebido®, Bayer, Germany) at the start of the study, after 6 weeks, and every 12 weeks thereafter. Before T initiation, progestagens were sometimes taken to suppress the menstrual cycle. In case of nontolerance, injections with intramuscular T esters (T decanoate 100 mg, T isocaproate 60 mg, T fenylpropionate 60 mg, T propionate 30 mg/mL) (Sustanon 250®, MSD, Netherlands) every 2 weeks were prescribed.

All trans women younger than 45 years (N = 40) received 50 mg of CA (Androcur®, Bayer) in combination with 4 mg of estradiol valerate (EV) (Progynova®, Bayer) daily. Patients older than 45 years (N = 13) received 50 mg of CA daily in combination with 100 μg/24 hours transdermal 17-β estradiol (E2) patch (Dermestril®, Besins, Belgium). In case of nontolerance, 2 mg of transdermal 17-β E2 gel twice daily (Oestrogel®, Besins) or 4 mg EV per day was given. Based on the decision of the mental health professional and patient, some trans women who favored a slower procedure and/or needed an extra diagnostic evaluation received a dual-phase protocol (N = 16). In the first phase, sex-specific features were suppressed by administration of CA 50 mg daily for about 3 months, and estrogens were added to induce feminization in the second phase. This study complied with the recommendations of the Declaration of Helsinki and was approved by the ethical

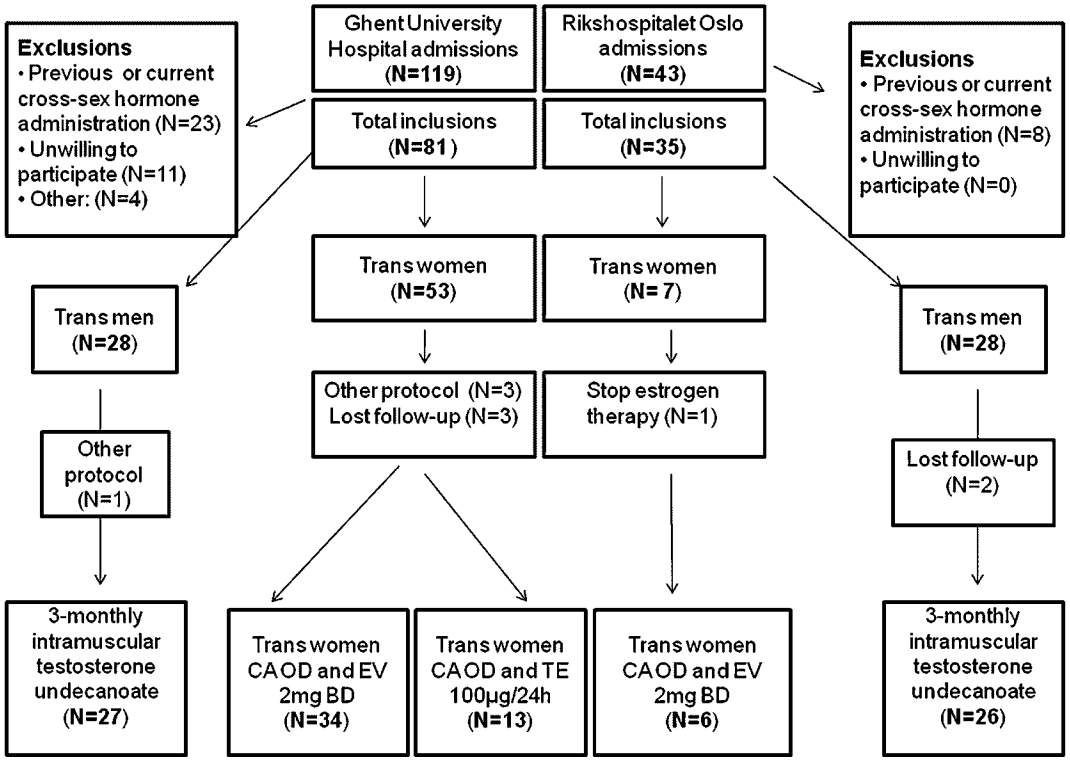

Figure 1 Subject enrollment

committee of the Ghent University Hospital and the University Hospital of Oslo. All participants gave written informed consent. Clinical trial number: NCT01072825.

Main Outcome Measures

Medical History and Examination

Descriptive data were collected from all individuals, including physical and psychiatric medical history, current and past medication use, familial medical history, and lifestyle factors such as smoking and alcohol consumption. Information was compared with data from medical files for accuracy and corrected if necessary.

Physical Parameters

Anthropometrics

Standing height was measured to the nearest 0.1 cm using a Harpenden stadiometer (Holtain Ltd, Crymuch, UK). Body weight was measured in light indoor clothing without shoes to the nearest 0.5 kg. Waist circumference, defined as the smallest abdominal circumference, and hip circumference, defined as the largest hip circumference, were determined to the nearest 0.1 cm.

Body Composition

Whole body lean mass and fat mass were measured using dual-energy X ray absorptiometry with Hologic Discovery (Hologic Inc., Bedford, MA, USA) in Belgium and with Lunar Prodigy Advance (GE, Madison, WI, USA) in Norway.

Acne

Clinical assessment of acne was performed every 3 months in each patient by the same endocrinologist with the Gradual Acne Grading Scale (GAGS) [13], a semiquantitative scoring system in which the total severity score is derived from the summary of six regional subscores. Each subscore was obtained by the score of the most heavily weighted lesion within each region (one for one or more comedones, two for one or more papules, three for one or more pustules, and four for one or more nodules) multiplied by the factor for each region (the factor for forehead and each cheek is 2, chin and nose is 1, and chest and upper back is 3).

Scores between 6 and 18, 20 and 30, and 31 and 36 were classified as mild, moderate, or severe acne, respectively.

Body Hair and Distribution

The effects of T therapy on hair growth and distribution of body hair in trans men was evaluated every 3 months using the modified Ferriman and Gallwey classification [14] in each patient by the same endocrinologist. This scale scores nine androgen-dependent areas on a five-point Likert scale (from 0 = no to 4 = very dense). A score for an androgen-dependent area of more than 8 indicated hirsutism. Androgenetic alopecia was assessed using the Norwood/Hamilton classification [15].

We were unable to evaluate the effects of CHT on hair growth and distribution of body hair in trans women because almost all of them underwent laser epilation during the course of the study.

Side Effects and Adverse Events

Clinical adverse events, including cardiovascular events, venous thrombosis and/or pulmonary embolism, osteoporotic fractures, abnormal liver function tests, hypertension, and death (including suicide), were recorded. We also evaluated whether hormone levels reached the target values for the desired gender. Development of erythrocytosis, hyperprolactinaemia, hypercholesterolemia, and hyperglycemia was also assessed.

We evaluated symptoms possibly related to hormonal status every 3 months, including hot flashes, night sweats, sleeping problems, fatigue, memory or cognition problems, mood swings, irritability, anxiety, low or high sexual desire, migraines, nail and gum problems, breast tenderness, joint pain, and muscle soreness using a four-point Likert scale (no, mild, moderate, or severe complaints).

Biochemical Determinations

Venous blood was obtained at baseline and at 12 months, and serum was stored at −80°C until hormones were analyzed in one batch. Blood samples for routine clinical parameters were drawn at the 3-, 6-, and 9-month time points.

Luteinizing hormone (LH), follicle stimulating hormone (FSH), sex hormone binding globulin (SHBG), insulin, dehydroepiandrosterone sulfate (DHEAS), and prolactin were measured by electrochemiluminiscence immunoassay (ECLIA) (Modular, Roche Diagnostics, Mannheim, Germany). The interassay CVs were as follows: LH 2.19%, FSH 2.55%, SHBG 2.8%, prolactin 4.8%, DHEAS 4.8%, insulin 2.3%. E2, estrone (E1), DHEAS, androstenedione, cortisol, and T were determined using liquid chromatography tandem mass spectrometry (AB Sciex 5,500 triple-quadrupole mass spectrometer; AB Sciex, Toronto, Canada). The serum limit of quantification was 0.3 pg/mL for E2 and 0.5 pg/mL for E1, and the interassay CVs were 4% at 21 pg/mL for E2 and 7.6% at 25 pg/mL for E1 [16]. Serum limit of quantification was 1 ng/dL (35 pmol/L) for T, and the interassay CV was 6.5% at 3 ng/dL. Hemoglobin, hematocrit (Hct), glucose, creatinin, and the liver enzymes glutamic-oxaloacetic transaminase (AST), glutamic-pyruvic transaminase (ALT), cholesterol (C), LDL-C, HDL-C, and triglycerides were measured using routine clinical chemistry methods.

Statistical Analysis

Descriptive statistics were expressed as means and standard deviations, or medians [first to third quartiles] for in case of a non-normal distributions. Statistical analyses of categorical variables were carried out using χ^2 and Fisher's exact tests as appropriate. Statistics of means in prospective data were carried out using the paired Student *t*-tests or Wilcoxon signed-rank tests when variables were not normally distributed. Between two groups, statistics of means was evaluated using independent Student *t*-tests and Mann–Whitney *U*-tests when variables were not normally distributed. Significance was set at $P < 0.05$ (two-tailed). Data were analyzed using SPSS software, v.21 (SPSS Inc., Chicago, IL, USA). For all analyses, missing values were excluded.

Results

General Characteristics

General characteristics of the study population are shown in Table 1. Trans women were significantly older than trans men at the time of presentation ($P < 0.001$). A significantly greater proportion of trans men were at the Oslo center, and the subjects were significantly older at the Ghent center.

Hormonal and Biochemical Changes in Trans Men

All trans men achieved T levels within the male reference range (321–1,005 ng/dL) during treatment (Table 2). Three trans men (5.7%) had T levels that exceeded the upper limit of 1,005 ng/dL (one at 3-month, one at 9-month, and one at 12-month time point of treatment). Hct levels

Table 1 Baseline characteristics of the study population

	Trans men		Trans women	
	Ghent (N = 27)	Oslo (N = 26)	Ghent (N = 47)	Oslo (N = 6)
Age (years)	27.3 ± 8.5	21.7 ± 5.1	31.7 ± 14.8	19.3 ± 2.4
Current smoker (%)	25.9	14.4	19.1	0
Former smoker (%)	33.3	30.8	38.3	0
Alcohol (U/week)	0 (0–0)	0 (0–0.75)	0 (0–7)	0 (0–0)
Height (cm)	163.4 ± 4.4	168.3 ± 6.0	178.4 ± 6.2	179.5 ± 3.8
Weight (kg)	65.7 ± 15.0	71.2 ± 15.9	76.1 ± 13.9	73.7 ± 18.5
BMI (kg/m^2)	24.5 ± 5.2	25.2 ± 5.5	23.9 ± 4.1	22.9 ± 5.7

Data are presented as mean ± standard deviation or median (first to third quartiles)
BMI = body mass index

gradually increased during T treatment. Two trans men developed erythrocytosis according to male reference ranges (Hct levels above 52%), one developed it after 9 months, and one after 12 months of treatment. T levels were within the male reference range in these men. Erythrocytosis was present in 20.1% of trans men according to female reference ranges (Hct levels above 48%). E2, E1, prolactin, and SHBG decreased significantly, whereas DHEAS, androstenedione, and cortisol levels were not influenced by T treatment. T treatment induced a less favorable lipid profile, as total C, LDL-C, and triglycerides increased, whereas HDL-C decreased (Table 2).

Hormonal and Biochemical Changes in Trans Women

All trans women achieved adequate gonadotropin and T suppression during antiandrogen and estrogen administration (Table 2). Two trans women (both on oral EV) who initially had an adequate T and gonadotropin suppression showed T levels within the normal male range at 12 months, possibly due to poor adherence to treatment. Gonadotropins, T, and androstenedione decreased during oral and transdermal estrogen therapy associated with CA, whereas E2 and E1 increased.

Trans women using oral EV, unlike those using transdermal estrogen, experienced a significantly increased SHBG, decreased DHEAS, and a trend toward increased cortisol levels. Prolactin levels significantly increased during both oral and transdermal estrogen treatments (Figure 2A) and during 12 weeks of CA treatment alone (Figure 2B).

CHT induced a more favorable lipid profile as total C and LDL-C decreased during both oral and transdermal estrogen treatments. HDL-C decreased during both forms of estrogen treatment, and triglycerides decreased during transdermal but not oral estrogen treatment. No significant differences were observed between trans women initially treated with CA plus estrogens compared with those initially treated with CA alone (data not shown).

Physical Changes

Trans Men

Total body weight significantly increased due to an increase in total lean mass, whereas total fat mass decreased. An android pattern of fat distribution was observed as the waist–hip ratio increased during treatment ($P = 0.02$), mainly due to reduced hip circumference (Table 3). As expected, Ferriman and Gallwey score significantly increased ($P < 0.001$), with a wide between-subject variability ranging from 2 to 28.

Trans Women

In contrast to trans men, total body weight remained unchanged for trans women, although they experienced an increase in total body fat mass and a decrease in total body lean mass. A gynoid pattern of fat distribution was induced in trans women as the waist–hip ratio decreased during treatment (Table 3).

Antiandrogen with estrogen treatment resulted in a significant increase (average 3.3 cm) in breast circumference at the nipple, with a wide inter-individual range of increase. Trans women using oral estrogens experienced similar changes in physical measures as those using transdermal estrogens (Table 3). No significant differences were observed between trans women treated with CA plus estrogens from start compared with those initially treated with CA alone (data not shown), although the former tended to have a larger breast circumference ($P = 0.06$).

Side Effects and Adverse Events

Trans Men

We recorded no deaths, cardiovascular events, osteoporotic fractures, venous thromboses, or

Table 2 Hormonal and biochemical changes in trans persons

	Trans men (N = 53)		Trans women (N = 53)			
	Intramuscular T undecanoate (N = 53)	*P*	Oral estrogens (N = 40)	*P*	Transdermal estrogens (N = 13)	*P*
Testosterone (ng/dL)						
Baseline	30.2 (20.4–39.9)	<0.001	517.5 (419.2–631.9)	<0.001	567.0 (484.8–678.2)	0.001
12 months	595.8 (481.1–715.7)		10.7 (8.3–14.3)		14.0 (12.4–17.2)	
Estradiol (pg/mL)						
Baseline	50.3 (24.2–99.3)	0.001	19.1 (15.3–24.9)	<0.001	23.6 (18.3–29.3)	0.001
12 months	29.4 (21.7–34.7)		56.5 (42.3–70.8)		95.4 (58.5–127.6)	
Estrone (pg/mL)						
Baseline	51.7 (30.3–80.9)	0.01	29.8 (24.1–40.1)	<0.001	45.8 (33.8–50.3)	0.028
12 months	45.5 (32.9–53.8)		360.9 (253.3–485.3)		57.8 (47.3–90.7)	
SHBG (nmol/L)						
Baseline	51.6 (23.0–81.2)	<0.001	31.2 (24.0–40.1)	<0.001	50.3 (36.0–59.7)	0.33
12 months	25.1 (19.7–34.0)		41.7 (25.8–50.7)		48.1 (29.0–90.7)	
LH (U/L)						
Baseline	6.7 (4.2–9.9)	<0.001	4.8 (3.7–6.4)	<0.001	5.5 (3.5–6.8)	0.001
12 months	2.4 (0.7–5.8)		0.1 (0.1–0.1)		0.1 (0.1–0.1)	
FSH (U/L)						
Baseline	4.8 (3.4–7.3)	0.02	3.5 (2.8–5.3)	<0.001	5.4 (3.5–6.8)	0.001
12 months	4.2 (1.2–6.6)		0.2 (0.1–0.4)		0.2 (0.1–0.4)	
Prolactin (ng/mL)						
Baseline	13.7 (9.3–19.9)	<0.001	8.1 (6.3–11.3)	<0.001	6.6 (4.1–9.3)	0.001
12 months	9.6 (7.9–14.4)		20.6 (16.7–28.9)		26.2 (18.6–36.2)	
DHEAS (μg/dL)						
Baseline	254.0 (170.1–351.4)	0.97	289.9 (253.1–401.7)	<0.001	285.9 (220.4–404.5)	0.42
12 months	257.6 (174.1–355.7)		236.8 (192.9–348.5)		275.6 (171.9–410.3)	
Androstenedione (ng/dL)						
Baseline	113.9 (90.9150.5)	0.42	101.2 (77.0–121.3)	<0.001	117.4 (94.6–161.4)	0.001
12 months	113.8 (80.2–151.9)		56.2 (43.4–76.4)		69.6 (55.9–106.7)	
Cortisol (μg/dL)						
Baseline	13.3 (8.9–16.0)	0.42	15.2 (12.9–18.7)	0.06	15.4 (13.4–19.9)	0.28
12 months	11.8 (9.4–15.2)		15.7 (12.8–20.8)		15.0 (11.8–18.9)	
Hematocrit (%)						
Baseline	40.8 ± 2.9	<0.001	45.2 ± 2.5	0.003	45.5 ± 1.7	<0.001
12 months	45.8 ± 3.0		42.0 ± 5.7		42.0 ± 2.3	
Fasting glucose (mg/dL)*						
Baseline	0.80 ± 0.1	0.28	0.83 ± 0.1	0.78	0.92 ± 0.06	0.25
12 months	0.77 ± 0.1		0.84 ± 0.1		0.88 ± 0.1	
Fasting insulin (mU/L)*						
Baseline	9.1 (5.8–16.3)	0.02	7.2 (4.9–9.7)	0.019	7.3 (6.6–18.7)	0.1
12 months	7.5 (5.2–13.2)		9.3 (7.2–11.3)		12.0 (9.1–17.0)	
Creatinin (mg/dL)						
Baseline	0.74 ± 0.1	<0.001	0.9 ± 0.1	0.001	0.93 ± 0.1	0.011
12 months	0.84 ± 0.1		0.8 ± 0.1		0.85 ± 0.1	
AST (U/L)						
Baseline	20.0 (17–23)	0.01	24.1 ± 9.2	<0.001	26.8 ± 8.3	0.013
12 months	24 (18–29.5)		17.7 ± 3.6		19.6 ± 4.8	
ALT (U/L)						
Baseline	16.0 (11.5–20)	0.02	25.0 ± 17.4	0.01	29.9 ± 15.3	0.12
12 months	20 (15–25)		18.6 ± 9.5		21.0 ± 10.1	
Total C (mg/dL)						
Baseline	171.9 ± 28.1	0.04	171.5 ± 32.7	0.001	227.2 ± 35.6	0.004
12 months	178.2 ± 30.6		152.3 ± 28.3		181.3 ± 20.6	
LDL-C (mg/dL)						
Baseline	98.4 ± 26.3	0.006	99.4 ± 29.0	0.03	138.3 ± 24.8	0.001
12 months	116.1 ± 28.9		92.3 ± 28.9		106.3 ± 20.4	
HDL-C (mg/dL)						
Baseline	56.3 ± 12.7	<0.001	52.9 ± 13.5	<0.001	58.2 ± 15.2	0.26
12 months	47.8 ± 10.7		45.7 ± 9.2		54.8 ± 15.7	
Triglycerides (mg/dL)						
Baseline	69.0 (51.7–89.5)	<0.001	79.5 (54.7–108)	0.1	87.0 (68.0–176.5)	0.03
12 months	81.1 (65.3–124.6)		70.8 (50–133.1)		85.0 (70.0–110)	

*Based on subsample of trans women (N = 44) or trans men (N = 23)
Data are presented as mean ± standard deviation or median (first to third quartiles). *P* value results from paired *t*-test or Wilcoxon signed-rank test
ALT = glutamic-pyruvic transaminase; AST = glutamic-oxaloacetic transaminase; DHEAS = dehydroepiandrosterone sulfate; HDL-C = HDL cholesterol; LDL-C = LDL cholesterol; FSH = follicle stimulating hormone; LH = luteinizing hormone; SHBG = sex hormone binding globulin

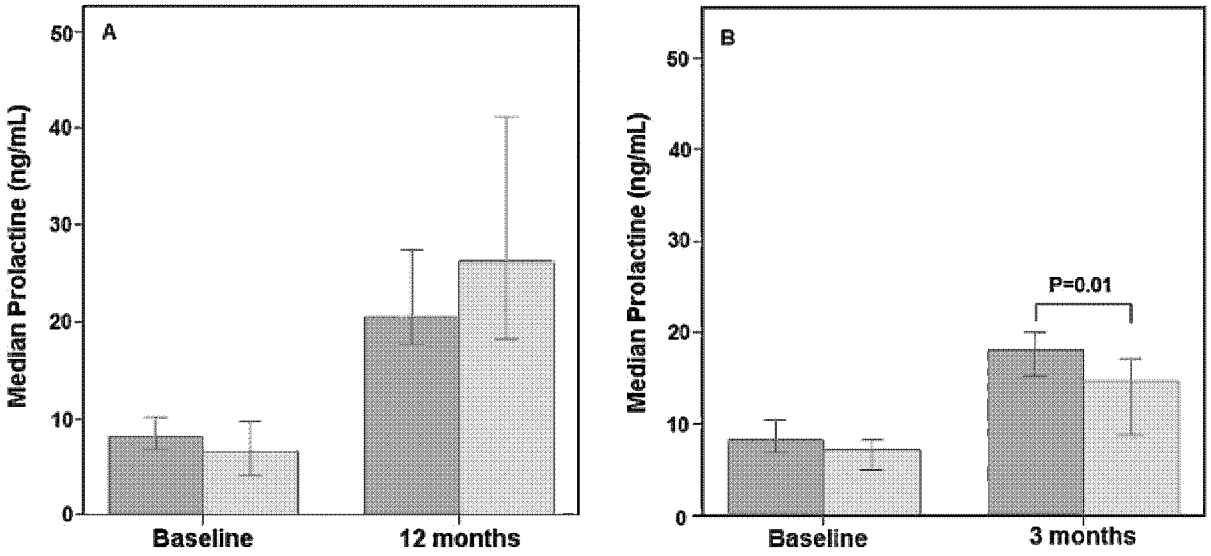

Figure 2 (A) Changes in prolactin levels during cyproterone acetate (CA) plus oral or transdermal estrogen treatment. Data are presented as median; error bars represent 95% confidence interval (CI). Dark grey square: CA plus oral estrogens; light grey square: CA plus transdermal estrogens. We observed a comparable increase in prolactin levels during both oral and transdermal estrogen treatment ($P < 0.001$ and $P = 0.002$, respectively). (B) Changes in prolactin levels during CA plus estrogens or CA alone. Data are presented as median; error bars represent 95% CI. Dark grey square: CA plus estrogens; light grey square: CA alone. We observed an increase in prolactin levels during CA + estrogen treatment ($P < 0.001$) and during CA treatment alone ($P = 0.009$). CA + estrogen treatment induced higher prolactin levels compared with CA treatment alone ($P = 0.01$).

pulmonary embolisms in trans men. Two were switched to short-acting intramuscular T esters after 9 and 12 months, respectively, of T undecanoate therapy mainly because of muscle and joint aches. Liver enzymes increased during T therapy, but only 1.9% of trans men had liver enzymes values exceeding twice the upper limit of normal according to female reference ranges. No subject had liver enzymes values exceeding twice the upper limit of normal according to male reference ranges.

Blood pressure increased slightly during treatment, but none of the subjects developed hypertension during our observation. Fasting insulin levels decreased ($P = 0.02$), and nobody developed type 2 diabetes. GAGS acne scores increased ($P < 0.001$), but the majority of trans men (94.3%) had mild acne lesions. The remaining 5.7% had moderate acne lesions after 12 months of therapy, and no individuals had severe or very severe acne lesions. Eighteen persons (34.0%) initiated topical or oral acne treatment.

Seventeen percent of trans men developed androgenetic alopecia. No indication of troublesome aggression, hostility, or sleep apnea was present. All trans men reported loss of vaginal bleeding during treatment. Spotting was reported in about one-third of participants, generally limited to the first 6 months of treatment (Figure 3).

Trans Women

Similar to trans men, no deaths, cardiovascular events, osteoporotic fractures, venous thromboses, or pulmonary embolisms were observed in trans women. One trans woman had to stop estrogen treatment due to major depression and was excluded from our analyses. Serum prolactin levels exceeding twice the upper limit of normal were observed in 15.7% and 3.9% according to the male and female reference range, respectively. One trans woman experienced galactorea, which spontaneously stopped after several weeks. Transient elevation of the liver enzymes exceeding twice the upper limit of normal was observed in 5.7% and 1.9% according to the female and male reference ranges, respectively. One trans woman developed hypertension during the study observation (defined as a systolic blood pressure above 140 mm Hg or diastolic above 90 mm Hg at three different time points). Fasting insulin increased during antiandrogen and estrogen treatment ($P = 0.005$), but no subject met criteria for diagnosis of type 2 diabetes. Two trans women using transdermal estrogen patches (15.4%) were switched to other

Table 3 Physical changes in trans persons

	Trans men (N = 53)		Trans women (N = 53)			
	Intramuscular T undecanoate (N = 53)	*P*	Oral estrogens (N = 40)	*P*	Transdermal estrogens (N = 13)	*P*
Weight (kg)						
Baseline	68.4 ± 15.5	0.01	73.3 ± 13.8	0.10	82.1 ± 13.1	0.99
12 months	70.6 ± 13.2		74.6 ± 14.3		82.1 ± 11.6	
BMI (kg/m^2)						
Baseline	24.8 ± 5.3	0.01	23.1 ± 4.2	0.42	26.1 ± 3.5	0.91
12 months	25.6 ± 4.4		23.7 ± 4.4		26.1 ± 3.4	
Total body fat mass (kg)						
Baseline	22.9 ± 11.4	<0.001	15.4 ± 7.4	<0.001	16.6 ± 5.4	0.01
12 months	19.9 ± 9.7		20.0 ± 8.1		18.7 ± 4.6	
Total body lean mass (kg)						
Baseline	43.0 ± 6.6	<0.001	5.6 ± 7.5	<0.001	62.6 ± 9.3	0.02
12 months	48.3 ± 5.6		5.3 ± 8.0		59.7 ± 8.1	
Waist circumference (cm)						
Baseline	80.3 ± 13.6	0.74	81.2 ± 10.1	0.21	91.6 ± 11.1	0.50
12 months	80.1 ± 11.2		79.7 ± 10.5		90.9 ± 10.1	
Hip circumference (cm)						
Baseline	97.3 ± 10.5	0.02	94.2 ± 9.3	<0.001	96.9 ± 7.8	0.07
12 months	95.4 ± 9.2		98.1 ± 9.3		100.4 ± 7.1	
Waist–hip ratio						
Baseline	0.82 ± 0.09	0.03	0.9 ± 0.1	<0.001	0.94 ± 0.06	0.07
12 months	0.84 ± 0.08		0.8 ± 0.1		0.91 ± 0.07	
Breast circumference (cm)*						
Baseline	—	—	92.9 ± 10.0	0.03	98.2 ± 9.0	0.09
12 months			95.7 ± 11.2		101.2 ± 9.0	
Systolic blood pressure (mm Hg)						
Baseline	111.5 ± 12.6	0.05	125.1 ± 13.8	0.005	131.6 ± 15.8	0.47
12 months	115.6 ± 11.7		118.8 ± 13.9		128.8 ± 15.5	
Diastolic blood pressure (mm Hg)						
Baseline	70.2 ± 10.5	0.18	76.8 ± 10.8	0.32	76.7 ± 9.0	0.43
12 months	72.5 ± 9.2		75.7 ± 10.6		79.7 ± 9.3	
Ferriman and Gallwey score						
Baseline	0 (0–2)	<0.001	—	—	—	—
12 months	10 (6–16)					
Acne score						
Baseline	2 (0–5)	<0.001	2 (0–7)	<0.001	0 (0–0)	1.0
12 months	7.5 (2–11.8)		0 (0–0)		0 (0–0)	

*Based on subsample (n = 20)
Data are presented as mean ± standard deviation or median (first to third quartiles) in case of non-Gaussian distribution; *P* value results from paired *t*-test or Wilcoxon signed-rank test in case of non-Gaussian distribution
BMI = body mass index

therapies because of skin irritation after 3 and 9 months of treatment, respectively.

Treatment-Related Symptoms

Trans Men

Treatment-related symptoms were investigated every 3 months in a subsample of trans men (N = 25). The vast majority of the trans men reported an increase in sexual desire (Figure 3). T treatment resulted in variable levels of voice deepening and an increased voice instability ($P = 0.024$). About 20% of trans men reported clitoral pain, with a peak incidence observed at 6 months of treatment. Symptoms of emotionality decreased ($P = 0.01$).

We observed no changes in symptoms of night sweats, hot flashes, abdominal pain, anxiety, breast tenderness, irritability, palpitations, joint pain, muscle soreness, headache, mood swings, fatigue, concentration difficulties, memory, or sleep-related problems (data not shown).

