Circulating FGF21 levels are related to nutritional status and metabolic but not hormonal disturbances in polycystic ovary syndrome

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Abstract

Objective: The aim of this study was to analyse relationships between plasma fibroblast growth factor 21 (FGF21) levels and nutritional status, and metabolic and hormonal disturbances in polycystic ovary syndrome (PCOS) women.

Design and setting: A cross-sectional study involving 85 PCOS (48 obese) and 72 non-PCOS women (41 obese) was conducted to evaluate the relationship between FGF21 levels and PCOS.

Methods: Anthropometric parameters and body composition were determined. In the fasting state; serum concentrations of glucose, androgens, FSH, LH, SHBG, insulin and FGF21 were measured.

Results: Plasma FGF21 levels were significantly higher in obese women compared with normal-weight women in both PCOS and non-PCOS subgroups (120.3 (18.2–698) vs 62.3 (16.4–323.6) pg/ml, P < 0.05 and 87.2 (12.9–748.4) vs 62.9 (18.0–378.8) pg/ml, P < 0.05 respectively). Additionally, circulating FGF21 levels were significantly higher in the obese PCOS subgroup compared with the non-PCOS subgroup (120.3 (18.2–698.0) vs 87.2 (12.9–748.4) pg/ml, P < 0.05). Circulating FGF21 levels were proportional to BMI (R = 0.27; P < 0.001), body fat mass (R = 0.24; P < 0.01) and percentage (R = 0.24; P < 0.01), as well as waist circumference (R = 0.26; P < 0.01). Additionally, plasma insulin and homeostasis model assessment of insulin resistance (HOMA-IR) values were related to FGF21 levels (R = 0.44; P < 0.001 and R = 0.19; P < 0.05 respectively). In multiple regression analysis, circulating FGF21 level variability was explained by HOMA-IR values and fat percentage, as well as waist circumference, but not correlated with oestradiol levels and free androgen index values.

Conclusions: Higher circulating FGF21 levels are related to nutritional status and insulin resistance independent of PCOS. Increased FGF21 is associated with metabolic but not hormonal disturbances.

Introduction

Polycystic ovary syndrome (PCOS) is a common and heterogeneous hormonal and metabolic disorder. The prevalence is estimated to be 5–7% according to the NIH/NICHD diagnostic criteria (1) and 10–15% according to the ESHRE/ASRM (Rotterdam) criteria in European women of reproductive age (2). The pathogenesis of PCOS is only partially explained. Numerous studies demonstrated family occurrence of this syndrome, suggesting a genetic predisposition. In some PCOS cases, insulin resistance and hyperinsulinaemia play a role (3, 4). Insulin resistance is related to excessive visceral fat distribution (5, 6), as well as inflammation and hormonal disturbances.
related to adipose tissue (6, 7). A recently discovered adipokine that may play a role in insulin resistance development is fibroblast growth factor 21 (FGF21) (8). FGF21 is expressed predominantly in the liver, pancreas and white adipose tissue (5, 6), and experimental studies show insulin-sensitising and lipolysis suppression properties (7, 9), mediated by adiponectin (10), and impaired by tumour necrosis factor alpha (TNFα) (11).

Increased circulating FGF21 levels in obese adult humans (12, 13), proportional to BMI values (12), were shown. In addition, plasma FGF21 levels were independently related to visceral obesity (14). Elevated plasma FGF21 levels were also found in obese adolescents, especially with high hepatic fat content (15). Increased FGF21 levels in obese children were associated with BMI values, but not with insulin resistance and non-alcoholic fatty liver disease (NAFLD) (16). Conversely, it has been suggested that obesity is associated with FGF21 resistance (17).

