The link between metabolic features and TSH levels in polycystic ovary syndrome is modulated by the body weight: an euglycaemic–hyperinsulinaemic clamp study

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Abstract

Objective: To evaluate the link among thyroid function, glucose/insulin metabolism and steroid hormones in women with polycystic ovary syndrome (PCOS), and to verify if the body mass index (BMI) might influence the interplay between PCOS features and subclinical hypothyroidism (SCH).

Study design: Case–control study conducted from January to December 2014.

Methods: One-hundred fifty-four young women with PCOS, according to Rotterdam criteria, and 88 controls were enrolled in an academic research environment. Anthropometric evaluation, hormonal and lipid assays, oral glucose tolerance test (OGTT) and euglycaemic–hyperinsulinaemic clamp were performed. Hirsutism was assessed with the Ferriman–Gallwey (FG) score.

Main results: SCH was found in 14% of PCOS subjects and in 1% of controls (P < 0.01). In PCOS women, TSH levels were directly correlated with fasting glycaemia, but not with other hormonal and metabolic parameters. When PCOS patients were classified on the basis of BMI, TSH levels significantly correlated with insulin secretion, insulin resistance, DHEAS and cortisol levels in obese PCOS women. Inverse correlations were found between TSH and both oestradiol and SHBG in the same group. In nonobese PCOS patients, only waist-to-hip ratio values were correlated with TSH. The prevalence of SCH was not different between nonobese and obese PCOS groups (14 and 15% respectively). However, SCH was associated with higher levels of insulin, DHEAS, cortisol and FG score only in the obese subgroup.

Conclusions: Our data confirm that the prevalence of SCH is increased in PCOS women. The presence of SCH is associated with endocrine and metabolic imbalances of PCOS, and the excessive body weight seems to promote this interplay.

Introduction

Polycystic ovary syndrome (PCOS) is characterized by the heterogeneous combination of menstrual irregularities, chronic anovulation and hyperandrogenism (1). The syndrome is also characterized by metabolic abnormalities. PCOS subjects often show insulin resistance and insulin circulating levels higher than BMI
Hypothyroidism is considered by several authors as a state of insulin resistance. Actually, the thyroid hormones may act as insulin agonists in muscle and as antagonists in the liver, thus creating a fine balance that is necessary to normal glucose homeostasis. A deficit or excess of thyroid hormones may account for a break in this equilibrium. In particular, it was demonstrated that hypothyroidism can lead to a decrease in glucose production and utilization, so that hypothyroid patients can experience both hypoglycaemia and insulin resistance (3). A defect in the functioning of the thyroid hormones has also been associated with weight gain and increase in body fat mass, hyperlipidaemia, decrease in the level of SHBG, increase in the conversion of androstenedione to testosterone and its aromatization to oestradiol (4). In addition, hypothyroidism may affect gonadal function and fertility, leading to delayed puberty onset and anovulatory cycles (5).

Based on the relationship between hypothyroidism, insulin resistance and reproductive disorders, it is conceivable that a possible imbalance in thyroid function may initiate, maintain or worsen the PCOS features. This is the reason why the association between hypothyroidism and PCOS has become object of interest for many authors in the recent years.

The prevalence of autoimmune thyroiditis and subclinical hypothyroidism (SCH) was found to be higher in PCOS women than in the general population (6, 7). Nonetheless, a clear relationship between insulin resistance and thyroid dysfunction has not been well established in these patients. Previous studies reported a reduction in serum TSH levels after metformin treatment in PCOS subjects (8, 9). On the other hand, some authors demonstrated a reduction in circulating androgens levels in PCOS subjects treated with a thyroid hormonal replacement therapy (10). However, little is known on the link between thyroid function and the complex endocrine–metabolic setting of PCOS. The aim of this study was to investigate the possible role of thyroid hormones in modulating peripheral insulin sensitivity, assessed by the gold standard method, euglycaemic–hyperinsulinaemic clamp. In order to verify if BMI might influence the interplay between PCOS features and hypothyroidism, we performed a subgroup analysis in normal-weight and obese patients. The investigation was focused on TSH levels, which represent the most reliable and feasible marker of thyroid function.

**Subjects and methods**

**Subjects**

This is a case–control study conducted from January to December 2014 at the Department of Obstetrics and Gynecology of the Università Cattolica Sacro Cuore, Rome. The study was approved by the institutional review board.