Trans Women

We examined treatment-related symptoms every 3 months in a subsample of trans women (N = 30) treated with 50 mg of CA daily in combination with estrogens from the start. A significant increase in breast tenderness, emotionality, low sexual desire, and hot flashes was observed ($P < 0.001$, $P = 0.001$, $P < 0.001$, and $P = 0.02$, respectively) (Figure 3).

We found no changes in night sweats, abdominal pain, anxiety, irritability, palpitations, skin dryness, joint pain, muscle soreness, headache,

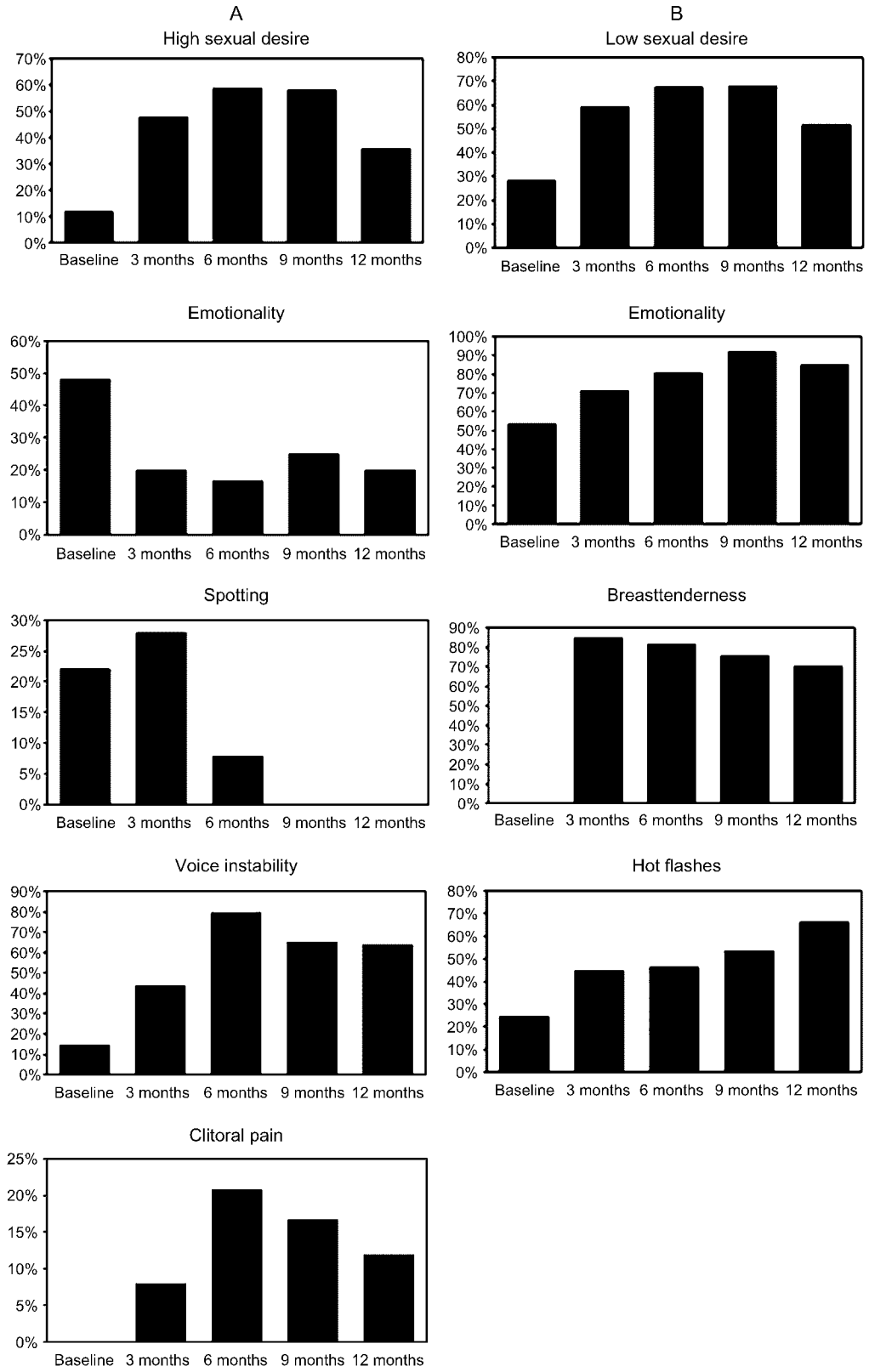

Figure 3 Prevalence of treatment-related symptoms in trans men (A) and trans women (B)

mood swings, fatigue, concentration difficulties, memory, or sleep-related problems (data not shown).

There were no significant differences in the presence of treatment-related symptoms at the 3-month time point between trans women treated with oral or transdermal estrogen. No significant differences were observed at the 3-month time point between trans women treated with CA plus estrogens from start compared with those initially treated with CA alone (data not shown), except for a higher prevalence of breast tenderness in those women treated with CA plus estrogens ($P = 0.001$).

Discussion

We presented the first multicenter prospective study describing the effects of the current CHT on hormonal and clinical changes, side effects, and adverse events in trans men and trans women. The main findings of our study were that our therapies (injections with T undecanoate every 3 months in trans men and 50 mg CA plus either 4 mg oral EV or 100 μg/24 hours transdermal 17-β E2 daily in trans women) were effective and safe. The majority of trans persons had desired levels of sex steroids according to the Endocrine Society Guidelines [1] and developed the secondary sex characteristics of the desired sex. Trans men experienced cessation of menses and developed a male body habitus with an android pattern of fat distribution, a deepening of the voice, an increase in lean mass, male pattern baldness, and both facial and body hair. Trans women experienced an increase in fat mass with a gynoid pattern of fat distribution and an increase in breast circumference.

None of the trans persons experienced severe adverse events such as cardiovascular events or death. These findings are in line with previous reports that demonstrated T treatment for trans men was effective and relatively safe in the short term [17–19]. The results in trans women confirm a recent study [10] showing a low risk for adverse events at short-time follow-up but contrast earlier reports that have indicated a high incidence of venous thrombosis and/or pulmonary embolism during the first year of CHT [17,19]. Current treatment modalities in trans women, including the avoidance of EE usage and using transdermal estrogens in older trans women, may therefore be less detrimental to the coagulation system. Indeed, Toorians and colleagues [20] described a higher activated protein C resistance with oral EE compared with oral EV and transdermal 17-β E2 usage. In addition, Van Kesteren et al. [17] reported a decreased incidence of venous thrombosis with the use of transdermal estrogens in older trans women.

Similar to Mueller and colleagues [18], we observed a small but significant increase in systolic blood pressure during T therapy. However, considering the small increase (about 3.5%) we observed, it is likely that sample sizes in other studies [21,22] were too small to detect a statistically significant difference. Importantly, no subject developed a clinically significant blood pressure increase during our study observation. T treatment also increased liver enzymes in our study, similar to the study from Mueller and colleagues [18], although the clinical relevance of this finding remains to be determined.

Previous studies have not investigated systematically the treatment-related symptoms during T therapy in trans men. However, the majority of trans men reported an increase in voice instability, acne, and sexual desire. Most acne cases were mild, and none was severe or very severe according to the GAGS. Nevertheless, about a third of our patients underwent acne treatment, indicating that even mild and moderate acne lesions were clinically relevant.

One trans woman discontinued treatment because of depression. Indeed, it has been previously reported that CHT increases depression risk [19]. However, others show that CHT lowers anxiety and depression scores possibly due to improvements in mental health after initiation of cross-sex reassignment therapy [23]. Similar to others [17,19], we found a transient elevation of the liver enzymes during antiandrogen and estrogen treatment, although, generally, a decrease in liver enzymes is observed [24].

Concerning the risk for hyperprolactinemia during CHT in trans women, our findings substantiate most other studies showing an increase in prolactin levels during administration of CA combined with estrogen therapy [1,17,25–28] and CA alone [25,27]. However, our previous retrospective follow-up studies do not show a further increase in the long term [5,29]. Moreover, the clinical relevance of increased prolactin levels during CHT remains undetermined. Although a few case reports of prolactinomas have been reported following CHT, none was reported in large follow-up studies, perhaps suggesting a low risk associated with CHT.

Trans women also reported treatment-related symptoms. Although some of them, such as breast tenderness and low sexual desire, were well-known from our clinical practice, subjects unexpectedly reported a significant increase in hot flashes during antiandrogen and estrogen treatment. Because we did not use validated questionnaires or objective measurements to analyze these symptoms, further exploration and characterization are needed.

Investigation of cardiovascular risk factors during CHT is important as recent studies show that trans women have an increased cardiovascular morbidity [30] and mortality [11,31] compared with the general population. Similar to previously published studies using EE plus CA, we found a reduction in LDL-C and an increase in fat mass and fasting insulin during the first year of CHT in trans women [21,32]. Estrogen therapy increases removal of LDL apolipoprotein B-100 [33], but the pathophysiological mechanism of increased insulin resistance during antiandrogen and estrogen therapy is not fully understood. CHT induces important changes in body composition, with an increase in fat mass, which may affect glucose metabolism. Sex steroids may also exert direct effects, as acute T withdrawal in men decreases insulin sensitivity in the absence of any detectable changes in body composition [34]. Augmentation of E_2 to supraphysiological levels may also induce insulin resistance through liver hyperinsulinemia and reduced GLUT 4 expression within the muscles [35].

In line with Dittrich and colleagues [10], we observed no increase in body weight, triglycerides, or blood pressure in trans women. These findings differ from a previously published study using EE plus CA [21] and are likely to be related to differences in type of estrogen used. Different types of estrogen exert divergent metabolic effects. EE has a stronger hepatic impact due to its 17α-ethinyl group, which prevents the inactivation of the molecule and results in a slower metabolism [36,37]. Additionally, the higher estrogen dosage in the study by Elbers and colleagues [21] may also contribute to differences in these health outcomes.

HDL-C decreased in trans women (in both oral and transdermal group), which was somewhat unexpected under estrogen therapy. A potential explanation for these findings may be found in the progestagenic effects of CA, as progestogens decrease HDL-C concentrations [33]. Decreased HDL-C levels have been previously shown in trans women using transdermal estrogen therapy [38,39]. Because the transdermal route avoids the first pass effect of the liver, it may have different metabolic effects than oral estrogen therapy. Decreased triglycerides were also observed with transdermal but not oral estrogen therapy. In addition, trans women using oral EV showed significantly higher E1/E2 ratio than those using transdermal therapy. A lower E1/E2 ratio has been reported in postmenopausal women receiving transdermal hormone replacement therapy [40]. No other differences were observed between these two modes of estrogen treatment, suggesting both are equally effective.

T undecanoate treatment decreased fat mass but induced a less favorable lipid profile and an android pattern of fat distribution in trans men. Although these changes were also seen in studies using two or three weekly injections of intramuscular T esters [41,42], most of the evidence suggests that T treatment is relatively safe at short- and medium-term follow-up [17–19]. However, outcome studies in trans men are generally performed in much smaller sample sizes and at younger ages compared with trans women. Large, long-term (>20 years) follow-up studies are needed to investigate the cardiovascular safety of T therapy in trans men.

As previously described [12], the age and sex ratio differed significantly between our two centers. This age difference should be kept in mind in future outcome studies as older age may be associated with a worse cardiovascular outcome [43].

The strengths of the present study were its relatively large sample size compared with most other prospective studies; the use of widely prescribed (but scientifically not well documented) treatment modalities; our detailed description of adverse events, side effects, and treatment-related symptoms; and the use of a mass spectrometry-based methodology to measure serum sex steroid levels in trans persons. Although we did not use a validated questionnaire to measure treatment-related symptoms, we were the first to examine these symptoms systematically. Validation of such a questionnaire may be valuable for future studies as no standardized assessment of symptoms exists presently. Secondly, our treatment protocol was to administer oral EV to trans women younger than 45 years and transdermal estrogen therapy to those over 45 years. This age discrepancy may have influenced the difference in treatment response between the two groups. In addition, some trans women initially received CA alone without concomitant estrogen use. This may have influenced

our results, although no significant differences were observed between these two groups. Moreover, the lack of difference between the groups may indicate that most relevant changes occur during the first 9 months of CHT. Thirdly, we did not have a blinded clinician to determine the clinical effects of the treatment, which may possibly induce a bias. Finally, we described average differences associated with each treatment, but we observed large between-subject differences in clinical outcome measures, which may be due to differences in sex steroid metabolism or sensitivity.

Conclusion

We observed that our current treatment modalities in both trans men and women were effective and carried a low risk for side effects and adverse events at short-time follow-up.

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SPECIAL ARTICLE

MAYO CLINIC

Ten Common Questions (and Their Answers) About Off-label Drug Use

Christopher M. Wittich, MD, PharmD; Christopher M. Burkle, MD, JD; and William L. Lanier, MD

Abstract

The term *off-label drug use* (OLDU) is used extensively in the medical literature, continuing medical education exercises, and the media. Yet, we propose that many health care professionals have an underappreciation of its definition, prevalence, and implications. This article introduces and answers 10 questions regarding OLDU in an effort to clarify the practice's meaning, breadth of application, acceptance, and liabilities. Off-label drug use involves prescribing medications for indications, or using a dosage or dosage form, that have not been approved by the US Food and Drug Administration. Since the Food and Drug Administration does not regulate the practice of medicine, OLDU has become common. It occurs in every specialty of medicine, but it may be more common in areas of medicine in which the patient population is less likely to be included in clinical trials (eg, pediatric, pregnant, or psychiatric patients). Pharmaceutical companies are not allowed to promote their medications for an off-label use, which has lead to several large settlements for illegal marketing. To limit liability, physicians should prescribe medications only for indications that they believe are in the best interest of the patient. In addition, health care professionals should educate themselves about OLDU to weigh the risks and benefits and provide the best possible care for their patients.

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The term *off-label drug use* (OLDU) is used extensively in the medical literature, continuing medical education (CME) exercises, and the media. It is a polarizing term because it can be associated with great benefit or harm to patients.[1] In addition, OLDU, along with allegations of pharmaceutical company promotion of OLDU, has been the cause of major lawsuits and historically large out-of-court legal settlements.[2-7] Therefore, all health care professionals have likely heard the term *OLDU* used, yet we propose that many have an underappreciation of its definition, prevalence, and implications. This article introduces and answers 10 questions regarding OLDU in an effort to clarify the practice's meaning, breadth of application, acceptance, and liabilities.

QUESTION 1: WHAT IS THE DEFINITION OF OLDU?

The most common form of OLDU involves prescribing currently available and marketed medications but for an indication (eg, a disease or a symptom) that has never received Food and Drug Administration (FDA) approval.[8,9] Hence, the specific use is "off-label" (ie, not approved by the FDA and not listed in FDA-required drug-labeling information). The term *OLDU* can also apply to the use of a marketed medication in a patient population (eg, pediatric), dosage, or dosage form that does not have FDA approval.

The current role of the FDA is to control which medications are available commercially. Historically, the Food, Drug, and Cosmetic Act of 1938 required only that a new medication be safe.[9] In 1962, the Kefauver-Harris Amendment mandated that FDA-approved new drugs also must have evidence that they are effective.[9] Therefore, the FDA approves new medications that have been shown to be safe and effective for specific indications (ie, "on-label" prescribing). The FDA does not limit or control how the medications are prescribed by physicians once the medications are available on the market. By definition, OLDU is prescribing for an indication, or employing a dosage or dosage form, that has not been approved through the FDA process.

Off-label drug use can be motivated by several factors. First, a medication may not have been studied and approved for a specific population (eg, pediatric, geriatric, or pregnant patients).[10] Second, a life-threatening or terminal medical condition may motivate a health care professional to give any treatment that is logical and available, whether approved by the FDA or not. Third, if one medication from a class of drugs has FDA approval, physicians commonly use other medications in the same class without specific FDA approval for that use for the same indication.[8,9] In addition, if the pathologic or physiologic features of 2 conditions are similar, a physician may use a medication approved for 1 of these conditions for both (eg, diabetes and metabolic syn-

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drome; psychiatric diseases such as anxiety and posttraumatic stress disorder).[8]

QUESTION 2: IS OLDU COMMON?

Indeed, OLDU is common. Radley et al[1] reported in 2006 that in a group of commonly used medications, 21% of prescriptions were for an off-label use. In certain subpopulations of patients, this rate may be even higher. For example, a study by Shah et al[11] found that 78.9% of children discharged from pediatric hospitals were taking at least 1 off-label medication. In addition, in a pediatric emergency department, the rate of OLDU was estimated to be 26.2%.[2] The off-label use of antidepressant, anticonvulsant, and antipsychotic medications is high and is more prevalent with increasing patient age.[12] In an intensive care unit, Lat et al[13] reported that 36.2% of medication orders were for an off-label use. In addition, β-adrenergic blocking agents are commonly prescribed for an off-label indication, and specialists may more commonly prescribe for off-label β-blocker use than primary care physicians.[10] In a headache specialty practice, Loder and Biondi[14] reported that off-label use accounted for 47% of prescriptions written.

QUESTION 3: CAN AN OLDU FOR A GIVEN DRUG BECOME A WIDELY ACCEPTED PRACTICE OR EVEN A STANDARD OF CARE?

Off-label drug uses can become widely entrenched in clinical practice and become predominant treatments for a given clinical condition. For example, tricyclic antidepressants do not have FDA approval as a treatment for neuropathic pain, yet this class of drugs is considered a first-line treatment option.[15] The use of aspirin provides another interesting example of OLDU. Aspirin was widely used before the introduction of the Food, Drug, and Cosmetic Act of 1938. Therefore, aspirin was grandfathered and approved as an existing drug without the rigorous testing that modern medications undergo. Currently, aspirin is FDA approved for use in patients with pain, fever, rheumatic diseases, cardiovascular diseases (eg, acute myocardial infarction, previous myocardial infarction, angina pectoris, and previous cerebrovascular disease), and a history of a revascularization procedure (eg, coronary artery bypass grafting and carotid endarterectomy).[16] However, aspirin does not have an indication for coronary disease prophylaxis in diabetic patients, yet guidelines recommend its use in these patients.[8] Therefore, aspirin prophylaxis for coronary disease in high-risk patients is an off-label use.

Elsewhere, medications are often prescribed for OLDU with poor or absent clinical evidence. Radley et al[1] reported that 73% of medications prescribed for an off-label use had poor or no scientific support. In critical care patients, OLDU was without adequate evidence 48.3% of the time.[13] Because OLDU is typically less critically evaluated than is on-label drug use, OLDU may be associated with an increase in medication errors.[17] Rinke et al[17] studied pediatric antidepressant drug use in a national error-reporting database and found that 77% involved off-label prescribing.

QUESTION 4: WHAT ARE SOME EXAMPLES OF WIDELY PRACTICED OLDUs?

There are examples of widely practiced OLDUs in every specialty of medicine (Table). Since the patient population in pediatrics is often excluded from clinical drug studies, examples of OLDU are especially abundant. For example, morphine has never received an FDA indication for pain treatment in children, but it is extensively used for this indication in hospitalized pediatric patients.[11] In another example, researchers discovered in the 1970s that the nonsteroidal anti-inflammatory agent indomethacin was efficacious as a medical therapy for closing a persistent, symptomatic patent ductus arteriosus in newborns.[18] Thus, a trial of indomethacin became the treatment of choice for many affected newborns in an attempt to avoid curative surgery. Indomethacin has never been approved for this indication and, as such, this use remains an OLDU. In addition, many inhaled bronchodilators, antimicrobials, anticonvulsants, and proton pump inhibitors are often used in the pediatric population without formal FDA approval.[30]

The FDA has attempted to lessen the gap between FDA approval and contemporary drug-prescribing practices in pediatrics through the FDA Modernization Act of 1997. This Act created incentives, including exclusive marketing and patent extension, for pharmaceutical companies to test medications on children.[31]

Medications for psychiatric disorders are also frequently used for unapproved indications.[12,32] Patients with psychiatric disorders are often excluded from clinical trials, and these disorders are inherently difficult to study. Moreover, there is often crossover in symptoms from disease state to disease state, which has lead physicians to use psychiatric medications approved for one psychiatric condition for additional unapproved indications. For example, selective serotonin reuptake inhibitors have been used off-label for rare or difficult-to-study disorders, such as borderline personality disorder, stuttering, pathologic gambling, and alcoholism.[16] Moreover, selective serotonin reuptake inhibitors (eg, paroxetine, sertraline, and fluoxetine) are considered first-line treatments for premature ejaculation, another off-label use.[33] In recent years, antipsychotic drug use

TABLE. Examples of Common Off-label Uses of Drugs

Category and drug	Off-label use(s)[a]
Allergy	
Diphenhydramine	Chemotherapy-related emesis, insomnia[16]
Anesthesiology	
Propofol	Intracranial hypertension
Dexamethasone, propofol	Postoperative nausea
Meperidine	Postanesthetic shivering
Cardiology	
Amiodarone	Supraventricular tachycardia[16]
Aspirin	Antithrombosis in atrial fibrillation, Kawaskai disease[16]
Atorvastatin, simvastatin	Extended-interval dosing for hyperlipidemia[16]
Indomethacin	Pharmacologic closure of patent ductus arteriosus[18]
Dermatology	
Azathioprine	Atopic dermatitis, pemphigus; psoriasis[19]
Biologic agents (eg, etanercept, infliximab, intravenous immunoglobulin, rituximab)	Alopecia areata, atopic dermatitis, Behçet disease, dermatomyositis, hicradenitis suppurativa, pemphigoid, pityriasis, vasculitis[20]
Gastroenterology	
Erythromycin	Gastroparesis[21]
Omeprazole	Reflux-related laryngitis[16]
Hematology/oncology	
Alendronate	Hypercalcemia of malignancy[16]
Dabigatran	Venous thromboembolism prophylaxis after orthopedic surgery[22]
Doxorubicin	Refractory multiple myeloma[16]
Furosemide (nebulized)	Dyspnea[16]
Rituximab	Idiopathic thrombocytopenic purpura, Waldenström macroglobulinemia[16]
Infectious disease	
Linezolid	Infective endocarditis[16]
Sulfamethoxazole-trimethoprim	Sinusitis[16]
Nephrology	
Acetylcysteine	Prevention of contrast nephrotoxicity[16]
Albuterol	Hyperkalemia[16]
Erythropoietin	Anemia of chronic disease[16]
Neurology	
Atenolol, metoprolol, propranolol	Migraine prophylaxis[10]
Isoflurane	Seizure, status epilepticus
Donepezil	Frontotemporal dementia[23]
Gabapentin	Bipolar disorder, diabetes, fibromyalgia, neuropathic pain symptoms, headache, hiccups, hot flashes, restless leg syndrome[24]
Lidocaine	Postherpetic neuralgia[24]
Tricyclic antidepressants	Bulemia, insomnia, irritable bowel syndrome, neuropathic pain symptoms[15,16,24]
Obstetrics	
Magnesium sulfate	Premature labor[16]
Volatile anesthetics (eg, enflurane, isoflurane, halothane)	Intraoperative uterine contraction
Pediatrics	
Amoxicillin (high dose)	Otitis media in children[16]
Atenolol	Hypertension in children[16]
Intranasal desmopressin	Nocturnal enuresis[25]

(continued)

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TABLE. Continued

Category and drug	Off-label use(s)[a]
Pediatrics (continued)	
Morphine	Pain in children[11]
Sildenafil	Pulmonary hypertension in children[16]
Pulmonary	
Volatile anesthetics (eg, enflurane, isoflurane, halothane)	Status asthmaticus[26]
Psychiatry	
Atypical antipsychotics (eg, risperidone, olanzapine, quetiapine)	Anxiety, dementia, eating disorders, obsessive-compulsive disorder, personality disorders, posttraumatic stress disorder, substance abuse[27]
β-Blockers	Social phobia, public speaking[28]
Citalopram	Alcoholism, fibromyalgia, irritable bowel syndrome, obsessive-compulsive disorder, pathologic gambling, stuttering[16]
Fluoxetine	Borderline personality disorder, diabetic neuropathy, fibromyalgia, hot flashes, premature ejaculation[24]
Trazodone	Insomnia in elderly patients[16]
Urology	
Sildenafil	Sexual dysfunction symptoms in women[29]

[a]This table is not comprehensive and is not intended as an endorsement of these off-label drug uses.

for unapproved FDA indications has increased. Alexander et al[32] estimated that the cost of off-label antipsychotic drug use in 2008 was $6.0 billion.

During the 1970s and 1980s, there was a proliferation of cardiac surgery to repair or replace diseased heart valves. Disease in many of these patients was the result of rheumatic abnormalities in patient populations with inadequate or no antibiotic drug treatment of infections earlier in their lives. In these patient populations, hemodynamic stability was of utmost concern during anesthesia, surgery, and the immediate postoperative course. Lowenstein[34] reported that high-dose morphine, combined with amnestic agents, could provide the type of stable anesthetic required for these patients and that the beneficial effects of the anesthetic would continue into the postoperative intensive care period. With the later introduction of the short-acting opioid fentanyl, it was infused in doses much greater than approved by the FDA, thus converting a short-acting drug into a long-acting drug. High-dose morphine- and fentanyl-based anesthetics, highly favored therapy for valve replacement surgery, were retained as core anesthetics with the introduction of coronary artery bypass graft surgery. Today, patients are typically brought to surgery much earlier in the disease course (hence, they tend to be more stable hemodynamically), and there is a focus on shortening stays in the intensive care unit after cardiac surgery. In addition, improvements in surgical technique have shortened operation times. For these reasons, high-dose opioid anesthesia is less common than in the past, although it is still used. These high doses of morphine and fentanyl have never been approved by the FDA, and, therefore, their use has always been off-label.

Postoperative nausea and vomiting in surgical patients can add to patient morbidity and the cost of health care. Postoperative nausea is common, occurring in nearly 70% to 80% of high-risk patients.[35] Because of this, practitioners have empirically explored a variety of antiemetic therapies. In patients at high risk for postoperative nausea and vomiting, bolus or infused propofol and bolus dexamethasone have gained favor as antiemetic regimens. However, these treatments have never been approved by the FDA for this indication. As such, they represent OLDUs.

QUESTION 5: IF EFFICACIOUS, WHY IS GOVERNMENT APPROVAL NOT OBTAINED TO CONVERT OFF-LABEL USES OF DRUGS TO ON-LABEL USES?

Obtaining a new FDA approval for a medication can be costly and time-consuming. To add additional indications for an already approved medication requires the proprietor to file a supplemental drug application, and, even if eventually approved, revenues for the new indication may not offset the expense and effort of obtaining approval.[8] Finally, generic medications may not have the requisite funding foundations needed to pursue FDA-approval studies.[8] For these financial reasons, drug proprietors may never seek FDA approval for a new drug indication.

QUESTION 6: DO PHYSICIANS EXPOSE THEMSELVES TO LEGAL VULNERABILITY FOR INCLUDING OLDUs IN THEIR CLINICAL PRACTICES, PARTICULARLY IF THE PATIENT EXPERIENCES AN ADVERSE REACTION RELATED TO AN OLDU?

Physicians have been involved in legal claims due to an adverse reaction related to a medication prescribed for an off-label use.[8,36] The legal theories used in these lawsuits include unregulated use of a research drug, failure to provide adequate informed consent for an OLDU, and medical negligence.[37] In developing legal precedents for off-label therapies, the courts have typically treated drugs and devices as coequals. As such, many of the courts' views on OLDU have evolved from decisions regarding off-label uses of medical devices.

Research vs Practice

The FDA makes it clear that it does not regulate the practice of medicine and that the federal Food, Drug, and Cosmetic Act of 1938 will not play a role in creating physician liability for OLDU.[38] However, the FDA requires stringent review before drugs and medical devices are involved in research to ensure that steps are taken to properly protect human study participants. When not classified as tools involved in research, medications can be prescribed and medical devices can be used in an off-label manner without FDA regulatory oversight. Regarding this point, during its evaluation of possible harm arising from placement of an orthopedic spine medical device, an Ohio appellate court stated that "the off-label use of a medical device is merely a matter of medical judgment and, as such, subjects a physician to professional liability for exercising professional medical judgment, but off-label use of a medical device is not barred by the U.S. Food and Drug Administration."[38,39] By way of legal precedent and similar FDA regulatory processes, the same standard would apply to OLDU.

Drawing a clear line of demarcation between a drug's use in research vs practice can often be difficult. Prescribing a drug in a new and yet untested manner does not alone brand it as an interest of research.[38] The National Commission for the Protection of Human Subjects of Biomedical and Behavioral Research has attempted to define whether a drug's use might be classified as a practice or research tool, and their definitions follow. The goal of medical practice is to "provide diagnosis, preventative treatment or therapy."[38] Research, on the other hand, is "designed to test a hypothesis, permit conclusions to be drawn, and thereby to develop or contribute to generalizable knowledge."[38] When not deemed research, legal claims brought solely on the basis of failure to gain adequate FDA approval before prescribing an off-label drug will likely be struck down. However, physicians may not be sheltered from other forms of liability theories.

