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Original Research

Hormonal status and bone turnover in adolescents with polycystic ovarian syndrome

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Abstract

Background: Problems with hormonal changes and the related variations in bone turnover in adolescents with polycystic ovarian syndrome (PCOS) have been of interest in terms of providing these patients with an opportunity to receive a prophylactic and precision-based treatment aiming to prevent early onset of osteoporosis. **Materials and methods**: Prospective comparative clinical trial—'case-control' type in Bulgarian populace of 36 female patients with PCOS and 42 healthy controls aged 12 to 18. The study protocol included a general section of anthropometric patient data, clinical section–including general and Ob/Gyn Medical History, ultrasound exam of the lesser pelvis and a lab section examining the serum levels of Follicle-stimulating hormone (FSH), Luteinizing hormone (LH), estradiol, Anti-Müllerian hormone (AMH) and bone turnover markers–osteocalcin and β-CrossLaps (bCTX), as well as Vitamin D. **Results**: A statistically significant high serum levels of the gonadotropic hormones were observed (LH — $p < 0.001$ и FSH — $p = 0.017$), AMH ($p < 0.001$) in patients with PCOS compared to the controls, while the estradiol ($p = 0.043$) and osteocalcin ($p < 0.001$) levels displayed a statistically significant lower values in patients with PCOS compared to the control group. AMH can be utilized as a surrogate marker for diagnosing patients with PCOS where the marker shows sensitivity — 94% and specificity — 69% with threshold value (cut-off) at $\geq$5.95 ng/mL (area under the curve 0.854, $p < 0.001$). Significant variance in Vitamin D serum levels between the two groups was not detected. **Conclusion**: Despite the hormonal characteristic of normogonadotropic normogonadism in adolescent patients with PCOS, the significantly lower values of osteocalcin demonstrated suppressed bone metabolism–bone formation, in particular–compared to the healthy controls, which can be interpreted as increased risk of insufficient bone accretion and risk of early onset of osteoporosis later in life.

Keywords: Policystic ovarian syndrome (PCOS); Bone turnover; Hormonal status

1. Introduction

PCOS is a heterogenic endocrine disorder that affects 1 in 15 women worldwide [1]. Usually the onset of the condition is during adolescence and the clinical presentation is characterized by anovulatory cycles (amenorrhea, opsomenorrhea, irregular menstrual cycles) combined with hyperandrogenism symptoms (hirsutism, acne, alopecia).

Due to the varied expressions of the syndrome, the European Society for Human Reproduction and Embryology (ESHRE) and the American Society for Reproductive Medicine (ASRM) have established that the diagnosis requires the presence of at least two of the "Rotterdam Criteria".

- Polycystic ovaries on ultrasound.
- Anovulatory menstrual cycles.
- Clinical or biochemical evidence of excess androgen [2,3].

One of the basic irregularities of this syndrome is the increased serum levels of LH and the imbalanced ratio of LH versus FSH. The paraclinical characteristic of PCOS patients is increased LH serum levels and normal FSH levels. It is considered to be due to increased release frequency of GnRH from the hypothalamus which leads to a hyperfunction of the theca cells [4]. The estradiol serum levels are similar to those of the healthy controls but still of lower values and they remain constant along the duration of the whole menstrual cycle. AMH is usually significantly higher in PCOS patients, regardless of obesity and evidence of excess androgen, although other studies have recorded no statistically significant variance between those patients and healthy controls [5,6]. The high diagnostic reliability of AMH as a surrogate marker complementary to the Rotterdam Criteria in diagnosing PCOS patients has been confirmed in multiple trials [7–12].

Most of the patients with PCOS display high BMI which prevents amenorrhea/oligomenorrhea-induced bone loss. The adipocytes and the stromal cells in the adipose tissue express P450 aromatase which helps adrenal and ovarian testosterone and androstenedione convert to 17β-estradiol and estrone. Second, the mechanical loading stim-

ulates the formation of bone tissue by reduction of the apoptosis and increase of the proliferation and differentiation of the osteoblasts and osteocytes via the Wnt/β-catenin signalling pathway.