In addition, the results of studies that assessed the effect of weight reduction on FGF21 levels are inconsistent. Results including decrease (18), no change (19) and increase (20) were all described. However, it should be noted that an increase in FGF21 levels was observed in patients when combined with very-low-calorie diet (VLCD) and fenofibrate therapy (20). The results of study in monozygotic and dizygotic young adult twins revealed that genetic factors moderately contributed to circulating FGF21 variability (21). Furthermore, high FGF21 levels were associated with higher triglycerides, glucose and insulin levels, and homeostasis model assessment of insulin resistant (HOMA-IR) values, and lower HDL cholesterol concentrations (21). Moreover, high FGF21 levels were related to higher liver fat deposit, but not subcutaneous or intra-abdominal fat content (21). In a population-based, prospective study, it has been found that plasma FGF21 levels increased progressively from normal glucose tolerance, through pre-diabetes, to diabetes and is a strong independent predictor of the development of diabetes (22). The clinical studies do not permit clarification whether FGF21 predisposes to the development of carbohydrate disturbances or is a mechanism of ineffective compensation. Some authors suggest the use of anti-FGFR1 antibodies (R1Mabs) that mimic the metabolic effects of FGF21 on obesity and type 2 diabetes treatment (23, 24, 25, 26, 27).

Excessive visceral fat depot and its hormonal disturbances seem to play a role in hormonal and metabolic disturbances in PCOS (28, 29, 30, 31, 32). As described earlier, the novel adipokine FGF21 may play a role in the PCOS-related metabolic disturbances. Thus, in addition to obesity and type 2 diabetes, PCOS seems to be a therapeutic target for anti-FGFR1 antibodies.

However, only two studies thus far have assessed the FGF21 levels in PCOS women and their results are contradictory. Gorar et al. (33) revealed higher serum FGF21 levels in PCOS than in non-PCOS women and showed, in multivariate discriminant analysis, that BMI, triglyceride, HOMA-IR and fasting glucose, with rise in FGF21, were found significant in PCOS. Additionally, a positive correlation between FGF21 and luteinising hormone (LH), as well as FGF21 and testosterone was found. Sahin et al. (34) failed to find any differences in serum FGF21 levels between PCOS and BMI-matched non-PCOS women or any association between FGF21 levels and BMI, waist circumference and HOMA-IR values.

Therefore, the aim of this study was to analyse relationships between plasma FGF21 levels and nutritional status as well as metabolic and hormonal disturbances in PCOS women.

Materials and methods

We conducted a cross-sectional, prospective study involving 87 hospitalised women with PCOS, (39 normal-weight and 48 obese) with self-reported stable body mass during the last 3-month period, referred from outpatient clinic after initial examination to the Department of Endocrinological Gynaecology from 2010 to 2011. The short-term (3 days), routine in Poland, comprehensive, diagnostic hospitalisation reimbursed by the National Health Service was not part of the study. The day of admission was set after contact of the patients at the first day of menstruation to schedule the admission for the hormonal diagnostics on the third to fourth day of the menstrual cycle.

The diagnosis of PCOS was based on the Rotterdam ESHRE/ASRM criteria from 2003 (35). Each woman with PCOS studied possessed all three of the Rotterdam criteria in order to decrease the heterogeneity of the study group. Seventy-two regularly menstruating women without PCOS (41 obese and 31 normal-weight) were recruited in the Metabolic Management Center due to obesity and in the Endocrinological Gynaecology Outpatient Clinic referred for contraceptive advice, for the control group. Patients suffering from Cushing’s syndrome, thyroid dysfunctions, androgen-secreting tumour, enzyme deficiency (21-hydroxylase in particular), decreased ovary reserves, amenorrhea and type 1 or type 2 diabetes
were excluded. Any pharmacological therapy, as well as smoking and alcohol abuse, were also among the exclusion criteria. The study was conducted after obtaining informed consent from each participant. The study protocol was approved by the Bioethical Committee of Medical University of Silesia.

Normal weight was defined as a BMI from 18.5 to 24.9 kg/m² and obesity as ≥30.0 kg/m². The characteristics of the study groups are presented in Table 1.

Each study subject was examined within 3 and 5 days of her menstrual cycle. Anthropometric measurements (body mass and height) were performed and BMI was calculated according to the standard formula. Body composition was assessed by the bioimpedance method using Bodystat 1500 (Douglas, Isle of Man). The bioimpedance method measured total body fat content based on the differences in conduction of electrical current through adipose and other tissues. The electrodes are placed on the patient’s hand and foot and pin on to the electrode measuring device. Venous blood samples of 15 ml were drawn in the mornings between 0800 and 0900 h, after an overnight fast (16 h). The blood samples were collected according to the manufacturer’s recommendations. Serum and plasma samples were frozen and stored at –70 °C until use.