Medical Malpractice: Informed Consent

No court decision to date has mandated that a physician must disclose, through an informed consent process, the off-label use of a drug.[40] Two arguments are often voiced by those who oppose any routine requirement for disclosure: (1) disclosure may unduly frighten patients and (2) the extensive burden placed on physicians to constantly review and communicate medication risk and benefit information may divert attention away from other more important patient care issues.[40]

Perhaps the most cited modern legal case involving the medical informed consent process is *Canterbury v Spence*.[41] The Canterbury court held that "the test for determining whether a particular peril must be divulged is its *materiality* to the patient's decision."[41] A material risk is one in which "a reasonable person, in what the physician knows or should know to be the patient's position, would be likely to attach significance to the risk or cluster of risks in deciding whether or not to forego the proposed therapy."[41]

Many courts have not considered OLDU to be an independent material issue requiring disclosure during the consent process.[38] A 1996 Ohio court held that off-label use of medical devices was a "matter of medical judgment."[38,42] According to the court, physicians may be subject to professional liability for medical negligence involving OLDU but will not be held liable for nondisclosure.[38,42]

The results of a 2006 nationwide poll on the public's view of OLDU may precipitate concerns for future court challenges not fully appreciated by previous legal opinion. Half of the poll's respondents falsely believed that a drug could be prescribed only for its primary FDA-approved use.[43] An almost similar percentage felt that physicians should be prohibited from prescribing drugs for off-label use. Nearly two-thirds of those responding felt that except for use in clinical trials, OLDU should be completely banned.[43] This is a remarkable aggregate response given that a considerable fraction of those responding negatively to OLDU had likely benefited from the practice at some point in their lives (although they were probably unaware).

Although many courts do not require physicians to disclose OLDU, patients may have a different belief and concern regarding their use. Whether these matters will develop into a greater expectation for adequate disclosure remains unknown. Some physicians have suggested that providing patients with information about OLDU may afford greater protection from future liability suits.[38]

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Medical Malpractice: Negligence

Medical malpractice is a broad term that includes the action of negligence. In fact, 4 elements of tort law dealing with negligence must be proved before liability can be found to exist: (1) the prescribing physician must have a duty to the patient, (2) that duty must be breached, (3) there must be some injury requiring compensation, and (4) there must be a causal link between the breech and that injury.

A physician's duty of care is defined as the same degree of care provided by other physicians practicing under similar circumstances. Use of off-label medication alone does not result in liability under negligence standards.[44] When a patient believes that he or she was harmed by an off-label use of a medication, it must be established that the prescribing physician deviated from the standard of practice.[38] Because the FDA prohibits manufacturers from sponsoring physician education for off-label use of their medications, physicians may find it difficult to establish how others in their field are using medications outside their FDA-approved uses.[37] As peer-reviewed published evidence focusing on a drug's off-label use grows over time, new standards of practice involving the off-label use of a drug begin to develop.[38]

To help determine whether the standards of practice are being met when prescribing medications for OLDU, physicians should first ask themselves several questions[38,45,46]: (1) Does the native drug have FDA approval? (2) Has the off-label use been subjected to substantial peer review? (3) Is the off-label use medically necessary for treatment? (4) Is the use of the medication nonexperimental? To mitigate the risk of liability, physicians should always prescribe off-label drugs in "good faith, in the best interest of the patient, and without fraudulent intent."[45] This 3-pronged approach to prescribing medications will also ensure that the tenets of the FDA's requirement are met; specifically, physicians prescribing medications for off-label use should "be well informed about the product, to base its use on firm scientific rationale and on sound medical evidence, and to maintain records of the product's use and effects."[47]

QUESTION 7: WILL INDEXED MEDICAL JOURNALS PUBLISH ARTICLES ON OLDU?

Reports on OLDU, particularly original observations, are not only tolerated by indexed medical journals but also may actually be encouraged. The most welcomed reports may follow several patterns, the 2 most common of which are described in the following subsections.

Reports to Evaluate New Drug Therapies Seeking FDA Approval

Before a drug use can be approved by the FDA, drug utilization for this specific application must undergo extensive studies of efficacy and safety in humans. The data from multiple phases of study are needed for the drug's proprietor to file a New Drug Application to the FDA. Studies of new drugs or studies involving expanded use of an existing drug are, by definition, "off-label" indications until FDA approval is obtained. These studies may take the form of phase 0 (pharmacokinetic and pharmacodynamic studies of subtherapeutic drug doses in small numbers of patients), phase 1 (small studies of drug pharmacodynamic properties in healthy volunteers), phase 2 (larger studies of drug pharmacology, safety, and efficacy in volunteers and patients), and phase 3 (large, randomized, multicenter trials of drug safety and efficacy; drug compared with a placebo or an existing treatment standard) trials.[48] In addition, phase 4 trials are completed after FDA approval to further delineate the drug's effects and adverse reactions.[48]

Although preliminary research on drug pharmacology and safety intended to support a petition for FDA approval may be important to the proprietor and the FDA, articles based on these data may be difficult to publish in competitive biomedical journals because the data may not be of interest to the journal's target audience. As such, initial research may not pass peer review because of journal priorities. However, as subsequent trials evaluate drug efficacy and safety using methods that mimic the drug's use in clinical practice, journals' interest in the research will be piqued. The more novel the therapy (eg, a new class of drug for a common application, in contrast to a "me too drug"), the more likely the research data will be competitive for publication in better-quality medical journals. In fact, journals may introduce the reports with editorials and engage in media promotion of the discoveries, both testaments to the value the journals place on the research.

Reports to Evaluate Off-label Uses, or Describe Adverse Effects, of Drugs Approved for Other Indications

As previously described, a large fraction of drug use is off-label, and these indications may even become the standard of care (see Question 3). In these instances, the FDA will have previously approved the drug for clinical practice but for an indication other than the one under question. Medical journals and their readers may have a keen interest in original observations related to this form of drug use. Articles may not only become accepted for publication but may also get journal promotion (editorials and media promotion) reserved for the highest-priority articles. Clearly, a journal's enthusiasm for these types of articles is coupled with the quality and statistical power of the data, the novelty of the obser-

vation, the generalizability of the results, and the relevance of the observations to the intended audience's interests. As such, a journal may publish OLDU articles on drugs' effects and adverse effects related to indications for which FDA approval may never be sought.

Prospective trials of drug use in humans must conform to federal regulations, be approved by the institutional review boards of all participating institutions, and be registered in one of many appropriate registries (eg, ClinicalTrials.gov) to be considered for publication in biomedical journals.[49,50] Retrospective OLDU observations in patients, whether of a drug's effects or adverse effects, also must have accompanying institutional review board approval before reporting the observations to a biomedical journal. However, the standards of approval for retrospective observations are much less stringent than for prospective research.

Indexed biomedical journals are less likely to publish review articles on drugs that are seeking FDA approval for a first use. Reviews with the best probability of getting published are those that describe novel drug mechanisms or success in treating conditions in which other drugs have limited efficacy. Articles primarily intended to support a marketing angle for the proprietor (ie, seeding reports)[51] have difficulty getting published in the most competitive medical journals. In contrast, journals may welcome review articles that address a widely applied OLDU. As information on a given OLDU grows, journals may even welcome updated reviews or new reviews that address novel aspects of the OLDU experience (eg, new information on a drug's effects or adverse effects, updates on the operant mechanisms of action, and articles on drug-use adherence and economics).

QUESTION 8: CAN SPEAKERS DISCUSS OLDU DURING ACCREDITED CME COURSES?

Speakers at accredited CME courses are allowed to discuss OLDU during their presentations. The Accreditation Council for Continuing Medical Education historically required that all discussions of OLDU be disclosed during the CME presentation. However, current Accreditation Council for Continuing Medical Education requirements state that all clinical presentations should be based on "evidence that is accepted within the profession of medicine."[52] If the discussion of OLDU conforms to this mandate, no specific disclosure is required.

QUESTION 9: CAN DRUG COMPANIES PROMOTE OLDU?

The 1938 Food, Drug, and Cosmetic Act gave the FDA the power to regulate promotional materials on medications.[53] Two provisions from the FDA prohibit most promotion of off-label uses of medications by pharmaceutical manufacturers and marketers. First, the FDA requires approval before distribution into interstate commerce of all medication labeling (including the package insert, print and broadcast advertisements, brochures, and patient education materials).[53] Second, the FDA prohibits "misbranding" of medications. Misbranding includes labeling a medication with misleading information, including off-label uses.[53]

Although pharmaceutical manufacturers are not allowed to promote off-label uses of medications, they are allowed to respond to unsolicited questions from health care professionals about off-label use and to distribute peer-reviewed publications regarding off-label use.[53] Responses to questions regarding off-label use must be completed by the manufacturer's medical affairs office and not their sales representatives, and interactions with the questioner must be documented.[53]

Historically, the 1997 FDA Modernization Act allowed manufacturers to distribute to health care providers peer-reviewed journal articles about unapproved uses of medications.[54,55] If a given drug company chose to engage in distribution of this type of information, it was required to submit an application for approval of that indication within a rigid and prespecified period. These requirements were subsequently revised in 2009 with the approval of new FDA guidelines.[53] The new guidelines clarified existing rules and allowed distribution of information on off-label uses by pharmaceutical manufactures if specific regulations were followed.[53] After 2009, pharmaceutical manufacturers could distribute information, including journal articles and textbook chapters, describing unapproved uses for their medications. The FDA demanded that the information in these OLDU publications be accurate, the relationship between the distribution of information and the sponsoring drug manufacturer be disclosed, and the published material not be edited or presented in an abridged form.[53] In addition, the manufacturer is no longer required to submit an application for approval for that indication.[53]

With the increase in direct-to-consumer marketing by pharmaceutical manufacturers, in 2010 the FDA introduced the Truthful Prescription Drug Advertising and Promotion (Bad Ad) Program. This program provides a mechanism by which health care professionals and patients can report illicit OLDU promotion to the FDA.

Despite regulations that ban pharmaceutical manufacturers and marketers from promoting OLDUs, some have ignored this mandate. In fact, one study found that off-label marketing by drug companies was one of the most common causes of

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Medicaid fraudulent claim investigations.[2,56] In addition, marketing of off-label uses has been the source of costly lawsuits and out-of-court penalties for pharmaceutical manufacturers. In 2012, GlaxoSmithKline paid a record $3 billion to settle a dispute, including alleged illegal off-label marketing involving paroxetine in children (approved only for use in adults), the antidepressant bupropion as a weight loss aid, and failure to report safety information about the antidiabetes medication rosiglitazone.[5] In 2012, Abbott paid $1.6 billion in penalties for alleged off-label marketing of valproic acid.[7] In 2009, Eli Lilly paid $1.4 billion in a settlement for alleged off-label marketing of olanzapine for dementia.[3] That same year, Pfizer paid $2.3 billion for alleged off-label marketing of 4 of its medications.[4]

QUESTION 10: WHAT IS THE DIFFERENCE BETWEEN OLDU AND ORPHAN USE OF DRUGS?

Orphan drugs are medications that are developed and used for rare, or orphan, diseases. Owing to a drug's limited clinical use for an orphan indication, it will typically generate insufficient profitability for the drug's sponsor to seek FDA approval for the narrow indication. As such, practitioners are typically forced to use medications in an off-label manner to treat orphan diseases. Therefore, orphan drugs are often a subtype of OLDU. However, in 1983, the FDA implemented the Orphan Drug Act, which offered incentives to pharmaceutical manufacturers that developed and marketed new drugs for rare diseases.[57] Incentives include tax breaks, exclusive marketing rights, and reduced drug application fees. In addition, the FDA has offered grants for the development of drugs for rare diseases. These measures have been successful in increasing the development of new, FDA-approved (ie, "on-label") drugs for orphan diseases.[57] Examples of off-label uses of medications for orphan disease include aspirin for Kawasaki disease and rituximab for Behçet disease.[16,20]

OLDU SUMMARY

Off-label drug use involves prescribing medications for an indication, or using a dosage or dosage form, that has not been approved by the FDA. Since the FDA does not regulate the practice of medicine, OLDU has become common. It occurs in every specialty of medicine, but it may be more common in areas of medicine in which the patient population is less likely to be included in clinical trials (eg, pediatric, pregnant, or psychiatric patients). Pharmaceutical companies are not allowed to promote their medications for an off-label use, which has lead to several large settlements for illegal marketing. To limit liability, physicians should prescribe medications only for indications that they believe are in the best interest of the patient on the basis of the most credible available evidence. In an era of global exchange of medical information, this approach to physician prescribing practices may have greater utility than restricting practices solely to indications approved by a US-based pharmaceutical labeling system. Health care professionals should continually educate themselves about OLDU to weigh the risks and benefits and provide the best possible care for their patients.

Abbreviations and Acronyms: **CME** = Continuing Medical Education; **FDA** = Food and Drug Administration; **OLDU** = off-label drug use

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Cognitive, Emotional, and Psychosocial Functioning of Girls Treated with Pharmacological Puberty Blockage for Idiopathic Central Precocious Puberty

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Central precocious puberty (CPP) develops due to premature activation of the hypothalamic-pituitary-gonadal (HPG) axis, resulting in early pubertal changes and rapid bone maturation. CPP is associated with lower adult height and increased risk for development of psychological problems. Standard treatment of CPP is based on postponement of pubertal development by blockade of the HPG axis with gonadotropin releasing hormone analogs (GnRHa) leading to abolition of gonadal sex hormones synthesis. Whereas the hormonal and auxological effects of GnRHa are well-researched, there is a lack of knowledge whether GnRHa treatment influences psychological functioning of treated children, despite the fact that prevention of psychological problems is used as one of the main reasons for treatment initiation. In the present study we seek to address this issue by exploring differences in cognitive function, behavior, emotional reactivity, and psychosocial problems between GnRHa treated CPP girls and age-matched controls. Fifteen girls with idiopathic CPP; median age 10.4 years, treated with slow-release GnRHa (triptorelin acetate—Decapeptyl SR® 11.25) and 15 age-matched controls, were assessed with a comprehensive test battery consisting of paper and pencil tests, computerized tasks, behavioral paradigms, heart rate variability, and questionnaires filled in by the children's parents. Both groups showed very similar scores with regard to cognitive performance, behavioral and psychosocial problems. Compared to controls, treated girls displayed significantly higher emotional reactivity ($p = 0.016$; Cohen's $d = 1.04$) on one of the two emotional reactivity task conditions. Unexpectedly, the CPP group showed significantly lower resting heart rates than the controls ($p = 0.004$; Cohen's $d = 1.03$); lower heart rate was associated with longer treatment duration ($r = -0.582$, $p = 0.037$). The results suggest that GnRHa treated CPP girls do not differ in

their cognitive or psychosocial functioning from age matched controls. However, they might process emotional stimuli differently. The unexpected finding of lower heart rate that was associated with longer duration of the treatment should be further explored by methods appropriate for assessment of cardiac health.

Keywords: central precocious puberty, gonadotropin releasing hormone analog, cognitive function, emotion regulation, psychosocial function, heart rate variability, puberty

INTRODUCTION

Central precocious puberty (CPP) is defined as the advent of an otherwise normal puberty before the age of 8 years in girls and 9 years in boys due to premature activation of the hypothalamic-pituitary-gonadal (HPG) axis (Nebesio and Eugster, 2007). The etiology of CPP is unclear and varies with gender. It is predominantly found in girls and while the majority of female CPP is idiopathic, in boys it is more frequently secondary to an organic cause (e.g., tumor; Choi et al., 2013). CPP incidence is age dependent. Danish data from 1993 to 2001 showed an incidence of 8:10,000 in girls aged 5–9 and 1–2:10,000 in boys aged 8–10 (Teilmann et al., 2005).

CPP is associated with early bone maturation and reduced adult height in the youngest cases. Pharmacological blockade of the gonadotropin stimulus with GnRH analogs (GnRHa), which leads to cessation of gonadal sex hormones production, is nowadays considered the standard treatment for CPP (Carel et al., 2009); the main treatment goals are an increase in adult height and prevention of psychological problems (Sonis et al., 1985; Johansson and Ritzen, 2005; Tremblay and Frigon, 2005). While research shows that treatment can positively influence adult height in treated girls, especially if started before 6 years of age (Carel et al., 2009), the effects in boys and with regard to psychological functioning are less explored. Both a recent consensus statement and an update on the usage of GnRHa in CPP strongly emphasize the need for more research regarding GnRHa effects on psychological functioning (Carel et al., 2009; Chen and Eugster, 2015).

GnRHa treatment can potentially influence CPP children's psychological functioning through several pathways. Firstly, postponement of the pubertal development by blockade of sex hormones production can reduce psychological distress associated with early biological maturation. Secondly, abolition of sex hormone influences on the developing brain may on its own have an effect on cognitive development. Finally, GnRHa can potentially influence cognitive development via GnRH receptors that are widely present in brain areas not related to reproduction (Skinner et al., 2009). Several human and animal studies suggest that GnRHa may indeed influence cognitive functioning. A decline in working and episodic verbal memory associated with GnRHa treatment has been observed in women with benign leiomyomata uteri and endometriosis (Grigorova et al., 2006; Craig et al., 2007). In an animal study, using an ovine model of pubertal development, prepubertal GnRHa treatment significantly affected emotion regulation capacity, reward seeking behavior, and emotional reactivity in young sheep (Wojniusz et al., 2011; Evans et al., 2012). Furthermore, GnRHa treatment significantly and sex-specifically affected hippocampus and amygdala gene expressions and altered amygdalae volumes in the same animals (Nuruddin et al., 2013a,b,c). In addition, possible effects of GnRHa on cardiac health have recently been postulated, following findings of increased prevalence of cardiovascular disease in prostate cancer patients treated with GnRHa (Tsai et al., 2007; Keating et al., 2010).

The consensus statements and findings from adult and animal studies warrant a broader investigation of cognitive and emotional functioning in GnRHa treated CPP children. In the current study we, therefore, compared CPP girls under GnRHa treatment to age-matched controls. We assessed children's cognitive function by using a comprehensive neuropsychological test battery consisting of paper and pencil and computerized tests. Additionally, we assessed cognitive, social, and behavioral function at home and school situations by employing questionnaires completed by the children's parents. Since animal twin studies indicated poorer emotional regulation capacity and higher emotional reactivity in GnRHa treated lambs compared to their untreated twins (Wojniusz et al., 2011; Evans et al., 2012), assessment of emotional processing was additionally included in the study. We employed the emotional flanker task (EFT) for the assessment of emotional reactivity (Bishop et al., 2004) and calculated vagally mediated heart rate variability (HRV) as a measure of emotional regulation capacity (Appelhans and Luecken, 2006; Thayer and Lane, 2009; Koval et al., 2013).

MATERIALS AND METHODS

Participants

Clinical records of girls with idiopathic CPP, treated with GnRHa between November 2009 and December 2011, either at the University Hospital Ghent or the University Hospital Brussels, were reviewed. CPP was defined according to the combination of the following three items: (a) the onset of breast development before the age of 8 years; (b) accelerated growth velocity in the months before diagnosis; and (c) advancement of bone age by at least one year compared to chronological age. In cases with uncertain diagnosis, a standardized LHRH test (applied in 12 out of 15 girls) yielding an LH peak above 4.5 U/l and the finding of an estrogenized uterus (corpus length/cervix length $>$ 1) on pelvic ultrasound were considered as additional evidence for the presence of CPP. A minimum age of 9 years (due to the complexity of the test package), treatment by GnRHa for at least 6 months, and 2–3 monthly clinical follow-up was mandatory to enter the study.

GnRHa treatment was adjusted in case of incomplete pubertal suppression as judged by physical examination and LH/FSH blood sampling or repeated GnRH testing. At the time of the study, puberty suppression was determined clinically and radiologically as successful in all patients based on Tanner stage (no progression of breast development), growth velocity (decreased as compared to pre-treatment), and bone age. Exclusion criteria were additional endocrine or other chronic diseases, which could influence cognitive and behavioral function; learning difficulties, defined as an IQ < 70; and non-European descent due to race/ethnicity based differences concerning age of pubertal onset (Biro et al., 2013). On the basis of these criteria, two girls out of 17 were excluded. Fifteen healthy controls, carefully matched for age, were recruited through flyers distributed in public places. All patients and controls gave their assent, and parents gave written informed consent. The study was approved by the ethical committees of both institutions; *Commissie Medische Ethiek UZ Gent* and *Commissie Medische Ethiek UZ Brussel*.

Procedures

Patients and controls were invited to either the University Hospital Ghent or Brussels. After assessment of medical history and physical examination including anthropometrics and pubertal staging by an experienced pediatric endocrinologist, bone age was assessed from an X-ray of the left hand and wrist by one single investigator (MC) according to the Greulich and Pyle method. All CPP patients and controls underwent neuropsychological assessments, an emotional reactivity test, and heart rate monitoring for calculation of HRV. Behavioral questionnaires were completed by parents. All neuropsychological tests were applied by one single psychologist (NC), experienced in pediatric clinical psychology, and trained in test administration and scoring, and consisted of a range of cognitive, behavioral, and neuropsychological assessments. Heart rate monitoring, EFT and computer based cognitive tests (CANTAB) were supervised by the same investigator (SW) in all participants. In total, tests took ~2.5 h to complete. The girls were offered two breaks and soft drinks in between the testing blocks and a small financial compensation for participation in the study.

Neuropsychological Tests and Questionnaires

Intellectual Level

An abbreviated version of the *Wechsler Intelligence Scale for Children-III* (*WISC-III*) was used to generate an estimate of general cognitive ability. Two verbal (Vocabulary and Information) and two performance subtests (Block Design and Picture Completion) were used. This short-form combination has been shown to have a high reliability (Atkinson and Yoshida, 1989).

Memory Tests

The *Rey Auditory Verbal Learning Test* [*RAVLT*; Dutch version: (Saan and Deelman, 1986)] was used to evaluate auditory-verbal memory. The recognition component was not assessed in this study. We derived five scores: Immediate Memory, Best Memory, Proactive Interference, Retroactive Interference, and Delayed Recall. We also computed two combined scores which are frequently used in studies that employ RAVLT: Learning Rate, reflecting the learning ability of the subject, and Total Learning, representing the capacity to recall and accumulate words across learning trials.

The *Continuous Visual Memory Test* (*CVMT*; Trahan and Larrabee, 1988) measures visual learning and memory, i.e., acquisition of information and retention over time (storage and retrieval). Acquisition or short-term memory included Immediate Memory and Proactive Interference scores, as well as Learning Rate score. Storage includes the CVMT Recognition score. Retrieval from long-term storage included Delayed Recall, Retroactive Interference, Best Learning, and Total Learning scores on both the RAVLT and CVMT.

Spatial Ability

The *Mental Rotation Test* in which the subject was asked to compare two 3D objects and state if they are the same images (non-mirror or mirror images) was an adapted version of the task used by Hugdahl et al. (2006), originally developed by Shepard and Metzler (1971). The test had 20 pairs of images, the subjects were judged on how accurately, and rapidly they could distinguish between the pairs. The task has not been specifically validated for use in children, however in our sample the children performed similarly to what has been observed in adults.

Executive Function and Attention

A selection of four tests from the *Delis-Kaplan Executive Function System* (Delis et al., 2001) was used to assess different aspects of executive functions; the *Trail Making Test*, the *Verbal Fluency Test*, the *Color Word Interference Test*, and the *Design Fluency Test*. Composite executive functioning and processing speed domain scores are expressed as mean of subscale *z*-scores.

Additionally a selection of four tests from the Cambridge Neuropsychological Test Automated Battery (CANTAB), provided by Cambridge Cognition Ltd. was used to further assess executive function and attention. CANTAB tests are computerized, giving higher chance to discover minor differences between the groups. Although CANTAB tests were originally developed to assess patterns of cognitive decline in adults, their applicability for usage in children in age group 5–12 has been previously confirmed (Luciana and Nelson, 2002). The following tests were included:

Choice reaction time (CRT) is a 2-choice reaction time test with stimulus and response uncertainty introduced by having two possible stimuli (left and right arrows) and two possible responses (left and right buttons). Mean correct response latency and percentage of correct responses were recorded as outcome measures.

Match to sample visual search (MTS) is a matching test, with a speed/accuracy trade-off. The subject is presented with a sample-stimulus figure, composed of four colored elements displayed in the middle of the screen. After a brief delay, a varying number of similar patterns (1, 2, 4, or 8) are shown

around the edge of the screen with only one of them matching the sample-stimulus pattern. The subject has to touch the matching pattern as fast as possible on the screen. Mean correct response time and percentage of correct responses were used as outcome measures.

Spatial working memory (SWM) tests subject's ability to retain spatial information and to manipulate remembered items in working memory. A number of colored boxes are shown on the screen. By process of elimination, the subject should find one blue "token" in each of a number of boxes. The number of boxes is gradually increased from three to eight boxes and the color and position of the boxes are changed from trial to trial to discourage the use of stereotyped search strategies. Total number of errors and SWM Search Strategy were used as outcome measures.

Stop signal task (SST) is a response inhibition test, giving a measure of an individual's ability to inhibit a pre-potent response. The subject is told to press the button that corresponds to the direction of the arrow presented on the computer screen, but, if they hear an auditory signal, they should withhold their response. There are five assessed blocks, each of 64 trials. The last four blocks were subjected to statistical analysis. The main outcome measure was the Stop Signal Response Time (SSRT), which is an estimate of the latency of the stop process. Additionally, the probability of inhibiting the response when signal occurred was calculated.

Parental Questionnaires

The *Behavior Rating Inventory of Executive Function* (BRIEF)—the parent version (Dutch translation Smidts and Huizinga, 2009) assesses children's cognitive and behavioral aspects of executive function in home situations. It includes eight non-overlapping clinical scales (Inhibit, Initiate, Organization of Materials, Shift, Working Memory, Monitor, Emotional Control, Plan/Organize) and two validity scales (Negativity and Inconsistency of responses)

The *Child Behavior Check List*—4–18 years (Dutch translation: Verhulst and Van der Ende, 2004) is a standardized measure of academic, social competence, and behavioral problems. The questionnaire is completed by parents and includes eight subscales: Withdrawn, Somatic complaints, Anxious/Depressed, Social Problems, Thought Problems, Attention Problems, Delinquent Behavior, and Aggressive Behavior. The first three subscales add up to the Internalizing Problems scale and the last two to the Externalizing Problems scale. Finally, the overall Total Problems scale consists of all items. Additionally a Social Competence scale is derived from items grouped into Activities, Social, and School constructs. For each scale, T-scores (mean = 50 $\pm$ 10) can be obtained. A clinical cut-off point on the Total, the Internalizing and the Externalizing score was set at $T = 60$.

Socioeconomic Indicators

Two socioeconomic indicators for parental occupations were used. An occupational class was constructed on the basis of International Standard Classification of Occupations (ISCO-08) (ILO, 1990) namely (1) managers and professionals; (2) technicians, clerks, and service workers; and (3) craft workers, machine operators, and elementary occupations. The number of years of formal education was divided into three groups: Secondary school, Higher education Short Type, and Higher Education Long Type or University.

Emotion Processing

The Emotional Flanker Task (EFT)

EFT was used to assess emotional reactivity. The task is an adapted version based on previous studies (Bishop et al., 2004). With an inter-trial interval of 1000 ms, on each trial, two faces and two houses were presented in horizontal and vertical pairs, respectively (**Figure 1**). Participants were instructed to decide as fast as possible whether the presented buildings were identical or not, and to respond by pressing a corresponding response button. They were informed that the faces presented in the periphery were irrelevant and didn't need to be attended to. If a participant did not make a choice within the first 4 s, the next trial was automatically presented. After five practice trials, participants were exposed to 207 trials, starting with three consecutive trials with neutral flankers to increase the effects of emotional flankers (Bishop et al., 2004). Out of the remaining 204 trials, target stimuli (houses) were identical in 50% of the trials; in 35% of all presentation trials flanker stimuli consisted of anxious faces, and in 65% of trials of faces with neutral expressions. The lower proportion of emotional flankers was chosen to increase the stimulus valence and resulting reactivity to these trials (Bishop et al., 2004).

The main outcome measure was a flanker-valence effect (FVE), which was calculated by subtraction of reaction times in the valence condition "neutral" from valence condition "anxious." Larger reaction time differences between distractor valences "neutral" and "anxious" were interpreted as higher emotional reactivity (Grose-Fifer et al., 2013). To avoid confounding biases caused by different processing of identical

FIGURE 1 | Emotional flanker task. In 207 trials, children were requested to decide as fast as possible whether two houses were identical or not. The faces were irrelevant for task solution and did not need to be attended to. The difference between reaction times in the presence of anxious and neutral faces (flanker valence effect) was used as a measure of emotional reactivity. Pictures of facial expressions were obtained from the Karolinska Directed Emotional Faces database (Lundqvist et al., 1998).

and non-identical target stimuli, behavioral analyses were done separately for both conditions. Only correct responses were analyzed.