Increase of BMI is associated with the increase of bone mineral density. It has been reported that the risk of osteoporosis-related fractures is lower in overweight women compared to those with normal or low values of BMI [13,14]. Ravn *et al*. [15] compared women with obesitas to same age women with normal weight and concluded that the former group had a significantly lower risk of developing osteoporosis. On the other hand, obesity can cause bone loss by stimulating the formation and excretion of proinflammatory cytokines-IL-6 and tumor necrosis factor-α, which stimulate osteoclastic activity [16,17].

Bone tissue is metabolically active during the whole human life cycle. Monitoring bone metabolism is achieved by assessment of bone formation markers (alkaline phosphatase, osteocalcin, collagen propeptides) and bone resorption markers (beta C-terminal cross-linked telopeptide of type I collagen, bCTX). Bone markers are highest at birth and remain so during childhood, reflecting the most rapid bone growth and most intense acting processes of modelling and remodelling of the bones. Gradual reduction of marker levels follows that period and only during the puberty growth spirt (12–13 years of age) does a new upswing occur with the peak between 10 and 13 years of age in girls [18,19].

Puberty is associated with accelerated bone metabolism [20–22]. After puberty follows a decline in bone marker levels (most pronounced between 30 and 50 years of age) and going into menopause marks an upswing in their values once more due to the direct effect of the menopause itself and the related hormonal deficit [23].

There is very controversial data when it comes to bone metabolism markers in PCOS patients as some authors report significantly lower values of bone formation markers and normal or lower levels of bone resorption markers. This data has not been confirmed by other studies conducted among adolescents with PCOS and where no significant variance has been noted compared to the control group in terms of bone metabolism and bone mineral density [24–28].

Vitamin D (Vitamin D - 1,25(OH)2 Vitamin D) plays a central role in bone formation and bone remodeling [29–31]. Multiple studies have asserted the importance of maintaining adequate Vitamin D serum levels to protect against bone fractures [32–37]. There is a number of studies that have not recorded a significant variance in Vitamin D serum levels in PCOS patients and controls [38–40], however, others have reported significantly higher levels in PCOS patients compared to healthy individuals of the same age [41,42]. Lagowska studied the Vitamin D serum levels of 77 patients with opso-(PCOS) and amenorrhea (primary and secondary) and of a control group consisting of women with normal menstrual cycle and discovered significantly lower values of Vitamin D in patients with hypomenstrual disorders compared to controls. The observed Vitamin D deficit in patients is associated with high levels of PTH, anovulation, hyperandrogenemia and infertility [43].

2. Materials and methods

This prospective trial "case-control" type studied 42 healthy "controls" and 36 patients with PCOS aged 12 to 18 for the period 2015–2019 in University hospital "Maichin dom", Sofia.

The PCOS patients included in the trial have met at least two of the three Rotterdam Criteria as established by ESHRE/ASRAM in 2003.

The control group included girls between 12 and 18 years of age with normal menstrual cycles, normal ultrasound findings–uterus and ovaries with no pathological deviations and normal or reference serum levels of the main lab indicators.

The exclusion criteria for both groups were: endocrine pathology with thyroid, adrenal origin; severe acute and chronic conditions; congenital conditions impacting the musculoskeletal system; primary amenorrhea due to Swyer syndrome, Morris syndrome, MRKH syndrome; use of oral contraceptives, corticosteroid drugs, hormonal drugs; pregnancy; refusal to participate in the trial.

The protocol of the trial included — general section of anthropometric patient data–age, height, weight, BMI; clinical section — general and Ob/Gyn Medical History, ultrasound exam, lab section-examining the serum levels of LH, FSH, estradiol, AMH and osteocalcin, β-CrossLaps and Vitamin D—to assess bone health.

Follicle-stimulating hormone (FSH — Reference Range Female, Follicular phase: FSH — 1–10 IU/L), Luteinizing hormone (LH — Reference Range Female, Follicular phase: LH — 2–10 IU/L) were measured in a serum of highly sensitive immunoradiometric assays (IRMA) using two monoclonal antibodies with kits from Immunotech, France.

Estradiol (E2 — Reference Range Female, Follicular phase: 9–550 pmol/L) is measured in a serum with Radioimmunoassay (RIA) with kits from Immunotech, France.

Anti-Müllerian hormone (AMH) was measured in a serum via manual Generation II (Gen II) ELISA method (Beckman Coulter, USA). The analysis is deemed the most sensitive today and uses two monoclonal antibodies from Diagnostic Systems Laboratories, USA. Analytical sensitivity detection limit of 0.08 ng/mL; intra-assay (3.4–5.4%) precision; inter-assay (4–5.6%) precision. Reference range method: girls–1–8.9 ng/mL.