### Laboratory procedures

Plasma glucose and lipids were estimated by colorimetric methods using commercially available test kits (Roche). Serum insulin concentration was determined by ELISA (DRG Instruments GmbH, Marburg, Germany) with a lower limit of sensitivity of 1.76 μIU/ml and intra- and interassay coefficient of variation (CV) values of 2.2 and 4.4% respectively. HOMA-IR index was calculated using the standard formula: HOMA-IR = fasting concentration of insulin (μIU/ml) × fasting concentration of glucose (mmol/l)/22.5.

Serum follicle-stimulating hormone (FSH), LH, oestradiol (E₂), total testosterone, androstenedione, DHEA-S and SHBG were determined by ELISA (DRG Instruments GmbH) with a lower limit of sensitivity 0.86 mIU/ml, 1.27 mIU/ml, 9.7 pg/ml, 0.083 ng/ml, 0.019 ng/ml, 0.044 μg/ml and 0.2 nmol/l respectively; the respective intra- and interassay CV values were 5.5 and 6.1% for FSH, 5.6 and 6.2% for LH, 4.7 and 7.8% for E₂, 3.6 and 7.1% for testosterone, 6.5 and 10.2% for androstenedione, 4.8 and 7.5% for DHEA-S and 5.3 and 9.0% for SHBG. The free androgen index (FAI) was calculated according to the standard formula (FAI = (total testosterone/SHBG) × 100%). The ELISA was also used for measurements of plasma FGF21 levels (BioVendor, Brno, The Czech Republic) with the lower limit of sensitivity of 7 pg/ml and intra- and interassay CV values were 3.5 and 3.7% respectively.

### Statistical analysis

Statistical analysis was performed by STATISTICA 9.0 PL (StatSoft, Cracow, Poland) Software and R Software environment. There were no missing data in the database. The results are expressed as mean values ± s.d. Distribution of variables was evaluated by the D’Agostino–Pearson test. Homogeneity of variances was assessed by the Levene test.

### Table 1 Patient characteristics.

<table>
<thead>
<tr>
<th></th>
<th>PCOS (n=85)</th>
<th></th>
<th>Non-PCOS (n=85)</th>
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<tbody>
<tr>
<td></td>
<td>All (n=85)</td>
<td>Normal weight (n=37)</td>
<td>Obese (n=48)</td>
<td>Normal weight (n=31)</td>
</tr>
<tr>
<td>Age (years)</td>
<td></td>
<td>25.4 ± 5.5</td>
<td>23.6 ± 4.6 a</td>
<td>26.8 ± 5.8</td>
</tr>
<tr>
<td>Body mass (kg)</td>
<td></td>
<td>80.7 ± 25.2</td>
<td>57.5 ± 7.1 b</td>
<td>97.7 ± 20.2 d</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td></td>
<td>28.6 (19.0–40.2)</td>
<td>20.5 (19.6–22.7)</td>
<td>35.1 (33.1–40.2)</td>
</tr>
<tr>
<td>Body fat (kg)</td>
<td></td>
<td>30.2 (15.4–56.3)</td>
<td>15.0 (12.6–19.7)</td>
<td>40.6 (33.4–56.3)</td>
</tr>
<tr>
<td>Body fat (%)</td>
<td></td>
<td>38.1 (24.2–51.1)</td>
<td>26.5 (24.2–31.0)</td>
<td>44.9 (41.9–51.1)</td>
</tr>
<tr>
<td>Waist circumference (cm)</td>
<td></td>
<td>89.8 ± 18.7</td>
<td>72.6 ± 6.9 b</td>
<td>103.7 ± 12.3 d</td>
</tr>
<tr>
<td>Total cholesterol (mg/dl)</td>
<td></td>
<td>176.3 ± 34.0</td>
<td>167.7 ± 28.1 a</td>
<td>183.2 ± 37.0</td>
</tr>
<tr>
<td>LDL cholesterol (mg/dl)</td>
<td></td>
<td>105.4 ± 34.9</td>
<td>93.8 ± 27.5 a</td>
<td>115.1 ± 37.6</td>
</tr>
<tr>
<td>HDL cholesterol (mg/dl)</td>
<td></td>
<td>45.7 ± 14.0 a</td>
<td>48.8 ± 12.4 a</td>
<td>43.8 ± 12.9 a</td>
</tr>
<tr>
<td>Triglycerides (mg/dl)</td>
<td></td>
<td>100.7 ± 55.2</td>
<td>77.2 ± 33.2 a</td>
<td>121.5 ± 61.4 a</td>
</tr>
<tr>
<td>Glucose (mmol/l)</td>
<td></td>
<td>5.1 ± 0.8</td>
<td>4.9 ± 0.4 a</td>
<td>5.3 ± 0.9 a</td>
</tr>
<tr>
<td>Insulin (μIU/ml)</td>
<td></td>
<td>10.6 (3.5–23.9)</td>
<td>8.4 (3.5–23.9)</td>
<td>12.9 (7.9–18.6)</td>
</tr>
<tr>
<td>HOMA-IR</td>
<td></td>
<td>2.3 (0.8–5.5)</td>
<td>1.8 (0.8–5.5)</td>
<td>2.8 (1.2–4.1)</td>
</tr>
</tbody>
</table>