Heart Rate and Heart Rate Variability (HRV)

HRV has been extensively used in psychophysiological research to assess emotion regulation capacity (Thayer et al., 2012). Heart rate (HR) and HRV were calculated from the inter-beat intervals (IBIs), recorded with a sampling rate of 1000 Hz, using the *Polar RS800*® monitor. After a *Polar* belt was placed around the participant's chest, she was seated in a comfortable chair and asked to relax for 10 min (baseline). Thereafter, she was led over to a computer station and performed the EFT. The recording was stopped after task completion. Altogether, 20 min IBI-recordings of 29 out of 30 participants were collected. IBI recordings of one CPP girl were invalid due to equipment failure. Prior to analysis, all recordings were cleared of artifacts using ARTiiFACT software (Kaufmann et al., 2011). A minimum requirement of 95% of artifact-free IBIs was set as an inclusion criterion. No participant exceeded the 5% artifact-threshold; however there was a significantly higher mean number of artifacts in the treatment group ($M = 7.1$, $SD = 4.4$) vs. controls [$M = 2.3$, $SD = 3.6$; $t_{(27)} = 3.2$, $p = 0.003$]. Five minute-periods of data from the baseline and the EFT conditions, respectively, were chosen for further analyses, according to the Task Force (1996) guidelines (1996). HR and Root Mean Square of Successive IBIs (RMSSD) were analyzed as time domain measures. Additionally, power spectral density of High (HF), frequency was analyzed using Fast Fourier transformation following guidelines of the Task Force (1996). Frequency spectrum data were normalized by logarithmic transformation. Recordings from baseline and during EFT were analyzed separately.

Statistical Analysis

SPSS (version 20) was used for statistical analyses. Due to a relatively low number of participants, resampling of data, applying bias-corrected and accelerated bootstrapping technique (5000 resamples) was used to control for data stability. For comparisons between the groups, independent sample *t*-tests were applied, while a paired sample *t*-test was used to assess the differences between repeated measurements. If differences between groups were significant, Cohen's *d* was calculated for effect size estimation. Partial correlations were used to explore the associations between treatment duration and cardiac measures and behavioral test while controlling for chronological age. Group differences in socioeconomic status were assessed by comparing the educational levels of children's parents using Fisher's exact test.

RESULTS

The clinical characteristics of CPP girls and controls are summarized in **Table 1**. Eleven out of 15 girls had started treatment with 11.25 mg intramuscular injection of GnRHa (Decapeptyl SR®) every 10th week, and 4 out of 15 girls with a 3.75 mg injection every 4th week. Patients were monitored regularly and their medication was adjusted in case of incomplete pubertal suppression as judged by physical examination and LH/FSH blood sampling or repeated GnRH testing; 11.25 mg every 8 weeks in three girls, 11.25 mg every 6 weeks in one girl, 11.25 mg every 10 weeks in two girls (from 3.75/per 4 weeks), and 11.25 mg every 12 weeks in one girl. At study entry (T1), 14 out of 15 girls had a Tanner score for breast development equal to or less than at the start of the treatment. Median duration of GnRHa treatment was 28 months (range: 8–57) at the time of the study. As expected, body height, BMI and bone age were still higher in treated CPP girls as compared to controls. Over the course of treatment, the difference between bone age (BA) and chronological age (CA) was reduced by 8.6 months, [$t_{(14)} = 2.2$, $p = 0.042$]. Whereas all control girls were healthy, one CPP girl suffered from chronic otitis media and one from hip dysplasia, independently of the CPP and GnRHa treatment.

Neuropsychological Findings

Table 2 summarizes the results of the neuropsychological assessment. The mean estimated IQ was 94 (range: 73–116) for CPP girls and 102 (range 81–125) for control girls; the difference was not significant. The estimated IQ scores were consistent with the school situation; 26/30 girls were attending an appropriate grade for their age. Two girls from the control group and two girls from the CPP group were delayed by 1 year at school. No associations were found between IQ scores and duration of GnRHa therapy. The statistical comparison of parental educational level (Fisher's exact test) showed no significant difference between groups (**Table 1**).

Regarding verbal (RAVLT) and non-verbal memory tests (CVMT), both groups performed very similarly, showing no significant differences. The four CANTAB tests targeting attention and executive function are sensitive to small differences in performance. Nevertheless, both groups showed very similar scores on all four tests, showing no significant differences. There were no significant between-group differences on the composite *z*-scores of cognitive executive function and processing speed except for the Trail Making Test-Number Sequencing, where CPP girls performed worse than controls [$t_{(28)} = 2.8$, $p = 0.01$, $d = 1.32$]. The BRIEF questionnaire scores showed no significant differences regarding parental reported executive function (**Table 3**).

Behavioral and Emotional Problems (CBCL)

Overall, the CBCL results (**Table 3**) showed that CPP girls did not have significantly more behavioral problems than controls and they displayed similar social competence. When compared to normal range ($T = 50 \pm 10$), the most elevated scores were observed within *internalizing problems* domain on *withdrawn*, *somatic complaints* and *anxious/depressed* subscales. Out of 15 CPP girls, two had elevated scores at a clinically meaningful level ($T > 60$) on all of these four scales apart from somatic complains where four out of 15 had a *T*-score > 60. Similarly, in the control group two girls showed elevated *T*-scores on each of the same scales.

TABLE 1 | Clinical characteristics of 15 girls with central precocious puberty (CPP) at the onset of gonadotropin releasing hormone analog therapy (T0) and at the moment of the study (T1), compared to 15 age-matched controls.

Clinical characteristics	CPP (T0)	CPP (T1)	Control	*p*
CA (years)	7.5 (4.4; 9.8)	10.4 (9.2; 11.8)	10.3 (9.1; 11.4)	0.877
Height (z-score)	0.9 (−0.7; 3,1)	0.8 (−0.4; 3.2)	0.3 (−2.8; 1.6)	0.023
Weight (z-score)	0.7 (−0.8; 2.4)	0.8 (0.0; 2.5)	−0.5 (−2.0; 1.2)	0.001
BMI (kg/m^2)	17.0 (15.0; 30.0)	18.0 (15.0; 34.0)	16.0 (13.0; 20.0)	0.055
BA (years)	9.4 (7.8; 13.0)	11.5 (10.0; 13.0)	9.3 (7.8; 12.0)	<0.001
Δ BA–CA	2.0 (0.3; 4.8)	1.3 (−0.4; 3.2)	−1.0 (−2.0; 1.7)	<0.001
Tanner stage M (n) and P (n)		M1 = 6; P1 = 4	M1 = 10; P1 = 14	
		M2 = 3; P2 = 5	M2 = 3; P2 = 0	
		M3 = 6; P3 = 5	M3 = 2; P3 = 1	
		M4 = 0; P4 = 1	M4 = 0; P4 = 0	
Socio-economic background		**CPP (T1)**	**Control**	
EDUCATION				
Mother: S/H1/H2		47/33/20%	23/62/15%	0.307
Father: S/H1/H2		33/47/20%	29/57/14%	0.842
OCCUPATION MOTHER				
Manager or professional		27%	69%	
Technicians, clerks and service workers		60%	31%	
Craft workers, machine operators, elementary occupations		13%	0%	
OCCUPATION FATHER				
Manager or professional		33%	38%	
Technicians, clerks and service workers		40%	31%	
Craft workers, machine operators, elementary occupations		27%	31%	

The values are presented as medians (min; max); CPP (T0), CPP group at the time of diagnosis; CPP (T1), CPP group at the time of study entry; CA, chronological age; BMI, body mass index; BA, bone age; Δ BA–CA, difference between bone age and chronological age in years; Tanner stage M(n) and P(n), Tanner stage for breast (M) and pubic hair (P) development; S/H1/H2, percentage of parents who fulfilled secondary school (S)/higher education short type (H1)/higher education long type (H2); p, significance level (independent sample t-test) of difference between CPP (T1) and Control for continuous variables and Fisher's exact test for education.

Emotional Reactivity

Mean reaction times in EFT for all four (2 × 2) conditions (range: 1062–1319 ms) were comparable to adult data of similar versions of this task (Bishop et al., 2004). In trials with non-identical targets (mismatch-condition) reaction times were generally slower, although not significantly, in both groups compared to identical target condition (data not shown). Interestingly, for the non-identical target condition the main outcome measure, calculated as difference in reaction times in presence of "neutral" and "anxious" faces (FVE), showed a significant distraction-related slow-down of motor response in the CPP group (FVE = 36.9 ms, $SD = 93.3$), whereas response facilitation was seen in controls [FVE = −42.7 ms, $SD = 75.5$; $t_{(28)} = -2.6$, $p = 0.016$, $d = 1.04$]. In trials with identical targets, the groups did not differ significantly in their distractibility, showing distraction related response facilitation in both the control (FVE = −8.3 ms, $SD = 68.6$) and the CPP group [FVE = −71.7, $SD = 83.4$; $t_{(28)} = 1.2$, $p = 0.238$].

Heart Rate and Heart Rate Variability

Under resting conditions, HR was significantly lower in the CPP group (HR = 76.4/min, $SD = 5.5$) as compared to controls [HR = 87.7/min, $SD = 10.9$; $t_{(27)} = 3.5$, $p = 0.004$, $d = -1.03$]. HRV parameters showed significantly higher values for the CPP group as compared to controls: RMSSD 72.4 ($SD = 19.1$) vs. 43.8 ($SD = 19.7$), $t_{(27)} = -3.9$, $p = 0.004$, $d = 1.44$, and Ln(HF); 7.5 ($SD = 0.6$) vs. 6.6 (SD = 1.0), $t_{(27)} = -3.1$, $p = 0.004$, $d = 0.95$. The same pattern was evident when participants performed the EFT paradigm (data not shown).

Effect of Treatment Duration

Partial correlation (controlled for age) between treatment duration and heart rate revealed that longer treatment duration was associated with lower mean heart rate, $r = -0.58$, $p = 0.037$ (**Figure 2**). No significant correlations between treatment duration and any of the HRV or EFT measures were found.

Post-hoc Power Analyses

In order to estimate the probability of falsely rejecting the null hypothesis, the power of the study was calculated *post-hoc*. The majority of the neuropsychological tests and questionnaires used in this study refer to norm data expressed in form of standardized, scaled or T scores, where a difference of more than one standard deviation from the population mean is considered to be clinically significant. Consequently, for independent sample

TABLE 2 | Cognitive function of girls with central precocious puberty (CPP) treated with gonadotropin releasing hormone analog therapy, compared to age-matched controls.

	CPP *N* = 15	Controls *N* = 15	*p*
IQ (WISC III)			
Total IQ	94.1 (12.1)	101.9 (12.0)	0.09
Performance	96.8 (7.8)	103.2 (11.2)	0.08
Verbal IQ	98.4 (10.7)	101.6 (9.4)	0.39
WISC information	10.9 (2.2)	10.7 (1.9)	0.80
WISC vocabulary	9.1 (2.6)	10.5 (1.7)	0.09
WISC incomplete pictures	7.7 (3.2)	10.1 (4.0)	0.07
WISC bloc design	8.5 (3.0)	9.8 (3.4)	0.30
MEMORY			
Verbal (RAVLT)			
Trial 1 (immediate memory)	6.2 (1.9)	7.1 (1.9)	0.23
Trial 2	9.0 (2.4)	10.2 (2.7)	0.22
Trial 3	9.8 (3.7)	11.7 (1.6)	0.08
Trial 4	12.3 (1.9)	13.2 (2.1)	0.21
Trial 5 (best memory)	12.7 (1.9)	12.9 (2.2)	0.80
Learning rate	6.5 (1.8)	5.9 (2.2)	0.38
Total learning	50.1 (9.3)	55.1 (8.5)	0.13
Proactive interference	5.7 (1.1)	6.1 (2.9)	0.70
Retroactive interference	11.7 (2.2)	11.3 (2.9)	0.62
Delayed recall	11.1 (3.4)	11.1 (2.7)	1.00
Visual (CVMT)			
Total score	108.7 (18.3)	114.4 (16.1)	0.40
Recognition	4.2 (1.8)	4.7 (1.2)	0.42
MENTAL ROTATION			
Number correct (out of 20)	14.6 (2.4)	14.3 (2.3)	0.77
Total time (s)	160.3 (63.3)	189.3 (62.3)	0.22
COGNITIVE EXECUTIVE FUNCTION			
Trail Making Test: shifting	9.3 (3.7)	11.4 (2.5)	0.07
Color-Word Interference Test: shifting	11.2 (2.4)	10.5 (3.0)	0.50
Color-Word Interference Test: interference	11.3 (2.2)	11.5 (3.5)	0.86
Verbal Fluency Test: shifting	12.9 (2.9)	12.6 (3.3)	0.77
Design Fluency Test: shifting	12.1 (2.1)	12.3 (3.2)	0.79
Composite *z*-score	−0.09 (0.9)	0.09 (1.1)	0.64
PROCESSING SPEED			
Trail Making Test: number sequencing	8.0 (3.3)	10.9 (2.2)	**0.01**
Trail Making Test: letter sequencing	9.4 (3.1)	9.4 (3.1)	1.00
Color-Word Interference Test: color reading	10.0 (2.6)	10.5 (2.7)	0.59
Color-Word Interference Test: word reading	11.2 (1.4)	11.3 (2.3)	0.85
Verbal Fluency Test: category fluency	11.7 (2.9)	12.1 (2.8)	0.72
Verbal Fluency Test: letter fluency	8.9 (1.9)	8.3 (2.1)	0.42
Design Fluency Test: filled dots	10.4 (3.0)	11.7 (2.0)	0.19
Design Fluency Test: empty dots	11.3 (2.7)	12.3 (3.9)	0.45
Composite *z*-score	−0.20 (1.1)	0.2 (0.9)	0.29
ATTENTION AND EXECUTIVE FUNCTION (CANTAB)			
Choice reaction time: correct responses (%)	98.4 (1.3)	99.0 (1.1)	0.18
Choice reaction time: mean latency (ms)	469.6 (107.9)	492.4 (112.1)	0.57
Match to sample visual search: correct responses (%)	98.1 (2.0)	98.9 (1.7)	0.23
Match to sample visual search: mean latency (s)	3.4 (0.7)	3.7 (1.0)	0.29
Spatial working memory: number of errors	30.7 (14.6)	26.5 (19.3)	0.50
Spatial working memory: search strategy	32.7 (5.4)	34.1 (6.2)	0.52
Stop signal task: successful stops (%)	47.3 (7.4)	50.1 (9.8)	0.30
Stop signal response time (ms)	205.8 (53.2)	214.9 (55.1)	0.64

IQ estimations are presented as standardized IQ scores (normative mean = 100, SD = 15). WISC subscales, Cognitive executive function and Processing speed are presented as scaled scores (normative mean = 10, SD = 3). CANTAB, Memory, and Mental rotation tests results are presented as raw scores; WISC, Wechsler Intelligence Scale for Children; RAVLT, Rey Auditory Verbal Learning Test; CVMT, Continuous Visual Memory Test; CANTAB, Cambridge Neuropsychological Test Automated Battery; p, significance level (independent sample t-test) of difference between CPP and controls. p < 0.05 are marked in bold.

t-test, statistical power (1–β) was found to be 0.75, based on an α level of 0.05 and a difference of one standard deviation between the groups (Faul et al., 2007).

DISCUSSION

The main objective of the study was to assess psychological functioning in GnRHa treated girls with idiopathic CPP as compared to age-matched controls. With respect to cognitive functioning, behavioral, and social problems, treated CPP girls do not differ from age matched controls. However, the significance of the results regarding emotional reactivity and emotional regulation capacity remains unclear. The interpretation of HRV findings, as a measure of emotional regulation capacity, is complicated by the fact that GnRHa may directly influence heart rhythm through GnRH cardiac receptors (Dong et al., 2011). Possible interpretations of our findings as well as methodological challenges are discussed below.

Cognitive Functioning and Psychosocial Problems

In contrast to previous reports on elevated verbal *IQ scores*, and/or accelerated school achievements in CPP girls (Galatzer et al., 1984; Ehrhardt and Meyer-Bahlburg, 1986), the GnRHa treated CPP girls' estimated IQ in the current study was within the normal range and somewhat lower, although not significantly, than that of controls (**Table 1**).

No significant differences between the CPP and the control group were seen with regard to *cognitive performance* neither on paper and pencil nor in computer based tests concerning memory, spatial ability, attention, and executive functions. Only in the Trail Making Test—Number Sequencing, assessing

TABLE 3 | Cognitive executive function and behavioral problems in girls with central precocious puberty (CPP) treated with gonadotropin releasing hormone analog therapy, compared to age-matched controls.

	CPP *N* = 14	Control *N* = 10	*p*
BRIEF			
Clinical scales			
Behavioral regulation index	51.8 (11.7)	47.6 (7.7)	0.33
Inhibit	52.1 (9.8)	48.9 (10.6)	0.47
Shift	52.7 (10.7)	46.7 (6.9)	0.14
Emotional control	50.1 (10.6)	48.3 (6.8)	0.65
Metacognition index	50.7 (10.1)	49.1 (10.4)	0.71
Initiate	50.5 (7.2)	46.5 (5.3)	0.15
Working memory	52.5 (11.0)	47.7 (9.0)	0.26
Plan/organize	49.1 (9.0)	49.7 (9.1)	0.88
Organization of materials	48.3 (7.7)	49.5 (11.2)	0.77
Monitor	48.7 (7.1)	50.3 (8.8)	0.62
Global executive composite	51.3 (9.8)	48.1 (10.5)	0.46
Validity scales			
Negativity	0.5 (0.9)	0.2 (0.6)	0.36
Inconsistency of Responses	2.0 (1.4)	1.9 (1.7)	0.90
	N = 15	*N* = 13	
CBCL			
Total social competence	44.5 (11.6)	47.0 (6.4)	0.49
Activities	41.4 (9.0)	44.5 (6.6)	0.31
Social	45.9 (7.4)	47.7 (6.3)	0.51
School	48.8 (9.1)	51.3 (6.1)	0.41
Internalizing problems	56.7 (10.9)	53.5 (11.1)	0.45
Withdrawn	55.2 (8.3)	55.2 (6.6)	0.99
Somatic complaints	58.8 (9.0)	54.8 (6.2)	0.18
Anxious/Depressed	58.1 (9.8)	56.3 (8.5)	0.61
Externalizing problems	48.6 (10.3)	45.7 (7.7)	0.41
Delinquent behavior	53.5 (5.7)	52.9 (5.6)	0.78
Aggressive behavior	53.0 (6.0)	51.2 (2.6)	0.33
Total problems	52.3 (11.5)	48.3 (10.2)	0.34
Social problems	54.1 (8.5)	52.7 (5.6)	0.62
Thought problems	53.7 (6.7)	52.7 (6.7)	0.68
Attention problems	55.7 (6.7)	55.7 (10.6)	0.99

Except for validity scales, all values are presented as T-scores (normative mean = 50, SD = 10). BRIEF, Behavior Rating Inventory of Executive Function questionnaire, parent version; CBCL, Child Behavior Check List questionnaire, parent version; p, significance level (independent sample t-test) of difference between CPP and controls.

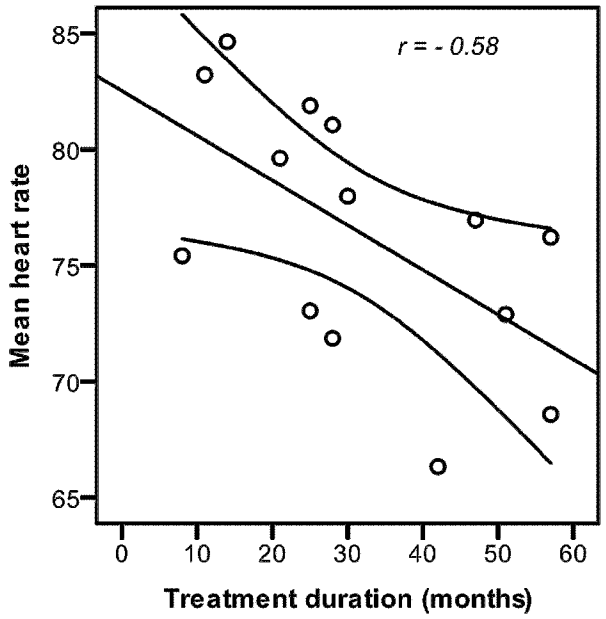

FIGURE 2 | Influence of treatment duration on heart rate. Longer GnRHa treatment duration was significantly correlated ($p = 0.037$) with lower resting heart rate (controlled for age) in girls with idiopatic central precocious puberty.

processing speed, the CPP group showed significantly poorer performance (**Table 2**). This finding is difficult to explain since neither the very similar Trail Making Test—Letter Sequencing, nor any other of the processing speed tests showed significant differences between the groups. Taking into account that the *p*-values were not corrected for multiple testing, it is possible that this finding is accidental. In line with this, the CPP girls' parents did not report any problems with regard to executive functioning as measured by the BRIEF questionnaire.

Behavioral problems and social competence were assessed with the parent version of the CBCL and showed no statistical differences between the groups (**Table 3**). Several earlier studies reported different levels of problems in CPP using the CBCL. Based on CBCL scores of 33 girls with CPP compared to age matched controls, Sonis et al. (1985) concluded that overall they could be described as troubled, depressed, aggressive, socially withdrawn, and moody (Sonis et al., 1985). In their results, the authors did not discriminate between the idiopathic and other types of CPP. Moreover, at the time of CBCL scoring, only 8 out of 33 girls received GnRHa treatment, which at the beginning of the 1980s was still considered to be experimental; the results did not discriminate between the treated and untreated girls. In a longitudinal study, Xhrouet-Heinrichs et al. (1997) followed 20 girls with CPP (15 of idiopathic type) of which 15 were treated with GnRHa for 2 years. The CBCL was filled out by parents at 1 and 24 months after diagnosis. Authors reported elevated *withdrawal*, *anxious/depressed*, or *aggressive behavior* scores in 30–40% of the girls. After 2 years, most of the same girls still displayed elevated scores. In contrast, in our study, only 2 out of 15 (13%) CPP girls showed elevated scores on the same scales, which was very similar to what we observed in age matched controls (2 out of 13). In a more recent study, Kim and Lee (2012) compared 34 girls with idiopathic CPP (mean age 8.1) to 39 same age controls; no information about whether CPP girls were treated was provided. The results showed that although CPP girls had more behavioral problems, no significant differences were observed between the two study groups in terms of clinically important scores.

While all of the presented studies differ with respect to the age of participants, treatment duration, and assessment time, it is worth mentioning that the most recent studies

show less psychosocial problems in CPP children than older ones. Although speculative, the decrease in psychosocial problems reported in recent studies (including this one) could be a result of less stigmatization of and more openness about this condition and perhaps the modified management of CPP with regard to treatment initiation and monitoring.

Emotional Processing

Emotional Reactivity

In the presence of fearful-faces in the mismatch-condition, CPP girls showed increased reaction times compared to neutral-faces, while the opposite was seen in the control group; in statistical terms this difference represented a large effect (Cohen, 1992). However, no significant differences between the groups were observed in the matched-condition.

In healthy samples, emotional stimuli can facilitate interference resolution, and enhance task performance by reducing reaction times (Levens and Phelps, 2008). In contrast, in vulnerable groups, increased reactivity toward emotionally intensive stimuli can be associated with increased interference between emotions and executive functions, leading to increased reaction times in a seemingly unrelated choice reaction task (for an overview see Mueller et al., 2011). Increased reaction time in the CPP group may therefore indicate higher distractibility by task-irrelevant stimuli (anxious faces) and increased interference with executive functions because processing of emotional (particularly negative) stimuli may impair executive motor control (de Houwer and Tibboel, 2010; Herbert and Sütterlin, 2011). Nevertheless, the fact that the same pattern was not observed in a matched-condition somewhat weakens such interpretation. While it is true that the mismatch-condition represents a higher cognitive demand, expressed by a tendency toward slower reaction times in both groups when compared to the matched-condition, we cannot firmly conclude that this minor difference in cognitive load is responsible for the diversity of outcomes. In summary, although part of the findings suggest differences in emotional reactivity between the groups, the results are not conclusive.

Cardiac Function and Emotional Regulation

GnRHa treated CPP girls had significantly lower resting HR and significantly higher HRV than controls. Resting HR was negatively correlated with treatment duration, i.e., longer GnRHa treatment was associated with lower resting HR (**Figure 2**), while no correlations between HRV and treatment duration were found. The results indicate large effects; Cohen's $d > 0.8$ and Pearson's $r > 0.5$ (Cohen, 1992).

The main goal of the heart rhythm recording was estimation of HRV as a proxy for cardiac vagal influence (Thayer et al., 2012). Consequently, the lower HR and higher HRV could suggest that treated CPP girls have better emotion regulation capacity and higher adaptability to changing contexts than controls. However, for such interpretation to be valid, a direct GnRHa effect on heart rhythm should be excluded. Such effect could be possibly mediated via GnRH receptors that have been found in cardiomyocytes (Kakar and Jennes, 1995). It has been shown in a murine model that GnRH can augment cardiomyocytes' contractile characteristics via a GnRH receptor/phosphokinase A-dependent (PKA) mechanism, while the opposite effect was observed after administration of GnRH receptor blocker (Dong et al., 2011). Although there is no direct evidence of such effects in humans, findings from other studies might be attributed to these cellular mechanisms; prolonged electrocardiographic QT intervals were recorded in GnRHa treated prostate cancer patients (Garnick et al., 2004), and GnRHa therapy in young women with symptomatic uterine leiomyoma, endometriosis, or candidates for *in vitro* fertilization led to significantly reduced peak flow velocity and cardiac index (stroke volume × heart rate; Eckstein et al., 1993).

A possibility of direct GnRHa effect on heart rhythm makes interpretation of the HRV results difficult, since HRV is only a proxy for central, prefrontally mediated inhibitory processes that are peripherally expressed through cardiac vagal influence (Thayer et al., 2012). At the planning stage of this study, the possibility of such interactions had not been described. If further confirmed, these findings can make applicability of HRV as a measure of emotional regulation capacity invalid in individuals receiving GnRHa treatment.

Assessment of Emotional Processing—Conclusions and Further Steps

Overall, our findings do not provide firm conclusions with regard to differences in emotional processing between the GnRHa treated CPP girls and age-matched controls. The diversity of the results suggests that more emphasis should be put on the investigation of emotion processing in future studies. In this respect both psychophysiological and experimental paradigms that tap in to the different domains of emotional processing and regulation (i.e., capacity, reactivity, recovery, and sensitivity) should be considered.

Methodological Considerations

Psychological functioning of GnRHa treated CPP girls may depend on a number of different mechanisms including direct effects of GnRHa on the brain, cessation of sex steroid influences, degree of exposure to the pubertal hormones before treatment initiation, the course of the CPP condition itself or psychosocial/educational environment. It is thus difficult to isolate the impact of GnRHa treatment on psychological functioning. The most appropriate study design to discriminate between GnRHa effects and other factors would be a randomized controlled trial (RCT). Since a RCT cannot be conducted due to ethical reasons, the most obvious alternatives include comparison of treated CPP children and controls matched for either chronological or biological age, in a cross-sectional or a longitudinal study. Comparison of cognitive development trajectories of non-CPP control and CPP-treatment groups through several measurement points, i.e., pre-, under- and post-treatment can provide most hints about GnRHa treatment impact on cognitive development. Nevertheless, while providing more information, the longitudinal design still cannot ensure proper isolation of GnRHa influence on brain development from the natural course of the condition, including pretreatment

sex steroid exposure. Furthermore, the question remains if matching should be done by chronological or biological age. It can be argued that matching by chronological age is not appropriate since CPP children's biological age is higher than that of their chronological age peers. Matching by biological age would ensure comparable levels of biological maturation between the groups, which theoretically could increase the likelihood that the observed cognitive differences are indeed related to the actions of GnRHa. On the other hand, development of cognitive functioning cannot be separated from environmental influences. The majority of the GnRHa treated CPP girls attend school classes that are appropriate to their chronological age and socialize with the same age peers. It is therefore, in our opinion, more ecologically valid to evaluate cognitive functioning in comparison to the same chronological age population.