N-MID Osteocalcin, β-CrossLaps and 25 Hydroxyvitamin D total (25OHD) were measured via Electrochemi-

luminescence immunoassay (ECLIA) of immunoassay analyzer "Cobas e411", Roche (Tables 1,2).

Table 1. Reference values for bone turnover markers in adolescents.

Age	N – MID Osteocalcin	β–CrossLaps
10–13 years old	0.519–2.415 ng/mL	49–167 ng/mL
14–17 years old	0.242–1.291 ng/mL	14–85 ng/mL
>17 years old		8–32 ng/mL

Table 2. Classification of 25OHD (vitamin D) status according to serum levels.

Serum levels of 25OHD	Classification of 25OHD status
<10 ng/mL	Severe Deficiency
10–20 ng/mL	Deficiency
20–30 ng/mL	Insufficiency
>30 ng/mL	Sufficiency (Normal serum levels)

Each girl was examined as per an individual program, in which a particular date was calculated for each exam on a case by case basis.

After a detailed current personal and family medical history was taken and recorded in a form (for each individual case), a final assessment was determined to evaluate the degree of development of secondary sexual characteristics according to the approved in literature and in practice in Bulgaria — Tanner scale — thelarche, pubarche, adrenarche. The presence or lack of hirsutism-moustache, under the chin, lower abdomen, sternum, and pubis — was evaluated.

Serum levels of LH, FSH, estradiol, AMH were measured during the early follicular phase of the menstrual cycle where the timing of the evaluation of the indicators was in accordance with the menstrual intervals or after an induced uterine bleeding by means of progestins (one tablet twice a day for 5 days-Lynestrenol per os). Samples were collected in the morning between 8 AM and 10 AM on an empty stomach after 30 minutes of rest before the venipuncture.

Transabdominal or transvaginal ultrasound of lesser pelvis — to determine the presence of follicles, the thickness of the uterine lining, uterine sizes. Exam was performed with MEDISON Accuvix V20 (Germany).

All data was entered and processed via statistical package IBM SPSS Statistics 25.0 (IBM Corp., Armonk, NY, USA). MedCalc Version 14.8.1 (MedCalc Software, Sofia, Bulgaria). Level of significance where null hypothesis is rejected is set to be $p < 0.05$. Descriptive statistics and graphical analysis were utilized as well as parametric and nonparametric methods to check the hypothesis.

3. Results

The tested clinical population involves 78 girls at an average age 15.93 $\pm$ 1.45 years in the interval between 12 and 18.

It can be seen on Table 3 that the two tested groups were statistically made equal in terms of the known confounding factor age which ensured correctness of the subsequent comparisons.

Table 3. Comparative analysis of the two tested groups by age.

Groups	Age		
	N	$\overline{X}$	SD
Control	42	15.69^a	1.62
With opsomenorrhea (PCOS)	36	16.11^a	1.43

Note: The similar letters in vertical show lack of a significant difference and the different ones — existence of such ($p < 0.05$).

The average age for menarche (first menstruation in life) of the whole sample was 12.38 $\pm$ 0.52 years in the interval between 11 and 13. It can be seen from Table 4 that the control group had a significantly lower age for menarche (12.19 $\pm$ 0.51 years) compared to the groups with opsomenorrhea/PCOS (12.53 $\pm$ 0.51 years).

Table 4. Comparative analysis of the two studies groups by menarche.

Group	Menarche		
	N	$\overline{X}$	SD
Control	42	12.19^a	0.51
With opsomenorrhea (PCOS)	36	12.53^b	0.51
Whole sample	78	12.38	0.52

Note: The similar letters in vertical show lack of a significant difference and the different ones — existence of such ($p < 0.05$).

Early menarche is associated with early establishment of ovulatory cycles. When the first menstruation occurs earlier than 12 years, 50% of menstrual cycles are ovulatory in the first year after menarche. With a late menarche, it can take eight to twelve years for all menstrual cycles to become ovulatory. Later onset of the first menstruation and menstrual disorders indicate impaired function of the HHO-axis and can be an important risk factor for insufficient and inadequate accumulation of bone mass.