*P < 0.05; †P < 0.01 and ‡P < 0.001 – normal-weight PCOS subjects vs obese non-PCOS subjects. §P < 0.001 and †P < 0.001 – normal-weight non-PCOS subjects vs obese non-PCOS subjects. ¶P < 0.05; †P < 0.01 and ‡P < 0.001 – obese PCOS subjects vs normal-weight PCOS subjects. *P < 0.01 and †P < 0.001 – obese PCOS subjects vs normal-weight non-PCOS subjects. §P < 0.01 – obese PCOS subjects vs obese non-PCOS subjects. ¶P < 0.001 and †P < 0.01 – PCOS subjects vs non-PCOS subjects. §P < 0.05 normal-weight non-PCOS vs obese non-PCOS.
Table 2 Serum concentrations of hormones and FGF21 in analysed groups of PCOS and non-PCOS.

<table>
<thead>
<tr>
<th>Hormone</th>
<th>PCOS</th>
<th>Non-PCOS</th>
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<tbody>
<tr>
<td></td>
<td>All (n = 85)</td>
<td>Normal weight (n = 37)</td>
</tr>
<tr>
<td>FSH (mIU/ml)</td>
<td>5.7 (4.4–7.4)</td>
<td>5.6 (2.6–8.8)</td>
</tr>
<tr>
<td>LH (mIU/ml)</td>
<td>10.0 (2.9–27.6)</td>
<td>8.7 (3.1–28.4)</td>
</tr>
<tr>
<td>LHFSH</td>
<td>1.7 (1.1–3.7)</td>
<td>1.6 (1.0–6.4)</td>
</tr>
<tr>
<td>Androstenedione (ng/ml)</td>
<td>2.2 (0.5–5.2)</td>
<td>2.8 (0.5–5.2)</td>
</tr>
<tr>
<td>DHEA-S (µg/ml)</td>
<td>2.8 (1.4–5.1)</td>
<td>2.8 (1.4–5.1)</td>
</tr>
<tr>
<td>FAI</td>
<td>3.1 (0.2–3.1)</td>
<td>3.0 (0.2–3.1)</td>
</tr>
<tr>
<td>Oestradiol (pg/ml)</td>
<td>41.9 (10.0–229.9)</td>
<td>43.6 (12.0–229.9)</td>
</tr>
<tr>
<td>SHBG (nmol/l)</td>
<td>23.0 (5.7–79.7)</td>
<td>30.1 (7.5–78.7)</td>
</tr>
<tr>
<td>FGF21 (pg/ml)</td>
<td>157.7 (16.4–698)</td>
<td>62.3 (16.4–323.6)</td>
</tr>
</tbody>
</table>

Quantitative variables were compared by two-way multivariate ANOVAs using the Duncan post-hoc test. The assessment of associations between variables was carried out using the multivariate linear regression and the backward stepwise procedure. Outliers were identified based on Cook’s distance values. The Cook–Weisberg test was used to test the residuals for heteroskedasticity. Model calculation was performed, including evaluation of multicollinearity, which was assessed using the variance inflation factor (VIF). The VIF should not exceed 5. Goodness of fit of obtained model was assessed using the F test and determination coefficient $R^2$. All the results were considered as statistically significant with a $P$ value of $<0.05$.