Finally, to gain mechanistic insights into the GnRHa effects on brain development, animal studies might provide further knowledge. Our group has previously conducted a twin sheep RCT where one of the twins had their puberty blocked with GnRHa. The results indicated that GnRHa might have influenced the development of cognitive functions related to emotion processing, while no clear effects on cognitive functions that did not involve emotional processing were found (Wojniusz et al., 2011, 2013; Evans et al., 2012; Nuruddin et al., 2013a,b,c; Robinson et al., 2014). While this study represented a delayed rather than precocious puberty model and translation of the results to humans should be made with caution, it suggests emotional processing as a potential area of GnRHa influence on the brain.

Sample Size and Limitations

Due to the low number of CPP patients receiving GnRHa treatment, only 15 CPP girls were included in this study, which can limit its statistical power. Nevertheless, *post-hoc* power analysis showed $1 - \beta$ to be 0.75, which gives a fair chance of rejecting the false null hypothesis taking into account a group difference of interest of 1 *SD*. We argue that with regard to most of the cognitive tests and questionnaires used in this study, particularly those with known norm data, 1 *SD* represents a boundary of what is a clinically interesting difference. Although more participants would increase the statistical power and possibility of discovering smaller group differences, in our opinion, the present study provides useful information and suggestions for future research areas in a field that to date has been rarely investigated.

Regarding experimental and physiological measures for assessment of emotion processing, in the hindsight, we did not fully succeed in our choice of methods. While the results of EFT were ambiguous, perhaps depending on motivational factors, and overall difficult to interpret, the HRV findings were possibly not even valid as a proxy of cardiac vagal influence (see Section Cardiac Function and Emotional Regulation). Alternative approaches that could be applied in future studies could include functional neuro-imaging techniques to detect subtle changes of emotion processing directly within the central nervous system rather than applying peripheral proxies. Alternative behavioral measures of emotion-related attentional processing could be obtained via more implicit approaches that are less confounded by motivational states (e.g., eye-tracking).

Conclusion

Overall, the findings suggest that GnRHa treated CPP girls do not differ in their cognitive functioning, behavioral, and social problems from the same age peers, at least, in settings that do not involve emotional processing. Although our findings with regard to emotional regulation and reactivity are inconclusive, they provide hints that CPP girls may differ in these areas from same age peers. We, therefore, suggest that future studies should to a higher degree emphasize investigation of emotional processing in a CPP population.

Finally, the differences in cardiac rhythm, expressed as lower HR in the CPP group and the fact that they were increasing with treatment duration, should be more closely followed up in the future, making use of methodologies that are appropriate for investigation of cardiac health.

AUTHOR CONTRIBUTIONS

SW, One of the designers of the study; collected the data with regard to CANTAB, HRV, and EFT tests; analyzed the data from these experiments and drafted the manuscript with regard to introductory part, methods and analysis of CANTAB, HRV, and EFT, and drafted the discussion. NC, One of the designers of the study; main responsibility for subject recruitment; collected and analyzed the data with regard to neuropsychological tests and behavioral questionnaires; drafted the parts of the article associated with neuropsychological tests and behavioral questionnaires. SS, Took part in the data analysis and interpretation of results with regard to HRV and EFT experiments; critically reviewed the data analysis and the manuscript. SA, Responsible for the design of neuropsychological test battery; critically reviewed the data analysis and the manuscript. JD, IG, JV, KD, SV, MCr, Participated in subject recruitment/treatment; critically reviewed the data analysis and the manuscript. CV, Participated in design of the study; critically reviewed the data analysis and the manuscript. MCo, One of the designers of the study; participated in subject assessment/recruitment/treatment; critically reviewed the data analysis and the manuscript. IH, Primary investigator; responsible for the design of the study; critically reviewed the data analysis, and the manuscript.

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RESEARCH ARTICLE

Off-label Medication Prescribing Patterns in Pediatrics: An Update

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ABSTRACT

OBJECTIVES: To describe the frequency of off-label drug use in 2014 as defined by the Food and Drug Administration (FDA)–approved age ranges in patients ≤18 years of age, to determine the rate of off-label drug use in 2014 by drug classification, and to compare current off-label medication usage rates with historical rates.

METHODS: This is a retrospective cohort study of an administrative database containing inpatient resource use data from January 1, 2014, to December 31, 2014. Patients ≤18 years of age receiving 1 of 76 selected commonly prescribed medications are included. Off-label drug use is defined as use in a patient younger than the lower limit of the FDA-approved age range for any indication or dosage form of that drug.

RESULTS: At least 1 drug was prescribed off label in 779 270 of 2 773 770 (28.1%) patient visits during the study period. Younger age, longer hospital stays, and mortality were associated with higher rates of off-label medication prescription. Off-label usage of certain medications differed between care settings. Rates of off-label medication use were higher in observational (45.5%), inpatient (53.9%), and ambulatory (54.2%) settings.

CONCLUSIONS: Although off-label drug use at major US pediatric hospitals is declining, 1 out of every 4 medications is not in accordance with FDA label indications for patient age. There exists substantial variation in off-label drug use among drug categories and encounter types. Although many commonly prescribed medications are FDA-approved for use in subpopulations of pediatric patients, studies of their safety, efficacy, pharmacokinetics, and optimal dosing are ongoing.

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The US Food and Drug Administration (FDA) requires that medications be tested for safety and efficacy at a specific dosage (and for a specific time period) before approval for clinical use in a particular population.[1] Use of medications outside of these parameters is considered "off-label" drug use. Children present challenges in clinical trials owing to scientific, clinical, ethical, and logistic concerns, which have previously limited and even discouraged the testing of medications in children.[1] Consequently, the majority of medications used in the care of children has historically been used off label without an adequate understanding of appropriate dosage, safety, or efficacy.[1]

Numerous legislative measures have been enacted to address the barriers in pediatric drug testing.[1,2] In 2002, the Best Pharmaceuticals for Children Act (BPCA) directed the implementation of methods for pediatric drug development, including identifying and prioritizing drugs needing pediatric study.[1] The BPCA priority list, first generated in 2004, is annually updated and identifies medications that are deemed critical to the treatment of children and adolescents and to the needs for pediatric therapeutics.[1] Passed in 2003, the Pediatric Research Equity Act (PREA) allowed the FDA to require pediatric studies of any product that was likely to be used in a substantial number of pediatric patients or that had meaningful benefits for children over existing treatments.[2] In 2012, the US Congress passed the FDA Safety and Innovation Act, aiming to ensure that pediatric evaluations are conducted earlier in the drug development process.[3] This act created accountability for the completion of pediatric studies under the BPCA and PREA. After the enactment of the BPCA and PREA, >500 pediatric-specific drug-labeling changes have been made.[3]

In 2007, Shah et al[4] published a retrospective cohort study in which they analyzed inpatient off-label drug use from January 1, 2004, to December 31, 2004, at 31 tertiary care pediatric hospitals in the United States. Results revealed that at least 1 drug was used off label in 78.7% of patients and that 40% of the total dollars spent on these medications was for off-label use.[4] Authors of recent studies have analyzed the off-label use of certain medication classes in subspecialty populations of children with similar findings.[5–7]

To our knowledge, no recent studies have been published to describe the frequency of off-label drug use in pediatric patients across the inpatient, observational, ambulatory, and emergency department settings. Our aim was to analyze current rates of off-label medication use in children in light of ongoing efforts to incentivize and promote projects that improve pediatric drug labeling.

METHODS

Data Source

In this retrospective cohort study, we used the Pediatric Health Information System (PHIS) data set from 2014 (accessed in November 2016). PHIS is a comparative pediatric database and includes clinical and resource use data for inpatient, ambulatory surgery, emergency department, and observation unit patient encounters for >45 children's hospitals in the United States. Blinded patient data are collected from >6 million patient encounters and include information about diagnoses, procedures, demographics, length of stay, and discharge dispositions of patients. Data are de-identified at the time of submission and before data extraction and analysis. Additionally, resource use data are subjected to a number of reliability and validity checks before data quality reports are generated.

Aims and Hypothesis

We aimed to describe the frequency of off-label drug use in 2014 as defined by FDA-approved age ranges in patients ≤18 years of age and to determine the rate of off-label drug use in 2014 by drug classification and encounter type using PHIS. Additionally, we compared rates of off-label drug use in pediatric patients in 2014 with previous rates. We hypothesized that although the specific drugs used off label have changed over time, the frequency of off-label drug use has not changed.

Eligibility

All patients ≤18 years of age at the time of presentation to any of the >45 hospitals submitting data to PHIS from January 1, 2014, to December 31, 2014 were eligible for inclusion. Those patients, prescribed 1 of 76 preselected medications on the basis of frequency, composed the study population (see below and Table 1). This study was

TABLE 1 Percentage of Off-label Usage for 2014 BPCA Priority List Medications, Stratified by Encounter Type

2014 BPCA Priority List Medications	Off-label Usage, %					
	Total	Clinical	Emergency	Ambulatory	Observational	Inpatient
Albuterol	29.5	17.8	28.5	27.6	29.1	33.0
Ampicillin	0	0	0	0	0	0
Azithromycin	7.1	0	3.3	4.5	8.9	8.9
Clindamycin	0	0	0	0	0	0
Epinephrine	0	0	0	0	0	0
Furosemide	0	0	0	0	0	0
Heparin sodium	0	0	0	0	0	0
Hydrocortisone	0	0	0	0	0	0
Ketamine	93.7	92.0	97.2	87.9	92.9	91.3
Lorazepam	65.0	42.6	45.2	46.9	60.4	67.2
Metoclopramide	0	0	0	0	0	0
Midazolam	0	0	0	0	0	0
Morphine sulfate	97.9	96.2	96.8	98.6	98.2	97.4
Pantoprazole	34.0	7.7	7.6	9.9	17.0	36.2
Prednisone	0	0	0	0	0	0
Sulfamethoxazole and trimethoprim	1.5	0	0.1	0.4	0.4	1.8

determined exempt by our institutional review board.

Independent Variables

Demographic variables for each patient included age, sex, race, and insurance type. The specific encounter type (inpatient, observational, clinical, ambulatory, emergency, or other) and length of stay were recorded. Patient discharge disposition was determined and specifically defined as admitted, discharged from the hospital (to home, to court, or to law enforcement), transferred (to another hospital or health care institution), died, or other (hospice or left against medical advice).

Dependent Variable

We categorized drug use, the dependent variable, as either appropriate for age or off label. We defined off-label drug use as use in a patient who was younger than the lower limit of the FDA-approved age range for any indication or dosage form of that drug. Drug use was considered appropriate if the patient was equal to or older than the lower limit of the FDA-approved age range of a drug regardless of indication or dosage form. Because of limitations of the PHIS database, we could not determine the specific indication for which a drug was prescribed.

The most commonly prescribed drugs were examined for off-label use, as represented by patient-level data within PHIS. The top 100 most commonly prescribed drugs were listed, from which 76 medications were ultimately examined. Twenty-four commonly prescribed agents were removed from the final list because they were determined to not be discrete therapeutic medications (see Supplemental Table 5).

The lower limit of the FDA-approved age range for each drug was determined on the basis of consensus definitions from 3 separate drug information resources: Micromedex, Lexicomp, and DailyMed. All but 13 of the 76 drugs that were analyzed achieved a consensus definition for the current lower limit of the FDA-approved age range in 2 of the 3 databases. When a consensus was not achieved, the lower limit of the FDA-approved age range was defined by a pharmacist at our institution (see Supplemental Tables 6). If a patient received a drug during 2014, regardless of the number of times the same drug was administered during the patient visit, it was counted as 1 prescription.

Data Analysis

Descriptive statistics were obtained and stratified by off-label status to summarize the study population. All study medications were classified into 1 of 7 categories: central nervous system (CNS); respiratory and/or ear, nose, and throat (ENT); cardiac; endocrine; fluid, electrolyte, nutrition, and gastroenterology; hematology and/or oncology; and infectious disease (ID). The frequencies and proportions of visits in which patients received an off-label drug prescription were generated for each medication under these categories. The percent of off-label usage was also summarized for the 2014 BPCA priority list medications, which were also on the study medication list and stratified by encounter type.

RESULTS

There were 2 773 770 patient visits (≤18 years of age) recorded in PHIS in 2014, during which 1 of the 76 selected drugs was prescribed. Of these visits, 779 270 (28.1%) included the prescription of at least 1 off-label drug. Characteristics of patients who were prescribed medications off label are listed in Table 2. Rates of off-label medication use were higher in the inpatient, observational, and ambulatory settings (53.9% [95% confidence interval (CI): 53.8%–54.0%]; 45.5% [95% CI: 45.3%–45.7%]; and 54.2% [95% CI: 54.0%–54.3%], respectively) compared with the emergency setting (10.3% [95% CI: 10.3%–10.4%]).

Rates of off-label medication prescription were similar despite patient sex, race, and insurance type. Younger patients had higher off-label rates when compared with their older counterparts. Specifically, prescriptions to neonates <28 days of age were off label 51% of the time (95% CI: 50.6%–51.3%). Infants 29 days to 1 year old received off-label prescriptions at a rate of 44.8% (95% CI: 44.7%–44.9%). Older patients, however, experienced lower rates of off-label medication prescription (21.5% [95% CI: 21.4%–21.6%] for children ages 13–18 years).

Longer patient stays (>1 day) were associated with higher rates of off-label medication usage (55.3% [95% CI: 55.1%–55.4%]) compared with visits of ≤1 day (22.8% [95% CI: 22.7%–22.8%] of visits). Regarding discharge disposition, patients who ultimately died had the highest off-label prescription rates (86.9% [95% CI: 85.9%–87.8%]), whereas patients who were discharged or transferred received off-label prescriptions at much lower rates (28% [95% CI: 27.9%–28.0%] and 38.1% [95% CI: 37.7%–38.6%], respectively).

There was substantial variation in off-label medication prescription by drug category (Table 3). CNS medications and respiratory and/or ENT medications had the highest rates of off-label use at 30.4% (95% CI: 30.3%–30.5%) and 24.9% (95% CI: 24.8%–25%), respectively. Medications in the ID category, by comparison, were prescribed off label at a rate of only 1.7% (95% CI: 1.7%–1.8%). Several medications were noted to have lower usage rates but nearly universal off-label prescribing. This was most notable within the CNS category. Dexmedetomidine, hydromorphone, and ketamine had overall usage rates of 2.5%, 2.3%, and 2%, with off-label usage rates of 98.7%, 95%, and 93.7%, respectively. Similarly, morphine was used in 12.2% of patients and was used off label 97.9% of the time. In contrast, ibuprofen was used in a relatively high percentage (23.5%) of patients and was used off label <1% of the time (Table 3).

Commonly prescribed 2014 BPCA priority list medications are further analyzed by prescription setting in Table 1. Although a number of these medications were used on label in all patients, variation in off-label usage was demonstrated for other medications on the basis of encounter setting. Albuterol had higher rates of off-label prescription in the emergency (28.5% [95% CI: 28.3%–28.7%]), observational (29% [95% CI: 28.5%–29.6%]), and inpatient settings (33% [95% CI: 32.7%–33.3%]) compared with in the clinical setting (17.8%

TABLE 2 Characteristics of the Study Population, Stratified by Off-label Status

	Overall (N = 2 773 770)	Off-label Usage (n = 779 270), n (%)	No Off-label Usage (n = 1 994 500), n (%)
Age			
≤28 d	87 324	44 506 (51.0)	42 818 (49.0)
29 d–1 y	639 652	286 666 (44.8)	352 986 (55.2)
2–5 y	759 503	160 836 (21.1)	598 667 (78.8)
6–12 y	776 361	177 579 (22.9)	598 782 (77.1)
13+ y	510 930	109 683 (21.5)	401 247 (78.5)
Sex			
Boys	1 505 561	451 678 (30.0)	1 053 883 (70.0)
Girls	1 267 900	327 466 (25.8)	940 434 (74.2)
Race			
White	1 510 569	460 818 (30.5)	1 049 751 (69.5)
African American	641 279	151 186 (23.6)	490 093 (76.4)
Other	505 786	137 089 (27.1)	368 697 (72.9)
Insurance type			
Public	1 602 570	423 162 (26.4)	1 179 408 (73.6)
Private	988 218	310 822 (31.5)	677 396 (68.6)
Other	171 856	42 588 (24.8)	129 268 (75.2)
Encounter type			
Inpatient	532 850	287 153 (53.9)	245 697 (46.1)
Observational	189 874	86 351 (45.5)	103 523 (54.5)
Ambulatory	442 818	239 915 (54.2)	202 903 (45.8)
Emergency department	1 451 426	149 969 (10.3)	1 301 457 (89.7)
Clinic visit	87 342	7543 (8.6)	79 799 (91.4)
Other	69 438	8337 (12.0)	61 101 (88.0)
Length of stay			
≤1 d	2 320 730	528 843 (22.8)	1 791 887 (77.2)
>1 d	453 040	250 427 (55.3)	202 613 (44.7)
Discharge disposition			
Discharged	2 647 285	739 921 (28.0)	1 907 364 (72.0)
Transferred	37 161	14 175 (38.1)	22 986 (61.9)
Died	4851	4215 (86.9)	636 (13.1)
Other	9971	1103 (11.1)	8868 (88.9)

[95% CI: 16.5%–19.1%]). Lorazepam was used off label at rates of 60.4% (95% CI: 58.6%–62.1%) and 67.2% (95% CI: 66.8%–67.6%) in the observational and inpatient settings, respectively, compared with 42.6% (95% CI: 38.8%–46.4%) in the clinical setting and 45.2% (95% CI: 43.6%–46.8%) in the emergency setting. Similar findings were noted for azithromycin, pantoprazole, and sulfamethoxazole and trimethoprim.

DISCUSSION

Our analysis of off-label drug use in children's hospitals during 2014 reveals that the majority of patient encounters included prescriptions for drugs in accordance with FDA label indications for patient age. Higher rates of off-label medication use in pediatric patients were associated with younger patient age, longer hospital stays, and encounters categorized as inpatient, observational, or ambulatory. Patients who died had notably higher rates of off-label medication prescription compared with patients who were discharged or transferred. Although we are unable to directly correlate off-label drug use with illness severity, with these results, it is suggested that children with more severe diseases are more likely to receive medications outside of FDA labeling indications.

These findings differ from those of previous studies in which off-label medication use was analyzed in pediatric patients.[4–10] In their study, Shah et al[4] similarly measured off-label drug use in a broad cohort of pediatric patients who were hospitalized as reported through PHIS in 2004. Shah et al[4] concluded that 78.7% of patients received at least 1 of the most frequently prescribed drugs off label. In contrast, with our multicenter study, we were able to capture not only children who were hospitalized but also clinical, emergency, ambulatory, and observational patient encounters. The inclusion of outpatient visits in the current study could, in part, explain the overall lower rates of off-label drug use when compared with those in the 2007 study. However, at 53.9% (Table 2), our inpatient off-label prescription rates were nearly 25% less than those described by Shah et al[4]. Furthermore, rates in the clinical setting were 8.6%, >50% lower than rates cited in a 2009 study by Bazzano et al,[9] in which outpatient data from the 2001–2004 National Ambulatory Medical Care Surveys were used to analyze off-label drug use in pediatric clinic patients. Although direct comparisons with previous studies are affected by differing definitions of off-label drug usage and medications analyzed, these comparisons reveal that drug labeling for children has increased while reinforcing the need for further pediatric-specific research.

In a smaller study published in 2010, Czaja et al[5] analyzed a cohort of PICU patients and found that 85% received at least 1 medication off label. Patients requiring intensive care often experience longer inpatient stays and may have a higher likelihood of mortality. Although not specifically analyzing PICU patients, we found that inpatient encounters, hospital stays >1 day, and patient mortality correlated with higher rates of off-label drug prescription (Table 2). In a 2010 single-center study, Phan et al[10] analyzed the frequency of off-label and unlicensed medication use in a pediatric emergency department, concluding that 26.2% of

medication orders in 2191 patients were off label. In our study, rates of off-label medication prescription in emergency settings were 10.3%; however, this number accounts only for those patients seen in the emergency department and not subsequently admitted. In 2012, Lee et al[7] found that antidepressant prescription in children and adolescents was on label only 9.2% of the time. In our study, we did not capture psychiatric prescriptions because they did not occur in high frequency. In contrast to our study, these investigations are limited by their smaller sample size and are focused on subspecialty care.

Off-label drug prescription rates continue to vary by patient age. In our study, neonates ≤28 days of age had the highest rates of off-label medication prescription. Similarly, in their study, Hsu and Brazelton[11] concluded that patients ≤27 days of age are the most lacking in FDA-approved medication guidelines.[12–27] These results contrast with findings in the study by Shah et al,[4] in which patient age >28 days was associated with higher rates of off-label drug use. Bazzano et al[9] found that children <2 years of age were more likely to receive medications off label. This finding is reiterated in the current study, indicating that drug safety and efficacy research in young children should remain a priority.

Our study reveals that there continues to be substantial variation in off-label drug use by drug categories, which is consistent with a previous study.[4] Medications that affect the CNS were used off label >30% of the time in the current study, whereas use of off-label anti-infective agents occurred <2% of the time, and steroid use was nearly always on label. This suggests that further investment in the research of analgesics, anesthetics, and sedatives in the pediatric population is warranted over other drug categories.

Off-label drug prescription of a number of medications was influenced by clinical setting in our study, as demonstrated in Table 1. Medications such as azithromycin, lorazepam, pantoprazole, and sulfamethoxazole and trimethoprim were used off label at notably higher rates in the observational and inpatient settings compared with in the clinical, emergency,

TABLE 3 Drug Classification, Frequency, and Proportion of Study Patients Prescribed Drug and Percent of Off-label Prescription

Commonly Prescribed Medications by Drug Classification	Patients in PHIS Prescribed Drug, *n* (%)	Patients in PHIS Prescribed Drug Off Label, *n* (%)
CNS	1 821 645 (65.7)	553 655 (30.4)
Acetaminophen	735 724 (26.5)	0 (0)
Bupivacaine	117 041 (4.2)	83 061 (71.0)
Dexmedetomidine	68 493 (2.5)	67 576 (98.7)
Diazepam	38 958 (1.4)	266 (0.7)
Fentanyl	449 284 (16.2)	97 361 (21.7)
Hydromorphone	62 330 (2.3)	59 206 (95.0)
Ibuprofen	652 754 (23.5)	4354 (0.7)
Ketamine	55 547 (2.0)	52 023 (93.7)
Ketorolac	220 696 (8.0)	19 785 (9.0)
Levetiracetam	40 676 (1.5)	987 (2.4)
Lorazepam	60 647 (2.2)	39 429 (65.0)
Meperidine	40 769 (1.5)	0 (0)
Midazolam	339 571 (12.2)	0 (0)
Morphine sulfate	338 584 (12.2)	331 332 (97.9)
Neostigmine	101 920 (3.7)	0 (0)
Oxycodone	94 012 (3.4)	53 417 (56.8)
Propofol	415 430 (15.0)	9239 (2.2)
Rocuronium	111 341 (4.0)	11 301 (10.2)
Sevoflurane	58 020 (2.1)	0 (0)
Succinylcholine	27 970 (1.0)	0 (0)
Vecuronium	32 106 (1.2)	4724 (14.7)
Respiratory and/or ENT	652 987 (23.5)	162 447 (24.9)
Albuterol	336 152 (12.1)	99 264 (29.5)
Chlorhexidine	61 226 (2.2)	0 (0)
Diphenhydramine	189 414 (6.8)	27 016 (14.3)
Fluticasone	48 573 (1.8)	381 (0.8)
Ipratropium bromide	126 516 (4.6)	0 (0)
Oxymetazoline	69 288 (2.5)	40 967 (59.1)
Phenylephrine	37 692 (1.4)	0 (0)
Cardiac	665 259 (24.0)	64 154 (9.6)
Atropine	28 843 (1.0)	0 (0)
Bupivacaine and epinephrine	70 588 (2.5)	49 299 (69.8)
Epinephrine	87 471 (3.2)	0 (0)
Lidocaine	463 803 (16.7)	0 (0)
Lidocaine and epinephrine	80 844 (2.9)	0 (0)
Lidocaine and prilocaine	31 431 (1.1)	0 (0)
Ropivacaine	25 954 (0.9)	15 520 (59.8)
Endocrine	652 823 (23.5)	0 (0)
Dexamethasone	453 142 (16.3)	0 (0)
Hydrocortisone	36 121 (1.3)	0 (0)
Methylprednisolone	58 120 (2.1)	0 (0)
Prednisolone	127 383 (4.6)	0 (0)
Prednisone	38 340 (1.4)	0 (0)

TABLE 3 Continued

Commonly Prescribed Medications by Drug Classification	Patients in PHIS Prescribed Drug, *n* (%)	Patients in PHIS Prescribed Drug Off Label, *n* (%)
Fluid, electrolyte, nutrition, and gastroenterology	1 398 749 (50.4)	164 517 (11.8)
Calcium gluconate	24 408 (0.9)	0 (0)
Docusate	41 216 (1.5)	5322 (12.9)
Famotidine	29 236 (1.1)	0 (0)
Furosemide	49 828 (1.8)	0 (0)
Glycerin	34 832 (1.3)	23 630 (67.8)
Glycopyrrolate	143 812 (5.2)	3479 (2.4)
Lansoprazole	42 270 (1.5)	10 536 (24.9)
Magnesium sulfate	40 016 (1.4)	0 (0)
Metoclopramide	29 910 (1.1)	0 (0)
Ondansetron	817 265 (29.5)	7583 (0.9)
Pantoprazole	27 668 (1.0)	9400 (34.0)
Phytonadione (vitamin K_1)	48 495 (1.8)	0 (0)
Polyethylene glycol-electrolyte solution	99 461 (3.6)	1720 (1.7)
Potassium chloride	71 281 (2.6)	69 832 (98.0)
Ranitidine	101 162 (3.7)	6498 (6.4)
Sodium bicarbonate	38 159 (1.4)	0 (0)
Sodium chloride	727 576 (26.2)	83 220 (11.4)
Sodium phosphate and sodium biphosphate	28 916 (1.0)	2570 (8.9)
Hematology and/or oncology	178 098 (6.4)	2099 (1.2)
Heparin	167 190 (6.0)	0 (0)
Thrombin	22 571 (0.8)	2099 (9.3)
ID	678 333 (24.5)	11 705 (1.7)
Amoxicillin	63 138 (2.3)	0 (0)
Amoxicillin and clavulanate	22 924 (0.8)	0 (0)
Ampicillin	61 330 (2.2)	0 (0)
Azithromycin	33 722 (1.2)	2382 (7.1)
Bacitracin	90 389 (3.3)	0 (0)
Cefazolin	186 461 (6.7)	6942 (3.7)
Cefotaxime	30 116 (1.1)	0 (0)
Ceftriaxone	119 479 (4.3)	0 (0)
Clindamycin	85 267 (3.1)	0 (0)
Erythromycin	49 823 (1.8)	0 (0)
Gentamicin	46 660 (1.7)	0 (0)
Nystatin	32 951 (1.2)	0 (0)
Ofloxacin	34 498 (1.2)	577 (1.7)
Piperacillin and tazobactam	35 007 (1.3)	2750 (7.9)
Sulfamethoxazole and trimethoprim	37 494 (1.4)	552 (1.5)
Vancomycin	58 677 (2.1)	0 (0)

and ambulatory settings. This may be driven by patient-level factors, such as severity of illness, that influence location of presentation and need for admission and provider-level factors, such as subspecialty training and familiarity with therapeutic alternatives.

Importantly, in 2016, 16 of the 76 most commonly prescribed pediatric medications in 2014 remained on the BPCA priority list for the same year[1] (Table 4). Furthermore, 22 of the commonly prescribed medications are on the most recent BPCA priority list (published in 2017). In our study, the rate of off-label usage of BPCA priority list medications ranged from 0% to 98.7%, suggesting that although a number of medications may be FDA-approved for use in many members of the pediatric population, there remain ongoing questions regarding pharmacokinetics, optimal dosing, safety, and efficacy.

The current study has limitations that require acknowledgment. First, the PHIS database captures only a portion of pediatric care delivered in the United States because the majority of children receive their care outside of tertiary and quaternary children's hospitals. Thus, our study is only reflective of prescribing in these settings, and rates may be higher or lower than true pediatric population rates. In addition, we were unable to determine specific indications for which medications were prescribed and could not determine the dose, form, or route of the medication. These limitations likely led to an underestimation of off-label drug usage in pediatric patients. For example, although albuterol nebulization is approved for ages ≥2 years, the metered-dose inhaler is approved only for age >4 years. Thus, the metered-dose inhaler given to a 3-year-old patient was considered on label in the current study, although, by FDA definition, it was truly off label. Similarly, we were unable to capture any patient who received a higher than approved dose or a medication for any indication for which it was not labeled. In our study, we only analyzed the most commonly prescribed medications to children in 2014. Less common disease states, such as psychiatric or oncologic disease, were not captured in our study because the medications employed to manage these illnesses were less commonly prescribed. It is possible that such medications are used off label frequently because of smaller patient populations, less opportunities for study, and/or fewer on-label alternatives.