It is discutable whether adipose tissue contributes to the accumulation of bone mass or by various mechanisms to lead to bone loss.

A significant difference has been found in terms of BMI, and 38.9% of the patients with PCOS were over-

Table 5. Frequency distribution of the participants in the study by abnormal values of BMI and study groups (Chi-Square Tests).

	Statistics	Groups		Total
		Control	PCOS	*p*
<18.5	N	1	1	
	%	2.40%	2.80%	
18.5–25.0	N	36	18	
	%	85.70%	50%	<0.001
>25.0	N	4	14	
	%	9.50%	38.90%	
Total	N	42	36	
	%	100%	100%	

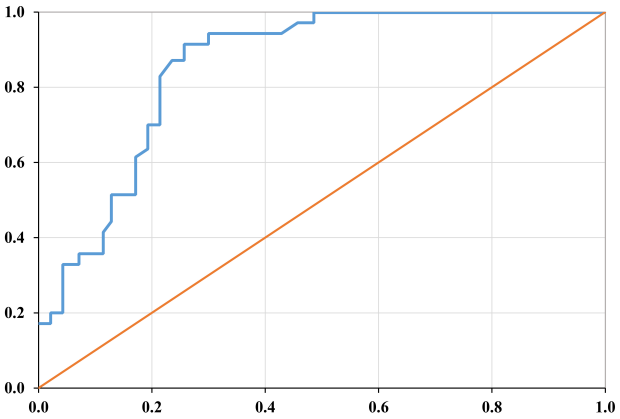

Fig. 1. ROC curve of AMH for determining its cut-off value when separating those having PCOS from the ones of the control group (area below the curve 0.854, $p < 0.001$). *Vertical axis — sensitivity, horizontal axis — specificity.

weight, but in the control group this percentage was 9.5% ($p < 0.001$) (Table 5).

In the group of patients with PCOS, there were statistically significant higher serum levels of gonadotrophic hormones (LH — $p < 0.001$ and FSH — $p = 0.017$), AMH ($p < 0.001$), compared to the control group, while the levels of estradiol ($p = 0.043$) and osteocalcin ($p < 0.001$) were statistically lower in those patients compared to the control group (Table 6).

In spite of the hormonal characteristics of normogonadotropic normogonadism in those patients, the significantly lower values of osteocalcin showed a suppressed bone metabolism and in particular–bone formation compared to the healthy control groups that may be interpreted as existence of enhanced risk of insufficient bone mass accumulation and risk of early onset of osteoporosis later in life.

The high diagnostic reliability of AMH as a surrogate marker complementing the Rotterdam criteria in the diagnostic process of patients with PCOS has been proven in numerous studies.

ROC curve analysis was applied to determine the cut-off values of AMH and asses the option to use it as a surrogate marker in the diagnostic process of patients with PCOS, supplementing the accepted Rotterdam criteria of ESHRE/ASRM. The criteria for optimization in the choice of cut-off value were high sensitivity and precision. According to the calculated values of validation criteria (Table 7).

In the patients with PCOS, sensitivity — 94% and specificity — 69% were ascertained, with cut–off value ≥5.95 ng/mL (area below the curve 0.854, $p < 0.001$) which showed the actual option that AMH is used as a surrogate marker in the diagnostic process in those patients (Table 7 and Fig. 1).

Vitamin D plays an extremely important role in calcium-phosphorus ($Ca^{2+} \times HPO_4^{2-}$) metabolism and provides constant serum levels of both elements for metabolic needs and bone mineralization. Vitamin D deficiency leads to secondary hyperparathyroidism associated with osteoclastogenesis and increased bone resorption exceeding osteoblast-mediated bone formation. The incidence of Vitamin D deficiency is 21.3% and of insufficiency — 54.5%. Globally, Vitamin D deficiency and insufficiency is widespread, ranging from 53.4% in South America to 81.8% in the Middle East. Vitamin D deficiency is higher in southern Europe than in northern Europe. In a study conducted in Bulgaria covering 2032 people, it became clear that the average population level of vitamin D (25(OH)D) in Bulgaria is 38.75 nmol/L. No statistically significant differences were found in serum levels of 25(OH)D depending on age, but females had lower mean values than males.