We studied 154 consecutive Caucasian women affected by PCOS, aged between 18 and 36 years, attending our divisional outpatient services. In accordance with the Rotterdam Consensus Conference (1), PCOS was diagnosed in the presence of at least two of the following criteria: irregular menstrual cycles (or amenorrhoea), clinical and/or biochemical evidence of hyperandrogenism and ultrasound assessment of polycystic ovary (≥12 antral follicles in one ovary or ovarian volume ≥10 cm³).

Eighty-eight normo-ovulatory and normoandrogenic women with similar age (median value: 25 years) and BMI (median value: 26 kg/m²) were enrolled as a control group for thyroid hormones levels. These subjects had regular menstrual cycles of 27–31 days and did not exhibit clinical and/or biochemical evidence of hyperandrogenism. Biochemical hyperandrogenism was defined as serum testosterone ≥0.6 ng/mL and/or androstenedione >3 ng/mL during the early follicular phase. Increased serum 17-OHP was defined as serum 17-OHP ≥1.2 ng/mL. Clinical hyperandrogenism was defined as the presence of hirsutism, acne and/or alopecia. The following conditions were the exclusion criteria: pregnancy; other endocrine dysfunctions as hyperprolactinaemia, diabetes or adrenal enzyme defects; manifest hypothyroidism or hyperthyroidism; previous thyroid surgery or use of thyroid medications and hormonal or hypoglycaemic drugs intake.

The subgroup analysis was performed after dividing our PCOS subjects into two groups: normal-weight and overweight women (group A: BMI <27 kg/m², 88 patients) and obese patients (group B: BMI ≥27 kg/m², 66 patients).

**Study protocol**

The clinical and laboratory workup was conducted during the early follicular phase (days 3–7) of the menstrual cycle. In amenorrhoic patients, menstrual bleeding was induced by medroxyprogesterone acetate (MAP) administration (10 mg/day for 5 days). The following anthropometric characteristics were measured: weight, height and waist...
and hip circumference for the determination of waist-to-hip ratio (WHR). Cutoff point for high WHR was set at 0.80 (11). The body mass index (BMI) was calculated as the ratio of weight (kilograms) to height² (square metres). Obesity was defined as a BMI ≥ 27 kg/m², as previously published (12, 13). The grade of hirsutism was evaluated using the Ferriman–Gallwey (FG) score (14). Patients were asked not to epilate for at least 1 month before each visit. Women with an FG score > 8 were considered hirsute. After fasting overnight for 10–12h, blood samples were collected for the following hormonal assays: LH, FSH, thyroid-stimulating hormone (TSH), free thyroxine (FT₄), prolactin (PRL), oestradiol (E₂), androstenedione (A), testosterone (T), dehydroepiandrosteronesulphate (DHEAS), 17-hydroxypregesterone (17-OHP), cortisol, sex-hormone-binding globulin (SHBG) and anti-Mullerian hormone (AMH). Subclinical hypothyroidism was defined as serum TSH levels above the upper limit of the normal range in the presence of normal values of thyroid hormones. The cutoff value of TSH was 2.8 μIU/mL, with an ultrasensitive assay, as established by the Hormonal Laboratory Unit of our university in the young population of our geographical area. Lipid assay was performed to measure total cholesterol, high-density lipoprotein cholesterol (HDL), low-density lipoprotein cholesterol (LDL), very-low-density lipoprotein cholesterol (VLDL) and triglyceride levels. On the following day, patients underwent a 75 g oral glucose tolerance test (OGTT). The OGTT was performed as follows: at 09:00h after overnight fasting, an indwelling catheter was inserted into the antecubital vein of one arm. Blood samples were collected basally and after ingestion of 75 g glucose in 150 mL water at 5, 30, 60, 90, 120 and 180 min. Insulin and glucose were assayed in all samples. Insulin and glucose responses to the glucose load were expressed as the area under the curve (AUC). The AUC was calculated by the trapezoidal rule method and reported as μIU/mL/180 min for insulin and as mg/dL/180 min for glucose. A normal glycaemic response to OGTT was defined according to the criteria of the National Diabetes Data Group (15). A normal insulinemic response to OGTT was defined by a threshold AUC value of 10000 μIU/mL/180 min, as described previously (16). On the same day, a transvaginal pelvic ultrasound was performed on each patient. The euglycaemic–hyperinsulinaemic clamp test was performed the day after OGTT, after a 12-h overnight fast, as described elsewhere (17). At 08:00h, an intravenous catheter was placed in the antecubital vein for the infusion of glucose and insulin. Another catheter was placed in the dorsal vein of the contralateral hand for blood withdrawal and was warmed to 65°C with a warming box. A primed constant infusion of insulin was given (Actrapid HM, 40 mIU/m² per min; Novo Nordisk, Copenhagen, Denmark). After reaching the steady-state velocity for the insulin infusion within 10 min to achieve steady-state insulin levels of approximately 694.5 pmol/L during the clamp (range, 555.6–868.1 pmol/L), a variable infusion of 20% glucose was begun via separate infusion pump. The rate was adjusted on the basis of plasma glucose samples drawn every 5 min, to maintain plasma glucose between 4.4 and 4.9 mmol/L. Total-body glucose utilization M (metabolic index) was determined between 60 and 120 min of the glucose clamp and was expressed as mg/kg body weight/min. A normal insulin sensitivity was defined by a threshold M value of 4.5 mg/kg/min (18).