Results

The characteristics of PCOS and non-PCOS subgroups, including metabolic parameters and hormonal profiles, are presented in Tables 1 and 2.

Plasma FGF21 levels were significantly higher in obese women compared with normal-weight women in both PCOS and non-PCOS groups. Additionally, circulating FGF21 levels were significantly higher in obese PCOS subgroup compared with the non-PCOS subgroup, but not in normal-weight PCOS subgroup compared with non-PCOS subgroup (Table 2).

Correlation between anthropometric parameters and FGF21

Circulating FGF21 levels were proportional to BMI, body fat mass and percentage, as well as waist circumference ($R=0.27; P<0.001; R=0.24; P<0.01; R=0.24; P<0.01$ and $R=0.26; P<0.01$ respectively).

Correlation between study hormones, glucose, insulin and HOMA-IR, and FGF21

The negative correlation between plasma FGF21 and serum E2 concentrations ($R=−0.18; P<0.05$) was found. There were no correlation between serum glucose concentration and plasma FGF21 levels. Serum insulin concentrations and HOMA-IR values were proportional to circulating FGF21 levels ($R=0.44; P<0.001$ and $R=0.19; P<0.05$ respectively).

Correlation between lipids and FGF21

There were no associations among total cholesterol, LDL cholesterol or HDL cholesterol and FGF21 levels. Only serum triglycerides concentrations were proportional to FGF21 levels ($R=0.29; P<0.001$).

Multiple regression analyses

In the multiple regression models, serum E2 concentration, FAF and HOMA-IR values and anthropometric parameters (BMI, waist circumference, body fat mass and percentage, alternatively), were included as independent explanatory variables for plasma FGF21 levels in all study groups. Circulating FGF21 level variability was explained by HOMA-IR values ($β=0.17; P=0.04$ in model 1 and $β=0.14; P=0.12$ in model 2), fat percentage ($β=0.15; P=0.04$ in model 1), as well as waist circumference ($β=0.19; P=0.03$ in model 2), but not by E2 levels and FAF values (Table 3).
levels are related to hepatic fat content and suggested that FGF21 is a marker of hepatic insulin resistance (15). However, we did not observe any associations between SHBG and FGF21 levels. In addition, contrary to the data published previously, we have shown that high FGF21 level was related to higher liver fat but not subcutaneous and intra-abdominal fat content (21). We found an association between FGF21 and waist circumference in multivariate regression analysis. Furthermore, our results indicate that adipose tissue is the main source of circulating FGF21 in obese women. This hypothesis is confirmed by the observation of an increase in FGF21 expression in visceral fat in obese subjects (20). It should be emphasised that the stronger association between FGF21 and HOMA-IR values than fat mass and waist circumference implies that higher FGF21 production and secretion may be an inefficient contraregulatory mechanism preventing insulin resistance development in obese subjects. This hypothesis is supported by the experimental studies that demonstrated that lipolysis suppression FGF21 properties are mediated by adiponectin (10) and impaired by TNFα (11).

In contrast to other studies, we did not observe any association between fasting serum glucose concentrations (21, 22, 38) or HDL cholesterol (21) and FGF21 levels. However, in accordance with the results published previously (21), our study demonstrated that insulin and triglycerides levels are proportional to circulating FGF21 concentrations. Interestingly, it has been shown that FGF21 levels increased during long-term treatment with fenofibrate in diabetic subjects (39). Additionally, FGF21 levels were elevated by twofold in hypertriglyceridaemic non-diabetic patients and increased by 28% during treatment with fenofibrate (40). It has been suggested that the effect is due to stimulation of FGF21 mRNA expression by PPARα receptors (41). Thus, we hypothesised that, in obesity, expression of FGF21 increased in adipose tissue, but decreased in the liver, and it may be a cause of lipid metabolism disturbances. However, this hypothesis required further studies.