Although more than a quarter of patient visits in our study included the use of at least 1 commonly prescribed medication off label, we were unable to determine whether use led to adverse events or whether the drug was effective in managing the condition for which it was

TABLE 4 BPCA Priority List by Year and Off-label Usage in 2014

BPCA Priority List 2017	BPCA Priority List 2014	BPCA Priority List 2004	Off-label Use in 2014, %
Albuterol	Albuterol	—	29.5
Ampicillin	Ampicillin	Ampicillin	0
Azithromycin	Azithromycin	Azithromycin	7.0
Clindamycin	Clindamycin	—	0
Dexmedetomidine	—	—	98.7
Diazepam	—	—	0.7
Epinephrine	Epinephrine	—	0
Furosemide	Furosemide	Furosemide	0
Heparin	Heparin	Heparin	0
Hydrocortisone	Hydrocortisone	—	0
Hydromorphone	—	—	95.0
Inhaled anesthetics (sevoflurane)	—	—	0
Ketamine	Ketamine	Ketamine	93.7
Levetiracetam	—	—	2.4
Lorazepam	Lorazepam	Lorazepam	65.0
Prokinetic drugs	Prokinetic drugs	Metoclopramide	0
Midazolam	Midazolam	—	0
Morphine	Morphine	—	97.9
Ondansetron	—	—	0.9
Pantoprazole	Pantoprazole	—	34.0
Prednisone	Prednisone	—	0
Sulfamethoxazole and trimethoprim	Sulfamethoxazole and trimethoprim	—	1.5
—	—	Piperacillin and tazobactam	7.9

—, not applicable.

prescribed. It is possible that these medications were used off label because no reasonable on-label alternative exists, further highlighting the importance of addressing gaps in pediatric-specific drug knowledge.

CONCLUSIONS

Our study reveals lower rates of off-label medication use in children compared with previous studies, suggesting the positive impact of FDA labeling initiatives. However, 1 out of every 4 patient visits were associated with off-label prescriptions, and variation in off-label medication use by drug class and clinical setting persists, highlighting the continued need for comprehensive drug development studies in which safety, efficacy, pharmacokinetics, and optimal dosing are evaluated in pediatric patients.

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human reproduction ORIGINAL ARTICLE *Reproductive endocrinology*

Functional ovarian reserve in transgender men receiving testosterone therapy: evidence for preserved anti-Müllerian hormone and antral follicle count under prolonged treatment

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STUDY QUESTION: Is the functional ovarian reserve in transgender men affected by testosterone therapy?

SUMMARY ANSWER: Serum anti-Müllerian Hormone (AMH) levels slightly decrease during testosterone treatment but remain within the normal range, suggesting preserved follicular ovarian reserve.

WHAT IS KNOWN ALREADY: Few small studies have investigated the impact of gender-affirming treatment on reproduction in transgender men. Conflicting results were reached concerning ovarian morphology and AMH levels in this context.

STUDY DESIGN, SIZE, DURATION: The study consisted of two arms. The first arm was a prospective pilot study, which enrolled 56 transgender men (median age 22.5 [interquartile range (IQR)—19–27.7] years), 27 of whom had polycystic ovary syndrome (PCOS), prior to the initiation of gender-affirming testosterone therapy. A structured assessment was conducted prior to, and at 3 and 12 months after treatment initiation. The second arm was a cross-sectional study that comprised 47 transgender men (median age 24 [IQR—20–31] years) who received testosterone for a median duration of 35 [IQR 13–62] months. The main outcome measures were serum AMH and antral follicle count (AFC) as indices of ovarian follicular reserve.

PARTICIPANTS/MATERIALS, SETTING, METHODS: The study was conducted at a tertiary center for transgender health. Gender-affirming therapy was administered according to standard practice. AFC was determined by pelvic (abdominal or transvaginal) ultrasound and blood collection for measurements of AMH, testosterone, estradiol, LH and FSH was performed at the designated time-points.

MAIN RESULTS AND THE ROLE OF CHANCE: Prospective arm for the entire group we observed a decrease of 0.71 ng/ml in AMH levels between baseline and 12 months ($P = 0.01$). When expressed in age-specific percentiles, AMH went from the 47.37th to the 40.25th percentile at 12 months ($P < 0.001$). In a sub-group analysis, a decline of 9.52 points in age-specific percentile was seen in subjects with PCOS ($P < 0.001$), while no changes were detected in the non-PCOS group. Testosterone treatment did not affect AFC over time in the entire cohort. In the sub-group analysis, a mean decrease of 5.0 follicles was detected between baseline and the 12 months assessment ($P = 0.047$) only in subjects with PCOS. In the cross-sectional study, AMH inversely correlated with age but not with treatment duration. Notably AMH did not deviate from the 50th age-specific percentile. Finally, four men fathered biological children after being under testosterone treatment for up to 12 years.

LIMITATIONS, REASONS FOR CAUTION: The limited sample size of the pilot study should be kept in mind. An additional limitation is the lack of a control group in the prospective study, as each participant served as his own control. Also, roughly 40% of the ultrasound examinations were performed transabdominally, potentially affecting the accuracy of the AFC measurements.
As study participants were quite young, our reassuring data may not apply to older transgender men, either because of an age-related decline in ovarian reserve or to possible long-term effects of testosterone therapy. Furthermore, the chances for fertility preservation may be more limited in subjects with PCOS.

WIDER IMPLICATIONS OF THE FINDINGS: This is an additional contribution to the emerging evidence that prolonged testosterone treatment may not be a major obstacle to later fertility potential in transgender men desirous of having children. Larger confirmatory studies, and particularly more with reproductive outcome data, are needed for evidence-based fertility counseling prior to treatment initiation in these subjects.

STUDY FUNDING/COMPETING INTEREST(S): This study received no funding. The authors have no competing interests to declare.

TRIAL REGISTRATION NUMBER: N/A.

Key words: transgender men / fertility preservation / gender-affirming therapy / anti-Müllerian hormone / antral follicle count

Introduction

A growing number of people worldwide identify themselves as transgender. In the USA alone, the estimated number of transgender adults is 1.4 million, or 0.6% of the population, and this figure may reach 1.3% among adolescents (Shields *et al.*, 2013; Flores *et al.*, 2016). There has been a common belief that transgender people may not desire to have biological children. Until recently, several legislations even mandated proof of incapacity to reproduce as a requisite for legal gender reassignment (Dunne, 2017). Since then, several studies have documented an earnest desire for reproduction among many transgender individuals about to undergo a gender-affirming process, and the general attitude in this respect is rapidly evolving (Auer *et al.*, 2018; Persky *et al.*, 2020).

Gender-affirming therapy in transgender men (those assigned female gender at birth but who self-identify as male) commonly consists of various testosterone preparations. Although successful pregnancies carried by transgender men have been reported (Light *et al.*, 2018), the long-term effects of testosterone therapy on fertility remain largely unknown. Consequently, several international medical organizations have recommend fertility counseling and consideration of fertility preservation before starting hormonal therapy (Coleman *et al.*, 2012; Ethics Committee of the American Society for Reproductive Medicine, 2015; Hembree *et al.*, 2017; Mayhew and Gomez-Lobo, 2020).

Despite the desire to have children, the majority of transgender men do not go through the process of preserving oocytes or embryos. A multitude of obstacles, including the high cost of the procedure, the emotional stress, the potential exacerbation of gender dysphoria caused by the ovarian stimulation required for oocyte retrieval, unwillingness to carry a pregnancy in the future and the difficulties involved in achieving a surrogate pregnancy, all significantly decrease the willingness to postpone the much-desired gender-affirming treatment initiation (T'sjoen *et al.*, 2013). Some of those who choose not to perform fertility preservation end up regretting their choice later in life and seek consultation concerning available options, but data concerning fertility at this stage, even if testosterone is withheld, are scarce.

Fertility preservation in transgender men is recommended prior to gender-affirming therapy in part for fear that testosterone might be deleterious to ovarian reserve. Some preclinical experience, particularly in primates, suggested that exogenously administered testosterone is a driver of follicular growth, leading to a polycystic ovary syndrome (PCOS)-like phenotype (Vendola *et al.*, 1998). At the same time, early investigations of transgender men yielded conflicting results on this matter, with some reporting the presence of prominent atretic follicles and others polycystic ovary (PCO) morphology as a result of testosterone treatment (Moravek *et al.*, 2020).

Among the available tests that may predict ovarian reserve (as indirect markers for fertility potential) are serum levels of FSH, estradiol, inhibin-β, and anti-Müllerian hormone (AMH). Additionally, ultrasonographic features of antral follicle count (AFC) and ovarian volume are also used for that aim (Grynnerup *et al.*, 2012).

AMH is a dimeric glycoprotein of the transforming growth factor β superfamily that is involved in growth and differentiation (Massagué, 1990). It is released from ovarian granulosa cells from birth until menopause, resulting in serum levels that are proportional to the number of early developing follicles, with small antral follicles (5–8 mm) making the greatest contribution to AMH blood level (Rajpert-De Meyts *et al.*, 1999; Jeppesen *et al.*, 2013). Very few studies using different treatment protocols have looked at AMH levels under testosterone treatment in transgender men, and these have had conflicting results, from dramatic suppression to almost no impact (Caanen *et al.*, 2015; Tack *et al.*, 2016; Taub *et al.*, 2020).

Given the scant and inconclusive information in the literature along with anecdotal reports of successful pregnancies in transgender men after transitioning, we hypothesized that testosterone treatment would significantly affect ovarian reserve without obliterating it altogether. Under this premise, we conducted this single-center prospective and cross-sectional study to assess the effect of testosterone treatment on ovarian reserve, using AMH and AFC as indices of future fertility potential in transgender men.

Materials and methods

Subjects and inclusion criteria

All subjects were recruited from the Transgender Health Center within the Institute of Endocrinology, Diabetes, Metabolism and

Hypertension at Sourasky-Tel Aviv Medical Center between 06/2014 and 07/2018. The study included transgender men (gender dysphoria diagnosed by qualified psychiatrist/psychologist/social worker) aged 16 years and older. None of the participants had undergone hysterectomy, salpingo-oophorectomy or gender-confirming surgery before or during the study. None of the subjects in the prospective study had borne children.

Study setting, design, sample size, and protocol

The study consisted of two arms. The first arm was a prospective study, which enrolled transgender men prior to the initiation of gender-affirming testosterone therapy, thus, allowing for a pre-treatment evaluation, and a structured assessment over the ensuing 12 months. Although data from the literature were conflicting, we assumed that testosterone treatment would result in a significant AMH decrease of at least 1 ng/ml over the course of a year, five times the average expected annual decrease (Seifer *et al.*, 2011), To demonstrate such an effect, a sample size of 28 was needed. However, as the nature of this pilot study was exploratory, we set out to augment enrollment, to up to double this number. The second arm was a cross-sectional, one time point study of a convenience cohort of transgender men who had already been under testosterone therapy at our center for varying amounts of time. Reproductive outcomes of subjects in this cohort, after initiation of treatment and until the latest clinic visit, were also documented.

For the prospective study, all subjects underwent a baseline assessment that consisted of anthropometric measurements, sex hormone and AMH determinations, and ultrasound assessment of endometrial thickness and AFC. Subjects were then requested to return for the same evaluation at 3 and 12 months after beginning treatment. The presence of PCOS at baseline was determined using the Rotterdam criteria (Rotterdam ESHRE/ASRM-Sponsored PCOS Consensus Workshop Group, 2004).

Treatment initiation consisted of either injectable testosterone enanthate (Testoviron Depot, Bayer, Germany) at doses ranging from 100 to 250 mg every 2 or 3 weeks, or applications of transdermal testosterone gel (Testomax, Perrigo, Israel) in daily doses of either 25 or 50 mg. For the cross-sectional study, all subjects underwent all of the above determinations at a single time point while receiving either testosterone enanthate, testosterone gel, or injections of testosterone undecanoate (Nebido, Bayer, Germany) 1000 mg every 12 weeks, for varying lengths of time.

The study received the approval of the local human studies ethical committee (approval number 496-14), and all participants gave written informed consent.

Serum hormone determinations

For baseline assessments, early follicular phase blood samples were taken in the morning after an overnight fast. After separation, serum was kept at −20°C until assayed. AMH was measured by an electro chemiluminescent immunoassay using the Elecsys Cobas e411 analyzer system (Roche Diagnostics, GmbH, Mannheim, Germany) with a lower limit of detection of 0.007 ng/ml (inter- and intra-assay variation 3.69% and 1.2%, respectively). AMH concentrations were expressed both in absolute values, and as age-specific percentiles derived from a total of 2226 healthy menstruating women, between the ages of 17 and 54 years (Anckaert *et al.*, 2016; Racoubian *et al.*, 2020). Under treatment, serum total testosterone concentration at trough level was determined using the Roche Elecsys Testosterone II assay for the Cobas e411 analyzer, with a lower limit of detection of 0.69–2.08 nmol/l (inter- and intra-assay variation 11.46% and 1.5%, respectively). Serum estradiol (inter- and intra-assay variation 8.58% and 2.4%, respectively, LH (inter- and intra-assay variation 6.77% and 13.1%, respectively, and FSH (inter- and intra-assay variation 4.5% and 4.2%, respectively) concentrations were measured by electro chemiluminescent immunoassays using the Immulite 2000 system (Siemens Healthineers Diagnostics, GmbH, Erlangen, Germany).

Sonographic parameters

Endometrial thickness (ET) and AFC (i.e. follicles 2–10 mm diameter) were determined by pelvic (transvaginal or transabdominal, according to the subject's preference) ultrasound (US), using the GE Voluson E6 Ultrasound Machine (General Electric Healthcare, Boston, MA, USA). In the prospective group, 30 exams were transvaginal and 26 were transabdominal. In the cross-sectional group, pelvic US was performed in 29 of the 47 participants, of which 20 were trans-vaginal and 9 were transabdominal. All examinations were conducted by two experienced gynecologists, with the same device, using a consistent protocol and the same image-processing software.

Statistical methods

Continuous variables were evaluated for normal distribution using histogram and QQ plot and reported as mean ±SD or as median and interquartile range (IQR), as appropriate. Categorical variables were expressed as frequencies and percentages. Univariate and multivariate generalized estimating equations models were used for repeated measures analysis.

The two-sample Student's *t*-test or the Mann–Whitney test was used for the comparison of continuous variables between transgender subjects with and without PCOS, as appropriate. As previously indicated, to fully adjust for the known decrease of AMH with age, AMH concentrations were also expressed as age-specific percentiles. To compute those, we used the published values for the 5–25, 25–50, 50–75, and 75–90 year, age-specific percentile brackets (Racoubian *et al.*, 2020). Assuming a linear progression of AMH values within any bracket, we then estimated the subject's percentile within his age-relevant bracket (the detailed computation can be found in the Supplementary data). Following this transformation, the one-sample Wilcoxon test was used to assess deviation from the age-specific 50th percentile. Correlations between age, duration of treatment, and AMH and AFC were performed through Spearman's correlation.

All statistical tests were two sided and $P < 0.05$ was considered as statistically significant. SPSS software (IBM SPSS Statistics for windows, version 25, IBM Corp., Armonk, NY, USA, 2017), and GraphPad Prism (version 8.0.0 for Windows, GraphPad Software, San Diego, CA USA) were used for statistical analyses and graph production.

Results

Prospective study

Baseline data

Fifty-six subjects enrolled in the prospective study, 27 of which fulfilled the criteria for PCOS. Five transgender men had the frank PCOS phenotype (oligomenorrhea, hyperandrogenism, and ultra-sonographic PCO), while the ovulatory (hyperandrogenism, PCO and regular menstrual cycles) and the non-PCO PCOS (oligomenorrhea, hyperandrogenism, and normal appearing ovaries on US) phenotypes were present in three and 19 subjects, respectively. The baseline clinical and biochemical data are presented in Table I. With the exception of AFC that was higher in subjects with PCOS, there were no differences in any of the variables between the two groups.

Effect of testosterone treatment on indices of ovarian reserve: AMH and AFC

As a reflection of treatment, testosterone concentrations rose to a median of 20 [12–24] nmol/l at 3 months, and 23 [13.8–37] nmol/l at 12 months, with no difference between subjects with PCOS and no PCOS. As a result of treatment, for the entire group, there was a significant ($P = 0.034$) decrease in AMH concentration over time in the generalized linear model, consisting of a non-significant decrease of 0.33 ng/ml between baseline and 3 months ($P = 0.158$), and a significant decrease of 0.71 ng/ml between baseline and 12 months ($P = 0.01$). This decrease in AMH over time corresponded to a change in age-specific percentiles of 7.12 points ($P < 0.001$), from a mean of 47.37 at baseline to a mean of 40.25 at 12 months. Again, at 3 months the decline in AMH, expressed as age-specific percentile, was only of 4.6 percentile points ($P = 0.135$). Considering older subjects, only two were 35 years of age or above. None of them had PCOS. AMH levels for the 35 year-old subject were 6.2 ng/ml (89th age-specific percentile) and 9.3 ng/ml (97.5th percentile) at baseline and after 12 months of treatment, respectively. The second subject was 40 years old at the beginning of the study, with a baseline AMH concentration of 1.9 ng/ml (53rd age-specific percentile), which went down to 1.1 ng/ml 1 year later (28th percentile for the age of 41 years).

When the analysis was repeated separately for subjects with or without PCOS (Fig. 1), it can be appreciated that the effect seen for the entire group was actually accounted for by a 9.52 AMH age-specific percentile decline between baseline and 12 months in subjects with PCOS ($P < 0.001$), while AMH did not change at all in subjects with no PCOS over the course of the study period.

We next analyzed the effect of testosterone treatment on the AFC over time. In contrast to AMH, the generalized linear model for the entire group did not indicate a significant reduction in the number of follicles over the course of a year ($P = 0.693$). When the analysis was run separately by PCOS status, although the model effect for this subgroup was not significant ($P = 0.115$), in subjects with PCOS a mean decrease of 5.0 follicles (from 17 to 12) was detected between baseline and the 12 months assessment ($P = 0.047$). In contrast, no such signal was present in subjects without PCOS, in whom a non-significant increase was seen, from 11.7 follicles at baseline to 13.4 at 12 months ($P = 0.312$ for the model effect and $P = 0.37$ for the baseline to 1 year comparison).

Effect of testosterone treatment on other variables

Endometrial thickness decreased by a mean of 1.41 mm by 12 months compared to pretreatment, from a mean of 6.76 ± 3.2 mm to 5.39 ± 2.9 mm ($P = 0.032$). Notably, by 12 months, 47% of subjects had an ET of ≥ 5.0 mm. Over the entire observation period, serum estradiol decreased by a mean of 120.78 pmol/L ($P < 0.001$). LH decreased also significantly by 3.0 IU/l ($P < 0.001$), while FSH remained unchanged.

Cross-sectional study

Demographic and clinical data

For this study, 47 subjects were recruited. The median age was 24 years [IQR 20–31]. By design, all subjects were under ongoing testosterone treatment. The median duration of treatment was

Table I Baseline characteristics of participants in the prospective study.

Variable	All n = 56	No PCOS n = 27	PCOS n = 27	P
Age (y)	22.5 [19–27.75]	21.0 [18–27]	23 [21.3–31.2]	0.41
BMI (kg/m^2)	24.05 [21.3–31.2] (54)	23.0 [20.95–26.75] (25)	26.7 [21.8–32.4] (27)	0.1
Testosterone (nmol/l)	1.3 [1–2.5] (55)	1.2 [1–1.9] (27)	1.4 [1–4] (26)	0.52
Estradiol (pmol/l)	289 [198–429] (55)	326 [198–532] (27)	260 [191–384] (26)	0.24
LH (IU/L)	4.75 [1.99–6.46] (55)	7.19 [3.2–11.9] (27)	6.32 [5.0–8.96] (26)	0.5
FSH (IU/L)	5.08 [3.58–7.1] (55)	6.0 [3.69–7.35] (27)	4.8 [3.12–6.79] (26)	0.34
AMH (ng/ml)	4.99 [2.8–6.53] (56)	5.02 [2.3–6.5] (27)	5.0 [2.8–7.5] (27)	0.62
AMH percentile	49.5 [17.5–37.75] (56)	34.0 [17–75.0] (27)	57.0 [16–75.0] (27)	0.5
AFC (n)	15.0 [10.0–20.0] (51)	11.0 [6.5–20.0] (25)	20.0 [11.0–20.0] (25)	0.047
ET (mm)	6.0 [4.45–8.5] (50)	6.0 [4.5–9.18] (25)	5.85 [4.15–6.95] (24)	0.4

PCOS, polycystic ovary syndrome; AMH, anti-Müllerian hormone; AFC, antral follicle count; ET, endometrial thickness.
Data are presented as median and [interquartile range] with the exception of FSH, which had a normal distribution and is expressed as mean (±SD). Comparisons were carried out between the subjects with PCOS and no PCOS by Mann–Whitney test, and a 2 sample *t*-test in the case of FSH. The number of actual values for each variable is indicated in parentheses.

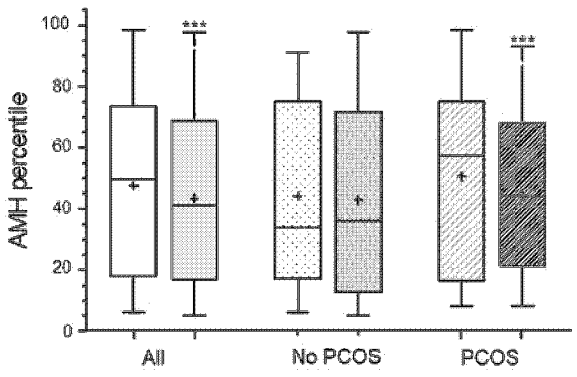

Figure 1. Anti-Müllerian hormone percentile under testosterone treatment in the prospective study. Data are shown for the entire group (n = 56, solid boxes), subjects without polycystic ovary syndrome (PCOS: n = 27, dotted boxes), and those with PCOS (n = 27, hatched boxes). For each group, baseline data are shown on the left and data at 12 months are shown on the right. The upper and lower limits of the boxes represent the 75th and 25th percentiles, respectively, the horizontal bars inside the boxes indicate median and the cross stands for the mean, while the whiskers indicate minimum and maximum. Pairwise comparisons were done on estimated marginal means from generalized linear models for repeated measures *** $P<0.001$. AMH, anti-Müllerian hormone.

35 months [IQR 13–62], with a range of 7–219 months. Thirty-six of them (77%) were treated for over 1 year.

The data are given in detail in Table II. Stratification by PCOS status was performed by reviewing data that preceded treatment, yielding a 30% rate of PCOS in this cohort. There were no significant differences in any of the continuous or categorical variables between the two sub-groups.

Ovarian reserve indices with prolonged testosterone treatment

In the entire cohort, AMH concentration correlated inversely with age r_s −0.33 ($P = 0.02$), but not with duration of treatment, in spite of the fact that age and duration of treatment were positively correlated, r_s 0.46 ($P = 0.001$). In fact, the median AMH age-specific percentile for the entire group did not significantly deviate from the 50th percentile (by one-sample Wilcoxon test).

When this cohort was analyzed by PCOS status, an inverse correlation between serum AMH and treatment duration did emerge, only in subjects with a history of PCOS, r_s −0.81 ($P < 0.001$).

AFC was by and large preserved, and was neither associated with age nor with treatment duration.

Reproductive outcomes

Four men fathered seven biological children (one actually had four children) after being treated with testosterone for between 3 and 12 years. None of them had PCOS. Three of them conceived spontaneously after discontinuing testosterone, and carried the pregnancies themselves. One subject, who had been under treatment since the age of 25 years, resorted to induction of ovulation and oocyte retrieval at the age of 36 years. In this case, a surrogate mother carried the pregnancy and delivered the baby. When he took part in the study, at the age of 34 years, his AMH concentration had been 0.79 ng/ml.

There were five men, aged 35 years or older at the time of the study, who had been under treatment for at least 3 years to above 10 years. None had fathered children. Three of them had a history of PCOS, and all three had age-specific AMH concentrations above the 50th percentile.

Discussion

Growing numbers of transgender individuals are now seeking gender-affirming therapy (Wiepjes *et al.*, 2018). This trend is particularly remarkable in youths (Zucker, 2017). Furthermore, among adolescents and young adults, there has been a well-documented reversal of assigned-at-birth-sex ratio, with young transgender males now representing a majority of youths presenting for gender-affirming therapy (Zucker, 2017; Segev-Becker *et al.*, 2020). Even though a vast majority of adolescents and young adults who request treatment do so long before parenthood becomes relevant, when questioned, many (~50%) express an earnest desire to have children, including a yearning for biological children (Auer *et al.*, 2018; Chen *et al.*, 2018; Baram *et al.*, 2019; Defreyne *et al.*, 2020). The rates of fertility preservation procedures are rising, even among young subjects (Defreyne *et al.*, 2020; Segev-Becker *et al.*, 2020), however, only a minority of them actually preserve gametes before initiating treatment. Among those who do, there is a clear and understandable preponderance of transgender women (Amir *et al.*, 2020; Pang *et al.*, 2020). Preserving fertility in transgender men is a major multifaceted challenge, hence, the interest in the potential ovarian reserve in subjects after chronic testosterone exposure.

Using serum AMH as an established proxy of follicular ovarian reserve (Dewailly *et al.*, 2014; Moolhuijsen and Visser, 2020), we show in a prospective study of testosterone treatment in a limited sample of transgender men, a significant decline over the course of 1 year both in absolute AMH concentrations, and as expressed in age-specific percentiles. However, this effect was entirely accounted for and restricted to subjects with PCOS in whom there was also an indication for a mean decrease of five follicles in the AFC over the same period. In subjects without PCOS, both AMH and AFC were unaffected by testosterone treatment over the course of 1 year.

These results are corroborated by the findings in the cross-sectional study. In this arm of the study, subjects who were under testosterone treatment for up to 219 months (median 35 and mean 48.2) had AMH concentrations that did not deviate from the 50th age-specific percentile. Furthermore, there was no correlation between the duration of treatment and prevalent AMH concentrations, which were determined by age only. Remarkably, a sizeable sample of this cohort fathered children after being under testosterone treatment for up to 12 years.

These findings for the entire cohort should be qualified for subjects with a previous history of PCOS, in whom a prolonged treatment duration was associated with lower AMH concentrations. At the present we do not offer a robust explanation for this dichotomous behavior. According to well-established evidence (Cook *et al.*, 2002; Pigny *et al.*, 2003), women with PCOS have higher serum AMH concentrations,

Table II Data for the subjects in the cross-sectional study.

	Age (years)	Treatment duration (m)	AMH (ng/ml)	AMH (percentile)	AFC (n)	Endometrial Thickness (mm)	Estradiol (pmol/l)	Testosterone (nmol/l)	LH (IU/l)	FSH (IU/l)
N	47	47	47	47	40	39	44	45	44	45
Mean	25.9	48.2	4.2	42	9.6	4.6	252	23.6	5.2	4.2
Median	24	35	3.1	33	10	4.5	198	21.3	3.7	3.9
SD	7.07	48.5	3.5	31.3	6.8	2.3	160	16	6.2	2.8
Minimum	16	7	0.14	2.5	0	0	73	1.9	0.1	0.3
Maximum	47	219	16.2	97.5	20	11	855	71.4	29.2	11.9
Interquartile range	20–31	13–62	1.8–6.1	12–69	2.5–14.0	2.6–6.0	149–323	10.5–71.4	1.1–7.1	1.7–6.0

and we were, thus, surprised not to find a difference at baseline between subjects with and without PCOS. This could obviously be due to the limited sample size of the study. However, upon reviewing the more recent literature on the topic, the relation between serum AMH and PCOS appears to be complex, and subject to modification by numerous factors such as BMI, fertility status, hyperandrogenism, and variations in the AMH and AMH Receptor 2 genes (Moslehi *et al.*, 2018; Gorsic *et al.*, 2019; Moolhuijsen and Visser, 2020). Nonetheless, as expected, subjects with PCOS did have a significantly higher baseline AFC, consistent with the nature of their condition. A larger decline in AMH, with a suggestion for some decline in AFC, while both remaining in the normal range, could simply indicate that chronic testosterone treatment levels off these baseline differences. Alternatively, although there were no differences in testosterone, estradiol, and gonadotrophin levels under treatment between the two groups, it could be hypothesized that the dysregulated gonadotrophin axis and its interaction with AMH secretion at baseline in subjects with PCOS could respond differentially to testosterone treatment. Taken together, these results suggest that testosterone treatment has limited impact on AMH secretion and AFC count, regardless of treatment duration.

Given the low rates of pre-treatment fertility preservation, along with the expressed desire for biological children, the issue of assessing the effect of testosterone treatment on ovarian integrity in transmasculine persons is of paramount interest.