In the control group, the following parameters were measured: TSH, FT₄ and androgens levels, to rule out cases of endocrine disorders or PCOS.

**Measurements**

Plasma samples for hormone determination were maintained at −20°C until they were assayed. TSH serum concentrations were assayed by an ultrasensitive chemiluminescent microparticle immunoassay (CMIA) designed to have an analytical sensitivity of ≤0.0025 μIU/mL (ARCHITECT, Abbott Ireland Diagnostic Division, Lisnamuck, Longford, Ireland). The serum AMH concentration was assessed using a second-generation ultrasensitive immunoenzymometric assay (Beckman-Coulter, Marseilles, France). The limit of detection, defined as the lowest detectable amount of AMH, was calculated at 0.08 ng/mL. All the remnant hormonal assays were performed with ECLIA (electrochemiluminescence immunoassays) kits (Roche Diagnostics). Glucose was measured within 24 h of blood collection using a glucose oxidase method (Beckman Glucose Analyzer; Beckman Instruments, Fullerton, CA, USA). Samples contained in test tubes lacking heparin were immediately centrifuged in a refrigerated centrifuge, and the sera obtained were stored at −20°C until assayed. Insulin assay was performed by RIA. The intra-assay and inter-assay coefficients of variation were <6% for all hormones. The intra-assay and inter-assay coefficients of variation were <3% for TSH.

Total cholesterol and triglyceride concentrations were determined by an enzymatic assay (Bristol, Paris, France). HDL concentrations were determined after precipitation of chylomicrons, VLDL and LDL (Roche), and VLDL was
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separated (as the supernatant) from LDL and HDL by lipoprotein ultracentrifugations. A magnesium chloride/phosphotungstic acid technique was used to precipitate LDL from the bottom fraction after ultracentrifugation. All lipids assays were performed according to standardized laboratory procedures, as previously reported.

Statistical methods

All the results are presented as median and interquartile range. Statistical analysis was performed by GraphPad Prism5 software. The distribution of the data was tested by the Kolmogorov–Smirnov test in order to verify whether the samples follow a specified distribution. We found that all variables were not normally distributed. The data from the study groups were compared using Mann–Whitney U test. The correlations between TSH values and the independent variables were evaluated with the use of Spearman’s rank correlation coefficient. We performed a multiple linear regression analysis to evaluate the influence of obesity on metabolic parameters. A P value of <0.05 was considered significant.

Results

A condition of subclinical hypothyroidism was found in 14.28% of PCOS women vs 1.14 % of controls (P<0.01). Both TSH and FT₄ median values were significantly higher in PCOS patients than in controls. PCOS: 1.79 (IQR, 1.085) and 11.6 (IQR, 1.8) respectively and controls: 1.3 (IQR, 0.77) and 10.55 (IQR, 1.5) respectively. P<0.01 for both the comparisons.

Table 1 Clinical, hormonal and metabolic characteristics in PCOS women with BMI values < (group A) and ≥ (group B) 27 kg/m². Data are expressed as medians with their respective range and interquartile range (IQR). The number of patients in each subgroup is indicated in parentheses.