Gorar et al. (33) showed positive correlations between FGF21 levels and serum LH and testosterone concentrations, while Sahin et al. (34) observed a negative correlation between FGF21 and DHEA-S levels. We did not observe any associations between FGF21 and gonadotropins as well as androgens, while a weak, negative correlation between FGF21 and E2 levels was found. The explanation of our finding is difficult. No study assessing the impact of sex hormones on FGF21 expression and secretion has been carried out. However, Persson et al. (42) reported a non-significant decrease in circulating FGF21

### Table 3 Multivariate regressions model examining the factors explaining FGF21 level variability in the combined group of PCOS and non-PCOS women.

<table>
<thead>
<tr>
<th></th>
<th>Model 1</th>
<th>Model 2</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>β coefficient</td>
<td>P</td>
</tr>
<tr>
<td>Oestradiol</td>
<td>−0.07</td>
<td>0.40</td>
</tr>
<tr>
<td>HOMA-IR</td>
<td>0.17</td>
<td>0.04</td>
</tr>
<tr>
<td>FAI</td>
<td>−0.03</td>
<td>0.70</td>
</tr>
<tr>
<td>Fat percentage</td>
<td>0.15</td>
<td>0.04</td>
</tr>
<tr>
<td>Waist circumference</td>
<td>–</td>
<td>–</td>
</tr>
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</table>

### Discussion

The results presented in this study demonstrate that circulating FGF21 levels are related to obesity independent of PCOS. To the best of our knowledge, there are only two studies that analysed the association between PCOS and circulating FGF21 levels. Most importantly, our study is the first performed in the European population. Contrary to our data, Sahin et al. (34) reported similar FGF21 levels based on age- and BMI-matched PCOS and non-PCOS women, and failed to find any association between FGF21 levels and markers of nutritional status (BMI and waist circumference) and HOMA-IR values. Gorar et al. (33) revealed higher FGF21 levels in PCOS with heterogeneous nutritional status compared with normal-weight, non-PCOS women, and frequently reported positive associations among FGF21 levels and BMI, as well as HOMA-IR values by multivariate discriminant analysis in clinical studies. It could not be excluded that demonstrated increased levels of FGF21 in the PCOS group in the Gorar et al. study is not a consequence of inclusion of only normal-weight controls. This hypothesis partially is confirmed by the results of the study conducted by Wang et al. (36), which demonstrated that higher FGF21 levels are related to insulin resistance in pregnant women, including a subgroup with a history of PCOS. Additionally, in the study by Wang et al. (36), BMI before pregnancy was also a factor raising FGF21 levels. In accordance with the last two studies mentioned, higher FGF21 levels in obese PCOS subgroups compared with non-PCOS subgroups in our study may be explained by a greater severity of insulin resistance and compensatory hyperinsulaemia in the first subgroup. This finding is also corroborated by the correlation analysis and multiple regression analysis in our study, and progressive increase in FGF21 levels from normal glucose tolerance, through pre-diabetes, to diabetes (22).

The SHBG level is a biomarker of hepatic insulin resistance related to liver fat accumulation (37). In this study, the lowest SHBG levels were observed in the obese PCOS group. It has been demonstrated that elevated FGF21
levels during oestrogen therapy in women treated before in vitro fertilisation.

Our results indicated that FGF21 may participate in metabolic but not hormonal disturbances, not only in PCOS women but also in non-PCOS obese women.

The limitation of our study is the size of study subgroups and the lack of separation of PCOS with normal weight for subgroups, with and without metabolic obesity, and the small subset of PCOS with significant hyperandrogenemia. Furthermore, the distribution of body fat and its visceral deposits were not directly assessed using DEXA or a CT scanner. Moreover, in this study, the interrelation between FGF21 and other adipokines was not analysed. We would like to acknowledge that our study group included the most severe phenotype of PCOS (phenotype A), which made our cohort more homogeneous. The strength of our study is the separation and comparison of obese and normal-weight subgroups.

In conclusion, the higher circulating FGF21 levels are related to nutritional status and insulin resistance independent of PCOS. Increased FGF21 level is associated with metabolic but not hormonal disturbances.

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
M Olszanecka-Glinianowicz conceived the study, and participated in its design and coordination, and writing of the manuscript. M Wikarek and A Brzozowska for their assistance and technical support. They also thank M Smertka, MD, PhD for language corrections.

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