Histopathologic studies of ovaries retrieved during oophorectomy of transgender men at the time of gender-confirming surgery have yielded conflicting findings. A frequent finding of a multi/polycystic histology was deemed, in several earlier reports, to be consistent with PCO morphology. In some studies, the presence of atretic follicles was also noted. In addition, a thickening of the tunica albuginea and stromal hyperplasia were also reported (Moravek *et al.*, 2020). More recently, however, three studies, that collectively included 145 transgender men under testosterone treatment for over 1 year, have revealed a fairly consistent picture of preserved ovarian follicular activity, along with a normal distribution of cortical follicles, mostly primordial, that had retained vital characteristics (De Roo *et al.*, 2017; Grimstad *et al.*, 2020; Marschalek *et al.*, 2020). In all three studies, no association between the number of follicles and the duration of testosterone treatment was noted. Remarkably, in one study, a substantial number of cumulus–oocyte complexes (COCs) retrieved were shown to have normal structural characteristics, and preserved IVM potential for cryo-preservation. Notably, the number of these COCs was significantly correlated with serum AMH levels (De Roo *et al.*, 2017). Contrary to an earlier rather bleak picture, these recent findings suggest that testosterone treatment has a limited impact on ovarian morphology and follicular activity, and that there might exist a potential for fertility preservation, even at the time of oophorectomy.

Given the correlation between serum AMH and functional ovarian reserve, other studies have looked at this hormone as a highly reproducible, non-invasive, and convenient means to assess such a reserve in testosterone-treated transgender men. In a first prospective study of 22 subjects, which employed a complex protocol of pituitary suppression with a GnRH analog for 8 weeks followed by testosterone administered together with an aromatase inhibitor for 16 weeks, there was a potent suppression of AMH compared to baseline (Caanen *et al.*, 2015). However, this study has been criticized for its unconventional protocol and its short duration. In another prospective study of 32 transgender men, that included both new users and subjects who were already under treatment, weekly injections of testosterone cypionate for 12 weeks had no effect on serum AMH concentrations (Taub *et al.*, 2020). Finally, in a retrospective study of 38 adolescents treated with incremental doses of testosterone for at least 6 months, there was no change in AMH between baseline and 1 year (Tack *et al.*, 2016).

A potential limitation of the current investigation, other than the limited sizes of the samples, is that it comprised a high percentage of subjects with PCOS, in whom the impact of testosterone treatment may be more substantial. Although not all studies go into this consideration, the rate of subjects with a history of PCOS in series of transgender men has been reported to be significantly higher than in the general population (Becerra-Fernández *et al.*, 2014). An additional limitation is the lack of a control group in the prospective study, as each participant served as his own control. Although the rate of decline in AMH concentrations over the course of 1 year is fairly well established (Seifer *et al.*, 2011), this was not directly documented in an untreated control group in our study. However, the fact that both AMH and AFC remained within the normal range in the cross-sectional subgroup confers further confidence that even in these subjects, the follicular ovarian reserve may still be clinically significant. Although the results of our study are reassuring, they were obtained from a fairly young population and may not be generalized to older transgender men. An

additional limitation is the fact that roughly 40% of the US examinations were performed transabdominally, potentially affecting the accuracy of the AFC measurements recorded for these subjects.

The strength of the study lies in the fact that it is the largest and most prolonged prospective study to date, and also the only one that comprises a separate cross-sectional observation of subjects already under treatment for prolonged periods of time, together with some positive reproductive outcomes. We believe our findings lend credence to the fairly novel notion that chronic testosterone administration does not obliterate follicular ovarian reserve in transgender men, and that the potential for fertility remains, even after years of treatment. Furthermore, this growing body of evidence provides the physiologic basis for the reports of pregnancies achieved by transgender men after prolonged testosterone treatment.

In conclusion, we contributed a new installment to the emerging evidence that prolonged testosterone treatment may not be a major obstacle to later fertility potential in transgender men desirous of having children. Further larger confirmatory studies, and particularly more studies including reproductive outcome, are needed for evidence-based fertility counseling prior to treatment initiation in these subjects.

Supplementary data

Supplementary data are available at *Human Reproduction* online.

Data availability

All data are incorporated into the article.

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Authors' roles

I.Y., K.T., M.S., Y.S., N.G., and Y.G. examined the patients and conducted all the clinical aspects of the treatment. G.M. and Y.S. conducted and interpreted the pelvic US exams. G.S. conducted the AMH measurements. I.Y. and K.T. collected the data and performed the statistical analyses. I.Y., K.T., and Y.G were responsible for primary study oversight and design, data analysis and interpretation, and primary writing of the manuscript. All the authors made substantial contributions in critically revising the manuscript. All the authors approved the final manuscript for submission.

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Conflict of interest

The authors have no conflicts of interest to declare.

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MANAGEMENT OF ENDOCRINE DISEASE: Long term outcome of central precocious puberty

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MANAGEMENT OF ENDOCRINE DISEASE

Long-term outcomes of the treatment of central precocious puberty

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Abstract

GnRH analogues (GnRHa) are the treatment of choice for central precocious puberty (CPP), with the main objective to recover the height potential compromised by the premature fusion of growth cartilages. The aim of this review was to analyze long-term effects of GnRHa on height, body weight, reproductive function, and bone mineral density (BMD) in patients with CPP, as well as the potential predictors of outcome. Because randomized controlled trials on the effectiveness and long-term outcomes of treatment are not available, only qualified conclusions about the efficacy of interventions can be drawn. GnRHa treatment appears to improve adult height in girls with CPP, especially if diagnosed before the age of 6, whereas a real benefit in terms of adult height is still controversial in patients with the onset of puberty between 6 and 8 years of age. No height benefit was shown in patients treated after 8 years. Gonadal function is promptly restored in girls after cessation of treatment, and reproductive potential appears normal in young adulthood. Data are conflicting on the long-term risk of polycystic ovarian syndrome in both treated and untreated women. Fat mass is increased at the start of treatment but normalizes thereafter, and GnRHa itself does not seem to have any long-term effect on BMI. Similarly, analogue treatment does not appear to have a negative impact on BMD. Owing to the paucity of data available, no conclusions can be drawn on the repercussions of CPP and/or its treatment on the timing of menopause and on the health of the offspring.

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Introduction

Puberty results from the reactivation of the hypothalamic–pituitary–gonadal (HPG) axis following the quiescent period occurring during childhood. It is characterized by an increase in the amplitude and frequency of the hypothalamic gonadotropin-releasing hormone (GnRH) pulses, which in turn promote follicle-stimulating hormone and luteinizing hormone secretion by the pituitary, leading to the activation of gonadal function (1).

Precocious puberty is clinically defined by the appearance of secondary sexual characteristics, i.e., Tanner stage II of breast development before the age of 8 in girls and the increase in testicular volume ≥ 4 ml before 9 years in boys (2, 3). Central precocious puberty (CPP) due to early activation of pulsatile GnRH secretion is the most common form (2). It occurs in ~1:5000–10 000 children, with a female-to-male ratio ranging from 3:1 to 23:1 (3). Females typically present with idiopathic forms, whereas in boys CPP is mostly due to organic lesions such as hypothalamic–pituitary congenital malformations, tumors, infections, infiltrative/inflammatory disorders, and iatrogenic or traumatic injuries (3). Genetic factors (mutations of KISS1, KISS1R, and MKRN3 genes (4)), secular trend, ethnicity, nutritional status, and environmental changes have all been involved in the pathogenesis of CPP (2, 3, 5), although their exact mechanisms of action remain to be elucidated.

Short stature caused by rapid advancement of skeletal maturation driven by premature exposure to sex steroids is

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the main unfavorable event associated with precocious puberty. Historical data on untreated patients with CPP show mean final heights ranging from 151 to 156 cm in boys and from 150 to 154 cm in girls, being the height loss inversely correlated with the age at the onset of puberty (2, 6).

Treatment is aimed at selectively and effectively suppressing gonadal steroid secretion through medical treatment or removing the underlying cause whenever possible to allow normal sexual maturation and statural growth (2, 3, 6). The avoidance of potential psychosocial problems derived from experiencing precocious puberty and undesirable behaviors like early sexual intercourse and substance abuse reported in some cohorts of patients may also be acknowledged as objectives of the treatment (2, 3, 7). The decision to treat depends on the age at onset of puberty, pace of pubertal development, estimated adult height, and psychological impact of the premature sexual development (2, 3). Treatment is undisputed in rapidly progressive forms, defined on the basis of clinical, radiological, and biochemical criteria (8), for the significant risk of short adult height, while it is not required in patients with nonprogressive or slowly progressive forms who were shown to achieve an adult height within the normal range without treatment (2, 3).

GnRH analogues (GnRHa) are the medical treatment of choice for progressive CPP (2, 3). They derive from a chemical substitution at position 6 and 10 of the native GnRH molecule, which increases its resistance to the enzymatic degradation and affinity to the GnRH–pituitary receptor leading to desensitization of the receptor, ultimately resulting in the inhibition of gonadotropin secretion and return of sex steroids to prepubertal levels (9). Several active principles and formulations are available. Depot formulations are generally preferred because of better patient compliance. Drug choice depends on physician experience, patient needs, and government regulations of drug prescription (6, 7). GnRHa is generally safe and well tolerated. Local events such as bruising, pain, injection reactions, and sterile abscesses are the most common side effects, followed by minor menopausal symptoms – that is, hot flushes, headaches, and nausea (7) – while anaphylaxis is extremely rare (10).

In the last decades, the widespread use of GnRHa has increasingly demonstrated its favorable effects on statural growth, although the net height gain (HG) associated with the treatment and predictors of long-term outcomes remains debated (2, 6, 7, 8), as no randomized controlled trials (RCTs) have been performed and growth estimation suffers from important methodological limitations, which will be discussed later. Moreover, concerns have been raised on the potential negative effects of treatment on weight and metabolic profile, bone mineral density (BMD), and reproductive function in adulthood (2, 3, 6). The aim of our review was to analyze long-term effects of GnRHa on height, body weight, reproductive function, and BMD in patients with CPP, as well as potential predictors of outcome.

A literature search was performed using the PubMed database (http://www.ncbi.nlm.nih.gov/pubmed) and entering the string 'precocious puberty AND (treatment OR GnRH analogues) AND (height OR body mass index OR bone OR fertility OR reproduction OR polycystic ovary syndrome)' with no date limits. Only original studies performed in humans, written in English, reporting data at the beginning and after the completion of treatment were considered for BMI and metabolic parameters, reproductive function, and BMD. Studies focusing on height were considered only if baseline values and adult heights were reported and data expressed as mean (absolute values or SDS) ±S.D. or S.E.M. for study comparison and calculation of treatment efficacy (i.e. HG). Additional articles were identified through a hand search of reference lists in the papers retrieved (Fig. 1).

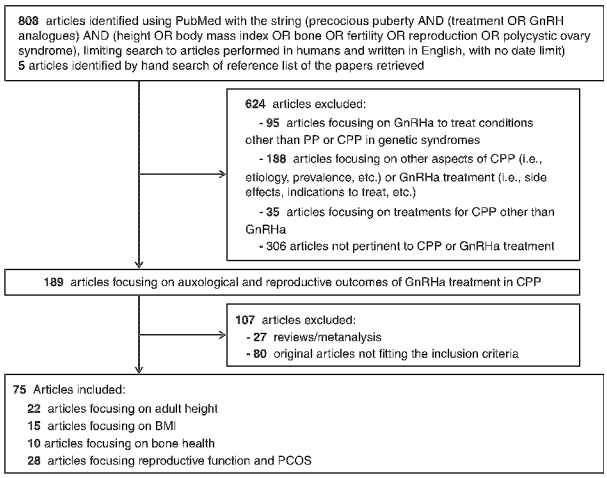

Figure 1

Methodological flow chart summarizing the main steps followed for the identification of articles of interest. CPP, central precocious puberty; GnRHa, gonadotropin-releasing hormone agonists; PCOS, polycystic ovary syndrome; PP, precocious puberty.

Outcomes of GnRHa therapy for CPP

Adult height

GnRHa have been extensively used since the 1980s in children with rapidly progressing CPP with the primary aim to restore genetic growth potential otherwise compromised by sex-hormone-driven premature closure of bone growth plates. The great majority of studies indicate some beneficial effect of treatment on statural growth, with limitations related to the absence of RCTs and the fact that the effects of GnRHa therapy have been traditionally analyzed by comparing the achieved adult height with predicted adult height at initiation of treatment or with adult heights of historical, untreated cohorts.

Bone age (BA) assessment is essential in the management of patients with CPP as it allows the identification of rapidly progressing forms of CPP with compromised predicted adult height requiring treatment. It is also important for monitoring treatment efficacy, as deceleration of BA maturation is a desired effect of treatment. Moreover, BA evaluation is valuable in defining the appropriate time for treatment discontinuation, because the best results in terms of adult height are achieved when treatment is discontinued at around 12–12.5 years in girls and 13.5 years in boys (11), although the optimal age for treatment interruption is not clearly defined by international guidelines. It should be pointed out, however, that BA assessment is affected by a great intra-observer variance, and Bayley and Pinneau tables, the reference standards for height prediction, have been validated for height prediction in normal children (11). In patients with CPP, height prediction based on both 'average' and 'advanced' tables is insufficiently reliable, especially when skeletal maturation is markedly advanced, and it is associated with a systematic overestimation of adult height (3.7–5.9 cm in girls, and even greater in boys in historical series) (2, 3).

The comparison of adult heights of treated CPP patients with those of historical cases is of limited value because data are derived from a small number of patients, usually the most severe, and do not take into account the influence of the secular trend on human growth over the decades. Moreover, studies are heterogeneous for patients – that is, chronological and BA at diagnosis and initiation of treatment and idiopathic vs organic forms of CPP – and treatment characteristics (2, 3).

Predictors of treatment outcomes also remain debated. Treatment efficacy appears to depend mainly on the age of CPP onset and treatment initiation with best outcomes reported in girls with onset of CPP before the age of 6 years and treated soon thereafter. BA advancement at the time of initiation of therapy, duration of therapy, mid-parental height, and height at the end of therapy have also been considered predictors of height outcome but with no definite conclusions reached on their appropriateness. The optimal age for treatment discontinuation is also questionable as several auxologic and treatment characteristics involved in post-treatment HG should be taken into account together with the psychological impact of the resumption of pubertal development on the patient and family (7, 8).

To clarify the impact of GnRHa treatment on statural growth and identify the most reliable predictors of height outcome in treated patients with CPP, 20 articles fitting the above-mentioned inclusion criteria were analyzed (Supplementary Table 1, see section on supplementary data given at the end of this article). For each of them, the following parameters, reported by authors or calculated from raw data, were recorded: number of enrolled patients; treatment type; target height (TH); chronological age, BA, and height at the beginning and end of treatment; HG (computed from the difference between adult and predicted height at diagnosis, according to average and/or advanced BA, as per authors' choice); and the difference between adult and predicted height at the end of treatment. Treatment efficacy was estimated from the comparison of adult height with predicted height at the beginning and end of treatment and/or adult height of historical, untreated patients, as well as the pace of bone maturation progression.

Data analysis demonstrated the efficacy of the various GnRHa formulations in halting BA progression, indicated by the statistically significant difference between BA and chronologic age at the end vs the beginning of treatment (Supplementary Table 1). The great majority of patients was female (female (F):male (M) = 947:90) and treated with triptorelin or leuprolide depot formulations administered monthly. Overall, the mean chronologic age at the start of treatment was 7.5 ± 1.2 years in females and 6.7 ± 1.5 years in males, with a mean BA of 10.3 ± 1.4 years in females and 10.6 ± 2.5 years in males. The mean chronological age at the end of treatment was 11.1 ± 0.9 years in females and 12.2 ± 1.8 years in boys, with a mean BA of 12.4 ± 0.7 and 13.6 ± 1.0 years respectively.

Mean adult height was significantly higher in treated patients than in untreated ones reported in an historical series adjusted for age at diagnosis (12, 13) (mean difference in adult height of 8.3 cm in girls and 13.7 cm in boys) (12) and in age- and sex-matched untreated study

controls (mean difference in adult height ranging from 3.3 to 8.9 cm) (12, 14, 15, 16).

The great majority of patients reached an adult height consistent with the TH (13, 14, 15, 17, 18, 19, 20, 21, 22, 23, 24, 25), a minority of them did not reach the TH (19, 26), and a very small portion of patients remained shorter than the predicted height before treatment (13, 27). Discordant results were obtained when comparing the efficacy of treatment in females and males. According to a study by Bajpai *et al.* (21), in patients treated with monthly triptorelin depot, mean HG was similar in females and males (mean HG: 6.4±2.4 cm vs 7.6±1.0 cm respectively). In contrast, Galluzzi *et al.* (13) reported a higher HG in boys (7.3±3.8 cm; $n=11$) than in girls (3.3±3.0 cm; $n=22$), with an achieved adult height SDS higher in the former than in the latter (0.13±0.91 vs −0.62±0.88; $P<0.001$). In general, HG was highly variable among studies depending on sample characteristics including the progression of pubertal development. Pubertal development was specified as rapidly progressing in some studies (17, 22, 24, 27, 28, 29), whereas in others pubertal progression was not detailed.

Several factors were postulated to influence the effects of GnRH treatment on statural growth. According to the majority of studies, earlier age at start of puberty (i.e. 5 years (15, 22)) and of treatment (i.e. 6 years (18, 20, 21, 23, 24, 25)) are associated with a taller adult stature. In a study by Klein *et al.* (24) performed in 98 patients (F:M=80:18) treated with histrelin or deslorelin, the average adult height and HG (14.5±9.9 cm vs 6.8±6.9 cm; $P<0.001$) were greater in girls with puberty onset <6 years of age than those with onset of puberty between 6 and 8 years of age. Few studies (12, 26) showed no correlation between HG and age at puberty onset or initiation of treatment, suggesting that girls with late onset CPP benefit from treatment similarly to girls with earlier pubertal onset (12). A longer treatment duration appears to be a positive outcome predictor in the majority (19, 20, 21, 24, 30), although not all (15, 23), of the studies, together with a short interval between pubertal onset and the start of treatment (24, 30), a great growth spurt after the end of treatment (12), low pre-treatment estradiol levels (30), and advanced BA at the start of treatment (14, 18, 19, 20, 21, 25). The impact of advanced BA at the start of treatment on adult height was not documented in the study by Brito *et al.* (30), whereas Carel *et al.* (12) found a negative association between the BA/statural age ratio at the onset of treatment and adult height suggesting that treatment is not capable of restoring a full adult height potential if started after an irreversible advancement of BA. This was also confirmed in a study by Kauli *et al.* (26) showing that therapy is more beneficial if started before BA exceeds 12 years.

Elevated height (or height SDS) before (16, 17, 19, 20, 24, 30) and at the withdrawal of treatment (12, 16, 17, 19, 20, 25, 30), as well as high TH (or TH-SDS) (17, 20, 21, 23, 30), has also been positively associated with adult height, supporting the primary influence of genetic factors in the determination of adult height even in patients treated with GnRHa (20).

Finally, BA at the end of treatment appears to be crucial on adult height, as it determines post-treatment growth potential (7). Indeed, the tallest heights were achieved by patients who stopped treatment at a BA of 12–12.5 years (20) or even <11.5 years (27), whereas continuing treatment after a BA ≥13 years negatively impacted on statural growth (20).

The efficacy of the various GnRHa in terms of HG appeared similar (24, 27, 31), except for a study (32) showing a higher adult height SDS in patients treated with leuprolide depot compared to triptorelin depot.

BMI and correlates of metabolic syndrome

Several studies reported an association between overweight and early/precocious puberty (5, 33) suggesting the involvement of various environmental, genetic, and biochemical factors (5, 7, 17, 34, 35) to explain this association. However, what remains to be clarified is whether it is the high BMI that results in precocious pubertal development or is it the latter that promotes the weight gain (33, 35).

Preliminary studies reported weight gain during treatment with GnRHa in patients with CPP, raising concerns for potential permanent obesity in adulthood (33, 35, 36). According to two independent studies (33, 37) analyzing normal-weight and overweight children separately, BMI–SDS during treatment increased in normal-weight children, whereas it remained stable in overweight subjects. The majority of long-term studies showed an increased prevalence of overweight and obesity in patients with CPP at diagnosis (22, 38), but no significant mean or individual BMI–SDS changes were shown at the end of treatment, irrespective of sex, age at puberty onset and at the start and discontinuation of treatment (15, 17, 22, 23, 24, 25, 30, 32, 39), and type of GnRHa (31). Recently, a study by Colmenares *et al.* (39) evaluated the effects of GnRHa in treated ($n=29$) and untreated ($n=8$) CPP patients and in treated ($n=14$) and untreated ($n=20$) rapidly progressing early puberty (EP), during a 3-year follow-up period. Treatment duration was ≥2 years.

At diagnosis, a higher BMI (*z*-score of 1.1±0.8 vs 0.6±0.7) and a higher prevalence of obesity/overweight (72.9% vs 35.3%) was observed in subjects with CPP when compared to those with EP. BMI *z*-score and obesity/overweight rates did not change significantly in girls with CPP or EP during 3 years of follow-up, regardless of treatment. Weight *z*-scores were higher at 3 years in treated than in untreated girls with CPP, while it was higher in untreated than in GnRHa-treated patients with EP at baseline, 1, 2, and 3 years. Both CPP- and EP-treated patients showed a reduction, although not statistically significant, in BMI *z*-scores and in obesity/overweight rate following treatment discontinuation, supporting the potential, although time limited, detrimental effect of GnRHa on weight. Indexes of glucose and lipid metabolism were in the normal range at diagnosis and remained unchanged during the follow-up period, independent of treatment.

Recently in a case–control study of a historical cohort, Lazar *et al.* (35) assessed the prevalence of obesity, the metabolic outcome (hyperlipidemia, diabetes, and hypertension), and the malignancy rate of former CPP GnRHa-treated and -untreated women between the third and fifth decades of life. The control group comprised women randomly matched for age, year of birth, and community clinic. Weight status of both GnRHa-treated and -untreated former CPP women resembled that of the general population from late adolescence to early-mid adulthood despite their above-average BMI at the onset of puberty. Permanent obesity was detected in women who were already obese in early childhood only. Moreover, weight gain of the treated CPP girls was not aggravated by GnRHa therapy. The incidence of obesity-related complications, such as metabolic dysfunctions and cancer comorbidities, were not increased in former CPP women, reassuring the health status of adult former CPP women.

Results from studies meeting inclusion criteria are summarized in Supplementary Table 2, see section on supplementary data given at the end of this article.

Reproductive function and risk of polycystic ovarian syndrome

The occurrence of menarche, or in some cases resumption of menses, after the discontinuation of either daily or long-acting GnRHa treatment was investigated by follow-up studies of girls in their late teens and women up to 56 years of age (Supplementary Table 3, see section on supplementary data given at the end of this article). The majority of the studies reported a 100% occurrence of menarche, with a few exceptions mostly related to CPP secondary to organic lesions such as hypothalamic hamartoma (40).

Spontaneous menses occurred 0–62 months after the end of treatment (mean 1.1±0.4 years); the mean duration of treatment varied widely among the studies, from 1 to 14 years. It was suggested that the longer time interval to menarche might be related to a longer duration of treatment and/or younger age at the start of therapy (40), but this hypothesis was not confirmed by other authors (41). Age at the discontinuation of treatment, BA, Tanner breast stage, or uterine development at the end of treatment, and the frequency of injections required to suppress the HPG axis function were all proposed as potential predictors of time interval to menarche, without consistency across studies. Interestingly, girls who had experienced menarche prior to GnRHa therapy showed a significantly shorter interval between the last injection and resumption of menses than those who had not experienced menarche before GnRHa treatment (~25 months vs 63 months) (19).

In the last decade, a subcutaneous hydrogel implant releasing histrelin continuously for at least 1 year has become available in the USA. Few reports of follow-up after histrelin implant treatment were published. Gillis *et al.* (31), evaluating a group of CPP patients treated with the monthly GnRHa and one with the histrelin implant showed that the mean time between the removal of the implant or last injection and menarche was shorter in the histrelin implant group. Fisher *et al.* (41) reported the resumption of puberty in 26 of the 30 girls treated with the histrelin implant, with occurrence of menarche 2–36 months after explantation in treatment-naïve and -non-naïve CPP girls, with an older age at explantation correlating with earlier menarche. In a recent study by Silverman *et al.* (43), menarche occurred in two patients, 9 and 2 months after the final explant respectively.

A great variability in the occurrence of regular ovarian cycles was so far reported in CPP-treated patients (44–96%; Supplementary Table 3), probably related to the heterogeneity of the study sample, type and duration of treatment, and follow-up. The highest prevalence of regular cycles (96%) was observed in 87 treated idiopathic-CPP girls during a 7-year follow-up period after the discontinuation of treatment (15). Jay *et al.* (43) described menstrual cycle lengths as becoming increasingly regular, from 41% in the first year post-menarche to 65% at 3 or more years post-menarche (44).

Fertility was reported to be normal in treated CPP girls, in contrast to the untreated ones. Supplementary Table 3 summarizes over 100 pregnancies reported in the

literature, with 97 uneventful pregnancies resulting in healthy children, five elective abortions, and 11 early miscarriages. In a recent study by Lazar *et al.* (45) assessing the reproductive outcome of former CPP women between the third and fifth decades of life, the frequency of pregnancy complications, such as early spontaneous abortion or pre-eclampsia, was comparable in the CPP women and controls. In the same study, spontaneous pregnancy was equally achieved by the treated CPP women and their control groups, while the percentage of women requiring ovulation induction and/or IVF was significantly higher in the untreated CPP group (33%) than in either the control (12.6%) or the CPP-treated groups (11.1%) (45). These findings suggest, according to the authors, a protective role for gonadotropin suppressive treatment on the reproductive outcome of CPP women.

PCO morphology detected by ultrasound (US) was reported in 0–37% of treated CPP girls (median 2%) (21, 36, 46, 47, 48, 49, 50, 51), with different lengths of post-treatment follow-up (up to 20 years), as summarized in Supplementary Table 4, see section on supplementary data given at the end of this article. Feuillan *et al.* (40) described the ovarian volume larger than normal at 4–5 years post-treatment, whereas another study including adult CPP-treated women showed the ovarian volume within normal range (52). Ovarian volume >10 ml was observed in 20% of CPP-treated patients (49, 50), a percentage similar to that found in age-matched, healthy controls (48). CPP patients with regular vs irregular menses showed no differences in the ovarian volume (22).

The development of signs and symptoms of polycystic ovarian syndrome (PCOS) in former CPP women is controversial. Data from the literature are limited, and the criteria used for PCOS diagnosis are not uniform among studies. While Heger *et al.* (22) observed a low incidence of PCOS (2%) based on Franks criteria, one study is supportive of the relationship between CPP and PCOS (48). In this cohort, which did not include a control group of untreated CPP girls, the prevalence of PCOS was 32% using the 2003 Rotterdam criteria and 30% using the Androgen Excess Society (AES) criteria. Moreover, the prevalence of hirsutism and biochemical hyperandrogenism was 23 and 48%, respectively, while irregular menses were present in 15% and PCO morphology in 37% of women. High prevalence of PCO morphology by US is also detected in normal young women (up to 33%) (53). Using the 1990 NIH criteria, Magiakou *et al.* (36) found that PCOS prevalence in CPP-treated young women was not different from that in the untreated ones (17.2 and 30.8% respectively), suggesting that GnRHa treatment does not predispose to PCOS development or menstrual irregularities.

Recently, in a large group of treated and untreated former CPP women aged 25–56 years, Lazar *et al.* (45) evaluated clinical signs potentially related to androgen excess, without performing hormonal assessment or imaging procedures. With these limitations, clinical signs of hyperandrogenism (acne/hirsutism with oligomenorrhea) were more frequent in CPP women than in controls with normal puberty matched for age and year of birth but not for BMI. The relative risk for the development of clinical hyperandrogenism with irregular menses was twofold higher in the untreated than the treated group. Moreover, among the treated women a small number had received cyproterone acetate with outcomes similar to those of GnRHa-treated women, suggesting that pubertal suppression itself may reduce the risk of PCOS rather than the kind of medical treatment. Study findings also suggest an association between CPP and ovarian dysfunction later in life, probably related to the underlying neuroendocrine dysfunction, manifesting as CPP and persisting into adult life. The reproductive outcome in early and mid adulthood was normal in the great majority of the patients studied. A high prevalence of fertility problems was present in the untreated CPP group only, suggesting that gonadotropin-suppressive therapy may have a protective effect on the reproductive outcome.