Table: 1st column Age, BMI, WHR, FG score, FSH, LH, E₂, A, T, DHEAS, FAI, FT₃, TSH, AMH, PRL, 17(OH)P, Cortisol, Total cholesterol, LDL cholesterol, HDL cholesterol, VLDL cholesterol, NEFA, Triglycerides, Fasting insulin, Fasting glucose, AUC insulin 180', AUC glucose 180', M clamp.

Significances: *P<0.01 for group B vs group A.

17 OHP, 17 hydroxyprogesterone; A, androstenedione; AMH, anti-Mullerian hormone; AUC, area under the curve; BMI, body mass index; FAI, free androgen index; FG, Ferriman–Gallwey; HDL, high-density lipoprotein; LDL, low-density lipoprotein; NEFA, nonesterified fatty acids; PRL, prolactin; SHBG, sex hormone-binding globulin; VLDL, very-low-density lipoprotein; WHR, waist-to-hip ratio.

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No differences were found in terms of clinical, hormonal and metabolic parameters in the comparison of PCOS women with SCH and euthyroid PCOS subjects. The exception was FG score, which was significantly higher in patients with TSH levels over the normal range \( (P < 0.05) \) (data not shown).

The Spearman’s analysis revealed a significant positive correlation between TSH and the basal glycemia \( (P < 0.05; R = 0.16) \), in the whole group of PCOS subjects. No further correlations were found in participants between TSH values and gonadotrophins, plasma androgens, AMH, SHBG and the parameters of insulin and lipid metabolism. The multiple linear regression analysis showed that the lipid profile (triglycerides, HDL, LDL and VLDL cholesterol levels), fasting glucose and insulin, the AUC-I after OGTT and the M value of clamp are influenced by the BMI value.

**BMI subgroup analysis**

Table 1 shows the clinical, hormonal and metabolic characteristics of the two subgroups of PCOS patients obtained after dividing the participants on the basis of the BMI (group A: normal weight/overweight and group B: obese). The obese group exhibited higher values of FAI and lower values of SHBG and AMH compared with patients with lower BMI \( (P < 0.01 \) for all the comparisons). No further statistically significant differences were observed in the hormonal assessment between the two groups of patients. Group B was characterized by significantly higher values of fasting insulin and glucose and increased insulin secretion during the OGTT with respect to group A \( (P < 0.01 \) for all the comparisons). The same group showed a blunted peripheral glucose utilization, as documented by the significantly lower values of M during the clamp than those of group A \( (P < 0.01) \). In the analysis of lipid profile, we found statistically significant differences in terms of triglycerides and total cholesterol, HDL, LDL and VLDL between the two groups \( (P < 0.01 \) for all the comparisons).

No differences were found in terms of FT4 and TSH between the two subgroups. Consistently, the rate of subclinical hypothyroidism was similar in groups A and B (13.63 and 15.10% respectively) and significantly higher compared with the corresponding control subgroups \( (0\% \) in normal weight–overweight controls and 2.86\% in obese controls, \( P < 0.01 \) for both the comparison; Fig. 1). Both PCOS subgroups showed higher values of TSH than corresponding control subgroups \( (P < 0.01) \).

TSH levels correlated with both stimulated insulin secretion \( (P < 0.01, R = 0.4) \) and peripheral insulin resistance in obese women \( (P < 0.05, R = -0.25) \), but not in the group of nonobese PCOS subjects. Furthermore, a statistically significant direct correlation was observed...
between the TSH values and both DHEAS ($P<0.01$, $R=0.33$) and cortisol ($P<0.05$, $R=0.26$). A statistically significant inverse correlation between TSH and oestrogens ($P<0.05$, $R=0.27$) and SHBG ($P<0.05$, $R=-0.25$) values was detected in the subgroup of patients with higher BMI (Fig. 2). By contrast, we failed to find any correlation between TSH values and hormonal and metabolic parameters in the subgroup of normal-weight patients. The TSH values directly correlated with waist-to-hip ratio in this group ($P<0.05$, $R=0.24$, data not shown).