The performed comparative analysis of the percentage of shortage of Vitamin D (values of Vitamin D below 30 ng/mL) in both groups and in total in the studied population showed that (Table 8).

No statistically significant difference between the studies group was found in terms of insufficiency of Vitamin D.

4. Discussion

In patients with PCOS, normogonadotropic normogonadism is observed, but nevertheless, they have disorders in the menstrual cycle characterized by prolonged intermenstrual intervals. The diagnosis PCOS was determined in observance of the requirements of ESHRE/ASRM — the patients to comply with at least two of the three Rotterdam criteria [2,3].

The average values of the gonadotropic hormones in those patients were higher compared to the control group but were within normal reference ranges (LH — 8.96 ± 2.7 UI/L and FSH — 6.71 ± 2.39 UI/L). Normal but statistically significant lower levels estradiol — 356.94 ± 157.91

Table 6. Comparative analysis of the control groups and the patients with PCOS according to the indicators osteocalcin, β-CrossLaps, LH, FSH, estradiol and AMH.

Indicators	Control groups			PCOS			p
	N	$\overline{X}$	SD	N	$\overline{X}$	SD	
Osteocalcin	42	39.36	20.68	36	23.84	8.83	<0.001
β-CrossLaps	42	1.03	0.4	36	0.94	0.28	0.44
LH	42	4.92	1.83	36	8.96	2.7	<0.001
FSH	42	5.23	1.91	36	6.71	2.39	0.017
Estradiol	41	444.46	207.31	36	356.94	157.91	0.043
AMH	42	5.68	3.25	36	10.11	2.67	<0.001

Table 7. Calculated values of validation criteria.

Reason	Cut-off value	Sensitivity	Specificity	Positive predictive value	Negative predictive value	% true answers
PCOS	≥5.95	94	69	72	94	81

Table 8. Comparative analysis of the percentage of shortage of Vitamin D in both groups.

Group	Patients with shortage of Vitamin D		
	N	%	Sp
Healthy control groups	25	59.5[a]	7.6
Girls with (PCOS)	16	44.4[a]	8.3

Note: The similar letters in vertical show lack of a significant difference and the different ones — existence of such ($p < 0.05$). Sp, Specificity.

pmol/L ($p = 0.043$) and statistically significant higher values of AMH — 10.11 ± 2.67 ng/mL ($p < 0.001$) are observed.

Franks and Bednarska report in their studies that the patients with PCOS are characterized by increased serum levels of androgens, LH and AMH, but by normal or low serum levels of FSH [2,4].

Tokmak *et al.* [5] publish in their study involving 90 girls, of which 43 with PCOS, and the remaining in healthy control groups, higher levels of AMH (10.1 ± 6.9 ng/mL vs. 9.4 ± 5.5 ng/mL, $p = 0.198$) in the patients with PCOS but without any statistically significant difference between the two groups. Their conclusion is that AMH is of higher levels in patients with PCOS in the period of puberty compared to the control group but it is not a good marker for diagnosis of this condition in the period of puberty-adolescence [5].

A prospective study performed by Park *et al.* [6] reports about significantly higher levels of AMH in patients with PCOS in the period of puberty — adolescence compared to healthy control groups. The study involves 220 patients with oligomenorrhea/opsomenorrhea, with PCOS and healthy control groups, and the patients with oligomenorrhea were without clinical or paraclinical evidence of androgenic excess. They find in their study increased levels of AMH in the patients with oligomenorrhea and in those with PCOS, having androgen excess (5.33 ± 0.47 ng/mL and 5.28 ± 0.26 ng/mL) — without any significant difference between them but with an existence of such between the two groups and the control group (3.05 ± 0.31 ng/mL).

The serum levels of gonadotropic hormones and estradiol are comparable with the results found by us as follows LH — 9.3 ± 0.7 UI/L, FSH — 4.8 ± 0.2 UI/L, estradiol — 208.88 ± 11.01 pmol/L in patients with PCOS and LH — 6.4 ± 1.0 UI/L, FSH — 5.4 ± 0.4 UI/L, estradiol — 171.07 ± 20.92 pmol/L in the patients with oligomenorrhea without androgen excess. According to this study, the serum levels of AMH may be included in the diagnostic criteria for PCOS, especially with a lack of opportunity for ultrasonography assessment of ovaries [6].