Limited data are available on the reproductive outcome of male patients treated for CPP (Supplementary Table 3). Three small studies showed normal gonadal function in former CPP male adolescents aged 15–18 years (19, 54, 55). Feuillan *et al.* (54) described a progressive increase in testicular volume, similar to controls after 2 years post-therapy, with normal gonadotropins and testosterone levels 1 year after the discontinuation of treatment. Bertelloni *et al.* (55) confirmed normal testicular function in adolescent boys after GnRHa therapy with full pubertal development and, normal testicular volume, gonadotropins, testosterone, and inhibin B levels into normal adult range. Even though paternity rates have not been reported, sperm analysis, performed in six patients, appeared normal for age (55). Following histrelin implant removal, a spontaneous increase in testicular volume was observed within 1 year of histrelin explantation in five boys with CPP (42).

Bone mineral density

Few studies assessed BMD in CPP during GnRHa treatment, showing minor or no changes in BMD parameters

(56, 57, 58). Boot *et al.* (56) found normal BMD for chronological age but decreased BMD for BA after 2 years of treatment with GnRHa.

Pasquino *et al.* (15) reported that both mean BMD lumbar spine and spine volumetric BMD at the discontinuation of treatment were significantly lower in treated CPP girls than in untreated controls. However, at the complete resumption of gonadal activity, both increased to levels similar to those detected in controls. In a more recent study, Magiakou *et al.* (36) showed BMD values adjusted for height were not different between GnRHa-treated and -untreated girls, evaluated at least 2 years after cessation of treatment. Alessandri *et al.* (59) evaluated bone mass, body composition, and bone remodeling in two groups of girls with idiopathic CPP, namely, one group assessed at diagnosis and a second group 3 years after GnRH agonist treatment. BMD and body composition were not affected by CPP, and GnRHa treatment did not seem to have a detrimental effect on the acquisition of bone mass. Heger *et al.* (22) described normal BMD for age in women after GnRHa treatment, with a 17% prevalence of osteopenia. In a small group of CPP-treated female adolescents, Bertelloni *et al.* (27) showed patients' BMD was not different from that of their mothers. In male patients, evaluated at adult height, BMD was similar to that found in a group of healthy young Italian men with normal pubertal development (55). The highest prevalence of osteopenia (45%) was observed by Tung *et al.* (60) in a small group of Taiwanese women, but a plausible explanation for the finding was not provided by the authors.

Conclusions

GnRHa therapy is efficacious in restoring the growth potential in the majority of children with CPP under the age of 6 years, although the estimate of HG is variable and difficult to assess because of sample heterogeneity, methodological limitations associated with height prediction, and the absence of randomized trials. Age at puberty onset, BA advancement, age at initiation, and duration of treatment are the most important outcome predictors, although their impact on adult height remains to be established. Above-average BMI is present at diagnosis at a high rate among children with CPP, but long-term GnRHa treatment does not appear to cause or aggravate obesity. Long-term data do not support adverse consequences of GnRHa therapy on BMD. Gonadal function is preserved after treatment cessation with normal reproductive potential. An association between CPP and ovarian dysfunction independent of GnRHa treatment was suggested, with a potential protective role of GnRHa therapy on the reproductive outcome. Data on the long-term risk of PCOS in CPP-treated and -untreated patients are conflicting although the majority of studies are not supportive of an association between GnRHa use and PCOS. Further studies to assess whether CPP has long-term implications on the general health status and the risk for premature ovarian failure and premature menopause in late reproductive years and to evaluate the impact of GnRHa therapy on the fertility, fecundity, and health of offspring are warranted.

Supplementary data

This is linked to the online version of the paper at http://dx.doi.org/10.1530/EJE-15-0590.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the review.

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PRESIDENTIAL MEMORANDA

Presidential Memorandum for the Secretary of Defense and the Secretary of Homeland Security Regarding Military Service by Transgender Individuals

NATIONAL SECURITY & DEFENSE

Issued on: **March 23, 2018**

★ ★ ★

Pursuant to my memorandum of August 25, 2017, "Military Service by Transgender Individuals," the Secretary of Defense, in consultation with the Secretary of Homeland Security, submitted to me a memorandum and report concerning military service by transgender individuals.

These documents set forth the policies on this issue that the Secretary of Defense, in the exercise of his independent judgment, has concluded should be adopted by the Department of Defense. The Secretary of Homeland Security concurs with these policies with respect to the U.S. Coast Guard.

Among other things, the policies set forth by the Secretary of Defense state that transgender persons with a history or diagnosis of gender dysphoria — individuals who the policies state may require substantial medical treatment, including medications and surgery — are disqualified from military service except under certain limited circumstances.

By the authority vested in me as President by the Constitution and the laws of the United States of America, I hereby order as follows:

Pl. Trial Ex. 221

Section 1. I hereby revoke my memorandum of August 25, 2017, "Military Service by Transgender Individuals," and any other directive I may have made with respect to military service by

transgender individuals.

Sec. 2. The Secretary of Defense, and the Secretary of Homeland Security, with respect to the U.S. Coast Guard, may exercise their authority to implement any appropriate policies concerning military service by transgender individuals.

Sec. 3. (a) Nothing in this memorandum shall be construed to impair or otherwise affect:

(i) the authority granted by law to an executive department or agency, or the head thereof; or

(ii) the functions of the Director of the Office of Management and Budget relating to budgetary, administrative, or legislative proposals.

(b) This memorandum shall be implemented consistent with applicable law and subject to the availability of appropriations.

(c) This memorandum is not intended to, and does not, create any right or benefit, substantive or procedural, enforceable at law or in equity by any party against the United States, its departments, agencies, or entities, its officers, employees, or agents, or any other person.

(d) The Secretary of Defense is authorized and directed to publish this memorandum in the *Federal Register*.

DONALD J. TRUMP

PLAN ID: XXXXXXXXXXXXXXXX Commented [A1]: Max 20 Characters

<<DATE>>
<<ENROLLEE>> and/or
<<LEGAL REPRESENTATIVE>>
<<STREET ADDRESS>>
<<CITY, STATE ZIP>>

NOTICE OF ADVERSE BENEFIT DETERMINATION

Dear <<ENROLLEE/LEGAL REPRESENTATIVE>>:

<<MANAGED CARE PLAN>> has reviewed your request for <<SERVICE and AMOUNT>>, which we received on <<DATE>>. After our review, this service has been:

<<PARTIALLY DENIED, DENIED, TERMINATED, SUSPENDED, REDUCED>> as of <<EFFECTIVE DATE OF ADVERSE BENEFIT DETERMINATION>>

We made our decision because:
(Check all boxes that apply)

☐ We determined that your requested services are **not medically necessary** because the services do not meet the reason(s) checked below: *(See Rule 59G-1.010)*

- ☐ Must be needed to protect life, prevent significant illness or disability, or alleviate severe pain.
- ☐ Must be individualized, specific, consistent with symptoms or diagnosis of illness or injury and not be in excess of the patient's needs.
- ☐ Must meet accepted medical standards and not be experimental or investigational.
- ☐ Must be able to be the level of service that can be safely furnished, and for which no equally effective and more conservative or less costly treatment is available statewide.
- ☐ Must be furnished in a manner not primarily intended for convenience of the recipient, caretaker, or provider.
  (The convenience factor is not applied to the determination of the medically necessary level of private duty nursing (PDN) for children under the age of 21.)

☐ The requested **service is not a covered benefit**.

☐ **Other authority** <<explain and cite authority>>

The facts that we used to make our decision are: <<explain>>
SAMPLE This determination of the Medical Director has been made based on medical necessity (as defined by Florida law – specifically see checked box above) and reflects the application of the Plan's approved review criteria and guidelines.
Clinical rationale: for clinician to write – see example for detail below – it would be different for each type of clinician

Pl. Trial Ex. 221

AHCA 007257

Example from eQHealth
Clinical Rationale for Decision: The patient is a ____ old with a history of gastroesophageal reflux disease and apnea. The patient is on an apnea monitor. Over the past month, the patient had four reported incidences on the monitor. No skilled interventions were required for these reported events. The patient is on oral _____ every 4 hours and requires positioning after meals. The patient is on two scheduled medications and as needed nebulizer treatments. The patient is currently attending ____ during the day. The request is for skilled nursing for 12 hours per day 7 days per week. The patient lives with his _____ and ____. The clinical information provided does not support the medical necessity of the requested services. The patient does not have any ongoing skilled interventions which would support skilled nursing. Additionally, the patient does not require nighttime monitoring by a skilled nurse.

You, or someone legally authorized to do so, can ask us for a complete copy of your file, including medical records, a copy of plan review criteria and guidelines, contract provisions, other documents, records, and other information relevant to the adverse benefit determination. These will be provided free of charge.

You may request these documents by contacting: <<Plan supplied contact information>>

Right to Request a Plan Appeal

If you do not agree with this decision, you have the right to request a plan appeal from <<MANAGED CARE PLAN>>. When you ask for a plan appeal, <<MANAGED CARE PLAN>> has a different health care professional review the decision that was made.

How to Ask for a Plan Appeal:

You can ask for a plan appeal in writing or by calling us. Your case manager can help you with this, if you have one. We must receive the request *within 60 days* of the date of this letter. (If you wish to continue your services until a final decision is made on your appeal, we must receive your request sooner. See the "How to Ask for your Services to Continue" section below for details.) Here is where to call or send your request:

<<MCO>>
<<MAILING ADDRESS>>
<<PHONE>>
<<FAX>>
<<EMAIL>>

Your written request for a plan appeal must include the following information:

- Your name
- Your member number
- Your Medicaid ID number
- A phone number where we can reach you or your legal representative

You may also include the following information if you have it:

- Why you think we should change the decision

- Any medical information to support the request
- Who you would like to help with your plan appeal

Within five days of getting your plan appeal request, we will tell you in writing that we got your plan appeal request unless you ask for an expedited (fast) plan appeal. We will give you an answer to your plan appeal within 30 days of you asking for a plan appeal.

How to Ask for an Expedited (Fast) Plan Appeal if Your Health is At Risk:

You can ask for an "expedited plan appeal" if you think that waiting 30 days for a plan appeal decision resolution could put your life, health, or your ability to attain, maintain, or regain maximum function in danger. You can call or write us (see above), but you need to make sure that you ask us to *expedite* the plan appeal. We may not agree that your plan appeal needs to be expedited, but you will be told of this decision. We will still process your plan appeal under normal time frames. If we do need to expedite your plan appeal, you will get our plan appeal resolution within 48 hours after we receive your plan appeal request. This is true whether you asked for the plan appeal by phone or in writing.

How to Ask for your Services to Continue:

If you are now getting a service that is scheduled to be reduced, suspended or terminated, you have the right to keep getting those services until a final decision is made in a plan appeal and, if requested, fair hearing. If your services are continued, there will be no change in your services until a final decision is made in your plan appeal and, if requested, fair hearing.

If your services are continued and our decision is upheld in a plan appeal or fair hearing, we may ask that you pay for the cost of those services. We will not take away your Medicaid benefits. We cannot ask your family or legal representative to pay for the services.

To have your services continue during the plan appeal, you MUST file your plan appeal AND ask to continue your services within this time frame:

File a request for your services to continue with <<MANAGED CARE PLAN>> no later than 10 days after this letter was mailed OR on or before the first day that your services are scheduled to be reduced, suspended, or terminated, whichever is later. You can ask for a plan appeal by phone. If you do this, you must then ***also*** make a request in writing. **Be sure to tell us if you want your services to continue.**

To have your services continue during the fair hearing, you MUST file your fair hearing request AND ask for continued services within this time frame*:*

If you were receiving services during your plan appeal, you can file the request for your services to continue with the Agency for Health Care Administration (Agency) **no later than 10 days** from the date on your notice of plan appeal resolution OR on or before the first day that your services are scheduled to be reduced, suspended, or terminated, ***whichever is later***.

What to Do if You Disagree with the Plan Appeal Decision

You will receive the result of the plan appeal process in a notice of plan appeal resolution (notice). If you still do not agree with our decision, or if you do not receive your notice on time, you can ask for a fair hearing.

How to Ask for a Fair Hearing:

When you ask for a Medicaid fair hearing, a hearing officer who works for the state reviews the decision that was made. You may ask for a fair hearing any time up to 120 days after you get our notice of plan appeal resolution. **You must finish your plan appeal process first.**

You may ask for a fair hearing by calling or writing to:

Agency for Health Care Administration
Medicaid Hearing Unit
P.O. Box 60127
Ft. Myers, FL 33906

(877) 254-1055 *(toll-free)*
239-338-2642 *(fax)*
MedicaidHearingUnit@ahca.myflorida.com

After getting your fair hearing request, the Agency will tell you in writing that they got your fair hearing request.

If you have questions, call us at <<PHONE>> or <<TTY NUMBER>>. For more information on your rights, review the Grievance and Appeal section in your Member Handbook. It can be found online at: <<WEB ADDRESS>>.

Notice of Nondiscrimination

<< INSERT NONDISCRIMINATION LANGUAGE>>

Commented [A2]: This can go before or after signature, as long as it's included in letter template.

Sincerely,

<<NAME>>
<<Medical Director or title of other professional who made the adverse benefit determination in accordance with Attachment II, Section VII.G.4 of the SMMC contract>>

PLAN ID: XXXXXXXXXXXXXXXXXXXX

Commented [EB1]: Max 20 Characters

<<HEALTH PLAN>>
<<STREET ADDRESS>>
<<CITY, STATE ZIP>>

<<DATE>>

<<ENROLLEE>> and/or
<<LEGAL REPRESENTATIVE>>
<<STREET ADDRESS>>
<<CITY, STATE ZIP>>

NOTICE OF PLAN APPEAL RESOLUTION

Dear <<ENROLLEE/ LEGAL REPRESENTATIVE>>:

On <<DATE PLAN APPEAL REQUEST RECEIVED>> we received your timely plan appeal request regarding <<PLAN>>'s Notice of Adverse Benefit Determination dated <<DATE OF NABD>>, NABD Number ACME-16-000156, <<PARTIALLY DENYING, DENYING, TERMINATING, SUSPENDING, REDUCING>> the <<SERVICE/ AMOUNT>> provided to <<ENROLLEE>>.

On <<DATE PLAN APPEAL PROCESS RESOLVED>>, after consideration of the information you provided to <<PLAN>> in support of your plan appeal, <<PLAN>> hereby <<PARTIALLY DENIES, DENIES, APPROVES >> your plan appeal. As a result, <<ENROLLEE>> will receive <<SERVICE/AMOUNT>>, effective <<DATE >>

Commented [EB2]: If applicable.

You, or someone legally authorized to do so, can ask us for a complete copy of your file, including medical records, a copy of plan review criteria and guidelines, contract provisions, other documents, records, and other information considered during the plan appeal process. These will be provided free of charge.

You may request these documents by contacting: <<Plan supplied contact information>>

Right to Request a State Medicaid Fair Hearing

If you do not agree with this decision, you have the right to request a Medicaid fair hearing from the state. When you ask for a fair hearing, a hearing officer who works for the state reviews the decision made during the plan appeal.

How to Ask for a Fair Hearing:

You may ask for a fair hearing any time up to 120 days after you get this Notice of Plan Appeal Resolution. Your case manager can help you with this, if you have one.

You may ask for a fair hearing by calling or writing to:

Agency for Health Care Administration
Medicaid Hearing Unit
P.O. Box 60127

Pl. Trial Ex. 221

AHCA 007263

Ft. Myers, FL 33906

(877) 254-1055 *(toll-free)*
239-338-2642 *(fax)*
MedicaidHearingUnit@ahca.myflorida.com

Field Code Changed

Your written request for a Medicaid fair hearing must include the following information:

- Your name
- Your member number
- Your Medicaid ID number
- A phone number where we can reach you or your authorized representative

You may also include the following information if you have it:

- Why you think we should change the decision
- Any medical information to support the request
- Who you would like to help with your fair hearing

After getting your fair hearing request, the Agency for Health Care Administration (Agency) will tell you in writing that they got your fair hearing request.

How to Ask for Your Services to Continue During a Fair Hearing:

If you were receiving services during your plan appeal, file the request for your services to continue with the Agency **no later than 10 days** from the date on this Notice of Plan Appeal Resolution OR on or before the first day that your services are scheduled to be reduced, suspended, or terminated, *whichever is later.*

If your services are continued and our decision is upheld in a fair hearing, we may ask that you pay for the cost of those services. We will not take away your Medicaid benefits. We cannot ask your family or legal representative to pay for the services.

If you have questions, call us at <<PHONE>> or <<TTY NUMBER>>. For more information on your rights, review the Grievance and Appeal section in your Member Handbook. It can be found online at: <<WEB ADDRESS>>.

Notice of Nondiscrimination

<<INSERT NONDISCRIMINATION LANGUAGE>>

Commented [EB3]: This can go before or after signature, as long as it's included in letter template.

Sincerely,

<<NAME>>
<<Medical Director or title of other professional who made the plan appeal decision and who was not the decision maker or the subordinate of the decision maker in the previous level of review, per 42 CFR 438.406(b)(2)(i)>>

STATE OF FLORIDA
AGENCY FOR HEALTH CARE ADMINISTRATION
OFFICE OF FAIR HEARINGS

FILED

Jun 01, 2020, 9:07 am

OFFICE OF FAIR HEARINGS

[redacted]

PETITIONER,

AHCA Case No.: 20-FH0855
Plan ID No.: [redacted]

vs.

SIMPLY HEALTHCARE PLANS, INC.,

RESPONDENT.
_____/

FINAL ORDER

Pursuant to notice, the undersigned convened a telephonic Fair Hearing on the instant case on April 29, 2020, at 9:00 a.m. EST.

APPEARANCES

For the Petitioner: [redacted]
Petitioner's Authorized Representative

For the Respondent: Angela Royster
Grievance and Appeals Associate
Simply Healthcare Plans, Inc.

STATEMENT OF ISSUE

The issue is whether Petitioner proved by a preponderance of the evidence that Respondent's decision to deny Petitioner's request for medical supplies was incorrect.

PRELIMINARY STATEMENT

Pl. Trial Ex. 221

All parties appeared telephonically. Petitioner's Authorized Representative [redacted] appeared on behalf of the Petitioner. Petitioner appeared to offer testimony. Carlos Sanchez appeared as a witness for Petitioner.

Angela Royster, Grievance and Appeals Associate for Simply Healthcare Plans ("Simply") appeared on behalf of Respondent. The following attended as witness for Respondent: Dr. Susan Ledbetter ("Dr. Ledbetter"), Medical Director for Simply, and Roberta Frank, Nurse Appeals Associate for Simply.

Sheila Gonzalez, Medical/Health Care Program Analyst for the Agency for Health Care Administration ("Agency" or "AHCA") and Charles Martin, Hearing Officer for AHCA, appeared as observers.

Oscar, interpreter number 351116 for Ciricom, and Veronica appeared to offer translation services for Petitioner.

Petitioner did not introduce any exhibits at the hearing. Prior to the hearing, Respondent sent to the Office of Fair Hearings and Petitioner a two hundred and sixty (260)-page evidence packet. The evidence packet included: a table of contents; Sequence of Events; a prescription for [redacted], dated [redacted]; Patient Information, dated [redacted] Petitioner medical record, dated [redacted]; Petitioner Identification Card; a Notice of Adverse Benefit Determination ("NABD"), dated April 1, 2020; Authorization Notes, dated March 31, 2020 to April 1, 2020; a letter from Respondent, dated April 7, 2020; an e-mail from Petitioner, dated April 5, 2020; a letter from Respondent, dated April 7, 2020; a Notice of Plan Appeal Resolution ("NPAR"), dated April 9, 2020; Member Appeals Notes, dated April 7, 2020, to April 9, 2020; Member Appeal Notes, dated April 13, 2020; an NPAR, dated April 13, 2020; Durable

Medical Equipment (DME) and Medical Supply Services Provider Fee Schedule for All Medicaid Recipients (2020) ("Fee Schedule") excerpt; and the Florida Medicaid Durable Medical Equipment and Medical Supply Services Coverage and Limitations Handbook (July 2010) ("Coverage Policy"). Absent an objection from the Petitioner undersigned admitted the two hundred and sixty (260)-page packet into evidence as Respondent's Composite Exhibit 1.

FINDINGS OF FACT

1. Petitioner is an enrolled member of Simply. Simply is a managed care organization contracted by the Agency to provide services to eligible Medicaid recipients in Florida.

2. Petitioner is [redacted]. *See* page 9[1] of Respondent's Composite Exhibit 1. Petitioner is diagnosed with [redacted] *Id.*

3. Petitioner requested medical supplies, specifically: [redacted]. Petitioner's request was denied in the NABD dated April 1, 2020. The NABD explained the basis of the denial as follows:

> We determined that your requested services are not medically necessary because the services do not meet the reason(s) checked below: *(See Rule 59G-1.010)*
> - Must be individualized, specific, consistent with symptoms or diagnosis of the illness or injury and not in excess of the patient's needs
>
> . . .
>
> The requested service is not a covered benefit.
>
> . . .
>
> The facts that we used to make our decision are: We cannot cover the personal care items as requested ([redacted]). You have many health problems ([redacted]. You have problems with [redacted] You have trouble [redacted]

[1] Respondent's evidence packet contains two hundred and sixty pages, which consists of two (2) unnumbered pages and two hundred and fifty-eight numbered pages. The pages referenced here refer to the page numbers as numbered in the packet.

> [redacted]. The doctor asked for [redacted] to help. We were not told you have [redacted] We were not told that you have any [redacted]. These care items are not covered by Florida Medicaid. They are not usually covered if you are [redacted]). These items could be covered with your over the counter benefit. You may need a different type of plan to help with these items (Long Term Care Services). This decision is based on the information provided and the Florida Medicaid Durable Medicaid Equipment Fee Schedule. Your Reference number is #110615917.
>
> *Id.* at 22 through 47.

4. Petitioner requested a plan appeal and received an NPAR dated April 9, 2020, and April 13, 2020, upholding the denial. *Id.* at 97 through 103 and 116 through 122.

CONCLUSIONS OF LAW

5. The Agency's Office of Fair Hearings has jurisdiction over the subject matter of this proceeding and the parties pursuant to Fla. Stat. § 409.285(2)(2019). This order is the final administrative decision of AHCA under Fla. Stat. § 409.285(2)(a).

6. This hearing was held as a *de novo* proceeding pursuant to Fla. Admin. Code R. 59G-1.100(17)(b).

7. Because Petitioner is requesting a new service, Fla. Admin Code R. 59G-1.100(17)(g) assigns the burden of proof to Petitioner. The standard of proof in an administrative hearing is a preponderance of the evidence. The preponderance of the evidence standard requires proof by "the greater weight of the evidence" (Black's Law Dictionary at 1201, 7th Ed.)

8. The Coverage Policy, incorporated by reference in Fla. Admin. Code R. 59G-4.070, governs requests for medical supplies under Florida Medicaid. The Coverage Policy provides the following:

> **Medical Supplies:** Medical Supplies are defined as medically-necessary medical or surgical items that are consumable, expendable, disposable, or non-durable and appropriate for use in the recipient's home.

. . .

Services Limited to Recipients Under 21 Years of Age:

Many durable medical equipment (DME) items and services are limited to recipients under 21 years of age.

To determine whether a service is available to all recipients or limited to recipients under age 21 years of age, refer to the DME and Medical Supply Services Provider Fee Schedules.

. . .

Authorized Prescribers of Durable Medical Equipment and Medical Supplies:

All durable medical equipment, medical supplies, and orthotic and prosthetic devices must be prescribed by the Medicaid recipient's:

- Treating physician, or
- Treating physician's physician assistant, or
- Treating physician's advanced registered nurse practitioner (ARNP), or
- Treating podiatrist.

The prescribing professional must include the date, his signature, and current professional license number or national provider identification number on each documentation of medical necessity when requesting DME and services or medical supplies.

. . .

Service Criteria:

All DME, medical supplies, and orthotics and prosthetic devices must be:

- Medically necessary, and
- Functionally appropriate for the individual recipient, and
- Adequate for the intended medical purpose, and
- For conventional use, and
- For the exclusive use of the recipient.

. . .

9. The Coverage Policy states the following with respect to acceptable documentation of Medical Necessity:

Acceptable Documentation of Medical Necessity

Medical necessity must be established for each service and documented, at a minimum, with the following:

- Written prescription not more than 12 months old, with the printed name and the dated signature of the recipient's treating physician or the treating physician's ARNP or physician assistant. The prescription can be received by the DME and medical supply provider before or after the DME service has been initiated, but the prescription cannot be dated more than 21 days after the initiation of service (date of service); or

- Current hospital discharge plan with the dated signature of the recipient's treating physician or the treating physician's ARNP or physician assistant that clearly describes the type of DME item or service ordered; or
- Certificate of Medical Necessity (CMN) not more than 12 months old, which includes the printed name and the dated signature of the recipient's treating physician or the treating physician's ARNP or physician assistant. Medicaid prohibits vendors from preparing sections of the CMN that are to be completed by the physician or authorized prescriber. The CMN cannot be dated more than 21 days after the initiation of service (date of service); and Plan of care, if a home health agency.

. . .

All documentation of medical necessity must include the type of medical equipment, services or consumable goods ordered, including the type, quantity, frequency and length of need ordered or prescribed. Prescribed oxygen services must include rates of flow, concentration, level of frequency, duration of use, and circumstances under which oxygen is to be used. If this information is not included, a new prescription that clarifies the order is required.

10. The Florida Medicaid Definitions Policy, incorporated by reference in Fla. Admin. Code R. 59G-1.010, defines "Medically Necessary" or "Medical Necessity" as follows:

The medical or allied care, goods, or services furnished or ordered must meet the following conditions:

- Be necessary to protect life, to prevent significant illness or significant disability, or to alleviate pain
- Be individualized, specific, and consistent with symptoms or confirmed diagnosis of the illness or injury under treatment, and not in excess of the patient's needs
- Be consistent with generally accepted professional medical standards as determined by the Medicaid program, and not experimental or investigational
- Be reflective of the level of service that can be safely furnished, and for which no equally effective and more conservative or less costly treatment is available statewide
- Be furnished in a manner not primarily intended for the convenience of the recipient, the recipient's caretaker, or the provider

The fact that a provider has prescribed, recommended, or approved medical or allied care, goods, or services does not, in itself, make such care, goods or services medically necessary or a medical necessity or a covered service.

11. The Fee Schedule, which is incorporated by reference in Fla. Admin. Code R. 59G-4.071, lists the medical supplies reimbursed by Florida Medicaid. The Fee Schedule include a column, labeled "Age", that denotes the age range covered for the service code. In this case, the service code requested is [redacted] and the age range identified as covered is [redacted] Petitioner is [redacted]. The Respondent denied Petitioner's request as the supplies are not covered under Florida Medicaid. As shown in the Fee Schedule, Petitioner's requested supplies are not covered for [redacted] age.

12. Therefore, upon consideration of the testimony provided, evidence submitted, and applicable policies, the undersigned finds that Petitioner did not prove by a preponderance of the evidence that Respondent's denial of medical supplies was incorrect.

IT IS THEREFORE ORDERED AND ADJUDGED THAT:

Respondent's denial is **AFFIRMED**. Petitioner's appeal based on Respondent's denial is **DENIED**.

DONE AND ORDERED this 1st day of June, 2020, in Tallahassee, Leon County, Florida.

Joseph Mabry
20-FH0855
2020.06.01 08:49:48
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JOSEPH MABRY, Hearing Officer
Agency for Health Care Administration
Office of Fair Hearings
2727 Mahan Drive, Mail Stop # 11
Tallahassee, FL 32308-5407
Office: (850) 412-3649
Fax: (850) 487-1423
Email: OfficeOfFairHearings@ahca.myflorida.com

NOTICE OF A RIGHT TO JUDICIAL REVIEW

A PARTY WHO IS ADVERSELY AFFECTED BY THIS FINAL ORDER IS ENTITLED TO JUDICIAL REVIEW, WHICH SHALL BE INSTITUTED BY FILING THE ORIGINAL NOTICE OF APPEAL WITH THE AGENCY CLERK OF AHCA, AND A COPY, ALONG WITH THE FILING FEE PRESCRIBED BY LAW, WITH THE DISTRICT COURT OF APPEAL IN THE APPELLATE DISTRICT WHERE THE AGENCY MAINTAINS ITS HEADQUARTERS OR WHERE A PARTY RESIDES. REVIEW PROCEEDINGS SHALL BE CONDUCTED IN ACCORDANCE WITH THE FLORIDA APPELLATE RULES. THE NOTICE OF APPEAL MUST BE FILED WITHIN 30 DAYS OF THE RENDITION OF THE ORDER TO BE REVIEWED.

Copies Furnished To:

[redacted]

Simply
MedicaidFairHearings@simplyhealthcareplans.com

AHCA Medicaid Hearing Unit
MedicaidHearingUnit@ahca.myflorida.com