Groups A and B were further divided on the basis of the presence of SCH (data not shown). We failed to find any significant difference between euthyroid and SCH women in the nonobese PCOS group. In contrast, in the PCOS group with high BMI, SCH was associated with higher FG score ($P<0.01$), DHEAS levels ($P<0.01$), cortisol levels ($P<0.05$) and AUC-insulin ($P<0.05$) in comparison with euthyroid women.

Discussion

The epidemiologic surveys conducted so far have reported a prevalence of SCH between 4 and 10% in the general adult population (19). This wide range might be due to differences in age, gender, dietary iodine intake and sensitivity of laboratory assays (20). In our university’s laboratory, the upper limit for TSH was set at 2.8 microIU/mL with an ultrasensitive assay. Under such conditions, SCH was diagnosed in 14.28% of PCOS patients in our study, a percentage that is considerably higher compared with the 1.14% prevalence in our control group. This finding confirms previous studies from literature, reporting an increased frequency of SCH diagnosis in women affected by PCOS (21, 22). A higher rate of SCH was found in the same proportion in both nonobese and obese PCOS subjects. It could be speculated that, beyond the body weight excess, the condition of PCOS may influence per se the thyroid function or vice versa.

The concept that subclinical thyroid disease may induce clinical consequences in the affected individuals is emerging over the past decades. Actually, this condition seems to be associated to a higher risk of developing atherosclerosis and coronary artery disease (19). Similarly, data from this study suggest that SCH might have some concrete consequences on the reproductive-endocrine system and the metabolic features also.

In the analysis of the whole group of patients, we found a direct significant correlation between TSH values and fasting glucose concentrations. This finding is not easily explained, as only few previous studies have specifically addressed the influence of subclinical hypothyroidism on glycaemic levels (3). Previous reports on thyroid function in PCOS women failed to find any correlation between TSH values and glycaemia (23). Nevertheless, some authors reported a predisposition to episodes of hypoglycaemia in diabetic patients with coexistent SCH (24). None of the participants in this study was affected by diabetes, and fasting glycaemic levels were in the normal range.

As expected, after dividing the patients on the basis of the BMI, the PCOS group with BMI ≥ 27 kg/m² showed significantly higher FAI and insulin levels, with lower indexes of insulin sensitivity, compared with the other group. These findings are in keeping with uncountable reports from literature (25, 26). Interestingly, in this group, we found several differences between women with normal TSH levels and women with SCH, and TSH levels correlated with several endocrine and metabolic parameters of the syndrome.

First, a statistically significant inverse correlation was observed between TSH and 17 β-oestradiol levels. Several lines of evidence strongly support a role of oestrogens in the thyroid physiology and pathology. By contrast, thyroid hormones are believed to take part in the endocrine and paracrine regulation of ovarian function. Free thyroid hormones are present in the follicular fluid and thyroid hormones receptors, mRNA and proteins are expressed in granulosa cells, cumulus cells (CC) and oocytes (27). Annelli Stavreus Evers and coworkers recently demonstrated that the TSH receptor expression in the ovary is upregulated by a gonadotrophin-driven cascade and downregulated by oestradiol (28). However, further studies are needed to cast a light on the interplay between SCH and ovulatory dysfunction in PCOS.

The inverse correlation between TSH and the hepatic production of SHBG confirms the results of the study by Dittrich and coworkers, which reported that women with PCOS and TSH > 2.5 mIU/L had significantly decreased SHBG concentrations in comparison with PCOS women with TSH < 2.5 mIU/L (29).

In our group of obese PCOS patients, we also found a direct correlation between TSH levels and both DHEAS and cortisol values. Consistently, these parameters were significantly higher in obese PCOS patients with SCH in comparison with their euthyroid counterpart. Several studies showed that hypothyroidism is associated with a mild, yet significant, adrenal insufficiency (30). However, the effect of thyroid hormones on the hypotalamo-
pituitary–adrenal (HPA) axis seems to be bimodal. Lizcano and coworkers recently evaluated the hormonal parameters in a group of 14 thyroidectomized women, due to thyroid cancer, before and after thyroid suppressive therapy with thyroxine (T₄). The authors concluded that both the conditions of hypothyroidism and iatrogenic subclinical hyperthyroidism are able to determine a hypersensitivity of ACTH to hCRH, with consequent increase of glucocorticoids secretion (31). These data match well with our finding of higher DHEAS and cortisol levels in obese PCOS patients with SCH in respect to the euthyroid ones and with the direct correlation between TSH levels and these hormones. It could be hypothesized that an upstream functional increase of ACTH secretion may mediate such effects.