In the group of patients with PCOS, we reported statistically significant lower values of osteocalcin ($p < 0.001$) but without a significant difference in the serum levels of β-CrossLaps compared to the healthy control group. In this group, a significantly higher percentage of above-norm values of BMI — 38.9% was reported.

In their study covering 298 patients with PCOS and 194 healthy control groups, Lingaiah *et al.* [25,26] report about statistically significant lower levels of osteocalcin in those patients but do not find any significant difference between the levels of β-CrossLaps and Vitamin D in both groups. In another of their studies, they report about a suppressed bone metabolism and low levels of both serum markers.

On the other hand, studies conducted in adolescent patients with evidence of PCOS report about a possible protective effect of hyperinsulinemia and increased androgenic level on bones and about lack of a significant difference between patients with PCOS and healthy control groups regarding bone metabolism markers and bone mineral density [27,28].

The high reliability of AMH as a surrogate marker supplementing Rotterdam criteria in the diagnostic process of patients with PCOS has been proven in multiple studies.

A high specificity and sensitivity (98% and 93%) are

reported by Saikumar *et al*. [7] in their study at cut-off value of AMH 3.34 ng/mL. Woo *et al*. [8] report about sensitivity (75.9%) and specificity (86.8%) with a higher cut-off value of AMH 7.82 ng/mL, Lin *et al*. [9] — 92% — specificity and 67% — sensitivity at cut-off value of 7.3 ng/mL.

Dewailly *et al*. [10] report higher specificity and sensitivity (92% and 97%) with a cut-off value of 4.9 ng/mL. Homburg *et al*. [11] report a higher specificity (98.2%) but lower sensitivity (60%) of AMH with cut-off value of 6.7 ng/mL.

In the study performed by us, we found high sensitivity — 94% and specificity 69% with cut-off value of AMH of 5.95 ng/mL, a positive predictive value of 72% and a negative predictive value — 94%. These differences in the cut-off value maybe result from the use of various kits for AMH. Cengiz *et al*. [12] reach the conclusion that where AMH is used separately and not in integrity with Rotterdam criteria, it is not a reliable indicator for determining or rejecting the diagnosis of PCOS.

The increase of BMI is related to an increase of bone mineral density. A lower risk of osteoporosis fractures is reported in overweight women compared to those of normal and especially sub-normal values of BMI [13,14].

Ravn *et al*. [15] compare women with obesity with women of normal body weight at the same age and reach a conclusion that in the first group, there is a significantly lower risk to develop osteoporosis. On the other hand, obesity may result in bone loss by stimulating the formation and release of pro-inflammatory cytokines-IL-6 and tumour necrosis factor-α that stimulate osteoclastic activity [16,17].

There are a number of studies in which, no significant difference between serum levels of Vitamin D is found in patients with PCOS and control groups [38–40] but in other studies, statistically significant higher levels with PCOS $_{\text{B}}$ compared to the control group [41,42] are reported.

Lagowska studied serum levels of Vitamin D in 77 patients with opso-(PCOS) and amenorrhea (primary and secondary) and a control group of women with a normal menstrual cycle and found significantly lower values in the patients with hypomenstrual disorders compared to the control groups. She relates the observed deficit of Vitamin D in patients with PCOS with increased levels of PTH, anovulation, hyperandrogenism and infertility [43].

No statistically significant difference was found between the patients with PCOS and the healthy control groups in terms of insufficiency of Vitamin D in the study conducted by us.

5. Conclusions

Despite the hormonal characteristic of normogonadotropic normogonadism in patients with PCOS in adolescence, the significantly lower values of osteocalcin showed a suppressed bone metabolism and in particular bone formation, compared to the healthy control groups which may be interpreted as existence of an enhanced risk of insufficient bone mass accumulation and risk of early onset of osteoporosis later in life.

Author contributions

DH—extraction and drafting of the manuscript, analysis of data, manuscript revision; DH and GK—design and revision, statistical analysis. All authors contributed to editorial changes in the manuscript. All authors read and approved the final manuscript.

Ethics approval and consent to participate

Ethical approval was granted by the Ethics Committee of Scientific Research at the Medical University-Sofia (ECSRMUS) — protocol NO. 2237, May 19th, 2015. Parent (legal guardian) has signed written informed consent form for participation in the trial.

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Conflict of interest

The authors declare no conflict of interest.

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