In the same group of PCOS patients, we found an interesting link between the thyroid function and the glucose and insulin metabolism. In particular, this study analyzed for the first time the relationship between TSH and insulin resistance assessed by the euglycaemic–hyperinsulinaemic clamp. Our results confirm that subclinical hypothyroidism is associated with an insulin resistance status, as suggested by the inverse correlation between the TSH value and the M during clamp. Furthermore, the obtained TSH levels directly correlated with the AUC-I, thus suggesting that thyroid hormones may have a role in modulating glucose-stimulated insulin secretion in PCOS. This contention is also supported by the finding of significant higher AUC-insulin values in obese PCOS patients with SCH compared with euthyroid participants. These data are consistent with the results published by Celik and coworkers in 2012. They observed in their study that women with PCOS and subclinical hypothyroidism showed significantly higher values of fasting insulin and a blunted peripheral glucose utilization, as documented by the significantly higher values of HOMA-IR compared with control patients (32). However, it remains to be determined if the relative TSH elevation observed in PCOS patients may be a cause or a consequence of insulin resistance. This aspect appears of particular interest due to the central role of hyperinsulinaemia and insulin resistance in the complex pathophysiology of PCOS. At the central level, hyperinsulinaemia seems to be involved in the dysregulation of luteinizing hormone (LH) secretion. At the peripheral level, insulin promotes ovarian androgen secretion by playing a synergistic role with gonadotrophins both directly and by stimulating insulin-like growth factor I (IGF-I) secretion; in the liver, it decreases serum SHBG synthesis. Furthermore, hyperinsulinaemia seems able to potentiate in vivo adrenocorticotropic hormone–stimulated adrenal androgen production in women with PCOS (33, 34). On the other hand, several studies have reported insulin resistance in patients with SCH due to decreased intracellular glucose utilization and reduced glucose transporter (GLUT4) translocation, decreased glycogen synthesis and reduced glucose oxidation (35). Several previous reports documented a TSH normalization after administration of insulin sensitizers (36). On the other hand, recent studies demonstrated that the correction of hypothyroidism with l-thyroxin supplementation might be helpful in the management of PCOS. In this regard, it has been reported that in obese diabetic rodents, treatment with thyroid hormones enhances insulin sensitivity and reduces hyperglycaemia and hyperinsulinaemia (37). In addition, thyroid hormones also seem to cooperate with catecholamines and reduce visceral fat mass with a consequent improvement in insulin resistance status (38). Based on these data, it could be useful to screen PCOS patients for subclinical hypothyroidism, as increased TSH levels in obese PCOS patients could be an additional risk factor for type 2 diabetes mellitus and cardiovascular diseases.

In the group of normal-weight patients, we failed to find any statistical correlation between thyroid function and the metabolic, clinical or hormonal parameters under evaluation. It could be borne in mind that, only in overweight PCOS patients, the ‘subclinical’ hypothyroidism may lead to clinically evident effects. In this intriguing perspective, the excess in body weight may play a permissive role.

**Conclusions**

This study confirms a high prevalence of subclinical hypothyroidism in PCOS patients according to our laboratory's upper limit of TSH set at 2.8μIU/mL. The normal range of our laboratory is different from those reported in other studies in literature. This could partially hinder the comparison with previous reports, thus representing an intrinsic limitation of this study. Furthermore, our results do not allow us to draw definite conclusions regarding the possible aetiological role of thyroid axis in PCOS syndrome. However, this is the first study assessing the link between thyroid and metabolism in PCOS by the gold standard method, euglycaemic–hyperinsulinaemic clamp, thus strengthening the contention that TSH levels are associated with insulin resistance and hyperinsulinaemia in the obese subjects affected by the syndrome.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

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Author contribution statement
Valeria Tagliaferri and Daniela Romualdi designed the study and drafted of the article; Maurizio Guido revised the article; Antonio Mancini helped in the conception of the study; Simona De Cicco, Christian Di Florio, Valentina Immediata and Chantal Di Segni carried out data acquisition and analysis and Antonio Lanzone approved the final version to be submitted.

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