A Prospective Study of the Prevalence of Nonclassical Congenital Adrenal Hyperplasia among Women Presenting with Hyperandrogenic Symptoms and Signs

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Context: The diagnosis of the polycystic ovary syndrome requires the exclusion of nonclassical congenital adrenal hyperplasia (NCAH).

Objective: Our objective was to evaluate the actual prevalences of 21-hydroxylase and 11β-hydroxylase deficiencies among women presenting with hyperandrogenic complaints.

Settings: This study was performed at an academic hospital.

Patients: A total of 270 consecutive unselected women presenting with hyperandrogenic symptoms were prospectively recruited.

Interventions: Basal and ACTH-stimulated 11-deoxycortisol and 17-hydroxyprogesterone concentrations were measured.

Main Outcome Measures: The prevalences of 21-hydroxylase and 11β-hydroxylase deficiencies were calculated, and the diagnostic performance of basal serum 17-hydroxyprogesterone levels for the screening of NCAH was evaluated by receiver operating characteristic curve analysis.

Results: Six of the 270 patients had 21-hydroxylase-deficient NCAH that was confirmed by CYP21 genotyping, whereas no patient was diagnosed with 11β-hydroxylase deficiency, for an overall NCAH prevalence of 2.2% (95% confidence limits 0.5–3.9%). According to receiver operating characteristic analysis, a single basal serum 17-hydroxyprogesterone determination has a 0.97 (95% confidence interval: 0.934–1.008) chance of detecting NCAH in hyperandrogenic women. In our experience, the most appropriate cutoff value for the detection of NCAH is a 17-hydroxyprogesterone above 1.7 ng/ml, showing a 100% sensitivity and a 88.6% specificity. Five of the six 21-hydroxylase-deficient NCAH patients carried a severe *CYP21* allele requiring genetic counseling and highlighting the importance of excluding this disorder among hyperandrogenic patients.

Conclusions: The prevalence of NCAH among hyperandrogenic patients from Spain is 2.2%. Basal serum 17-hydroxyprogesterone measurements have an excellent diagnostic performance, yet the cutoff value should be established in each laboratory to avoid false-negative results. **(*J Clin Endocrinol Metab* 93: 527–533, 2008)**

The polycystic ovary syndrome (PCOS) is possibly the most common endocrine disorder in premenopausal women (1), with prevalences in the 6–7% range reported worldwide (2, 3). PCOS is first a hyperandrogenic disorder that is characterized by a constellation of signs and symptoms, including clinical and/or biochemical hyperandrogenism, ovulatory dysfunction, and polycystic ovarian morphology (4).

Although the criteria for the diagnosis of PCOS have been a

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Abbreviations: AURC, Area under the receiver operating characteristic curve; NCAH, nonclassical congenital adrenal hyperplasia; PCOS, polycystic ovary syndrome; ROC, receiver operating characteristic.

matter of intense debate for decades, all the current definitions [the 1990 National Institute of Child Health and Human Development criteria (5), the 2003 European Society of Human Reproduction and Embryology/American Society of Reproductive Medicine definition (6), and the 2006 Androgen Excess Society evidence-based definition (7)] require the exclusion of specific disorders whose clinical presentation may mimic that of PCOS, including androgen secreting tumors, hyperprolactinemia, Cushing's syndrome, and nonclassical congenital adrenal hyperplasia (NCAH) (5–7).

Congenital adrenal hyperplasia is an autosomal recessive disorder caused by mutations in the genes encoding the enzymes involved in the adrenal synthesis of cortisol (8). The resulting decrease in cortisol production causes an increase in the secretion of ACTH, thereby stimulating the production of adrenal steroids up to and including the substrate for the defective enzyme. The excessive synthesis of precursor steroids due to the increase in ACTH secretion leads to androgen excess in 21-hydroxylase (encoded by the *CYP21* gene) or 11β-hydroxylase (encoded by the *CYP11B1* gene) deficiencies (8). The latter may also cause deoxycorticosterone excess and hypertension.

The 21-hydroxylase and 11β-hydroxylase deficiencies occur because of homozygous or double-heterozygous mutations affecting both alleles of *CYP21* or *CYP11B1*, respectively (8). The clinical presentation is variable and depends on the magnitude of the enzymatic dysfunction caused by the mutations. When these mutations cause a complete or almost complete deficiency of the enzymes, the classical presentation is that of virilization in female newborns, which may also present in both sexes with salt wasting and hyperkalemia because of deficient mineralocorticoid action in 21-hydroxylase deficiency, or with mineralocorticoid excess and hypertension in 11β-hydroxylase deficiency (8).

When one of the *CYP21* or *CYP11B1* alleles carries a less severe mutation, some enzyme activity is retained, and the clinical symptoms appear later in life, and frequently around puberty, causing NCAH (8). Of note, hypertension is rare in nonclassical 11β-hydroxylase deficiency (9).

The clinical presentation of NCAH in females is often indistinguishable from other hyperandrogenic disorders such as premature pubarche and PCOS, explaining the requirement mentioned previously of excluding these specific genetic etiologies in hyperandrogenic women. However, there are scarce data at present regarding the actual prevalence of NCAH among these patients.

In the present study, we have systematically evaluated the prevalences of 21-hydroxylase and 11β-hydroxylase deficiencies in a relatively large series of consecutive women presenting with hyperandrogenic complaints, including hirsutism, acne, androgenic alopecia, and menstrual dysfunction.

Patients and Methods

Patients and evaluations

From April 1998 to February 2007, a total of 270 consecutive women referred to the clinical practice of one of the authors (H.F.E.-M.) for evaluation of hyperandrogenic symptoms and signs, including hirsutism, acne, androgenic alopecia, and menstrual or ovulatory dysfunction, were recruited for the present and other studies. Women previously diagnosed with NCAH were excluded to avoid a positive selection bias.

All the patients underwent a diagnostic protocol that included anthropometric and clinical evaluations, basal serum androgen levels, a metabolic evaluation consisting in fasting glucose and insulin levels, and an ACTH-stimulation test.

Hirsutism was defined by a modified Ferriman-Gallwey score above 7 (10). Menstrual and ovulatory dysfunction were defined by the presence of oligomenorrhea, cycles longer than 35 d, or amenorrhea, absence of menstrual bleeding for at least three usual cycle lengths (11), or, in women presenting with regular menstrual cycles, by lack the of ovulation according to body temperature charts and/or by serum progesterone levels less than 4 ng/ml during the luteal phase of the menstrual cycle.

Samples were obtained between d 5 and 10 of the menstrual cycle, or during amenorrhea after excluding pregnancy. After 12-h overnight fasting, samples were obtained for the measurement of fasting glucose and insulin, prolactin, total testosterone, cortisol, 11-deoxycortisol, 17-hydroxyprogesterone, androstenedione, dehydroepiandrosterone-sulfate, and SHBG. A 250-μg iv bolus of 1–24 ACTH (Synacthen; Ciba-Geigy, Basle, Switzerland) was injected, and blood samples were obtained 60 min later for the measurement of cortisol, 11-deoxycortisol, 17-hydroxyprogesterone, and androstenedione. Samples were immediately centrifuged, and serum was separated and frozen at −20 C until assayed.

Informed consent was obtained from all the participants or from the legal representatives of minors, and the ethics committee of Hospital Universitario Ramón y Cajal approved the study.

Assays

Direct RIA was used to measure 11-deoxycortisol and 17-hydroxyprogesterone in duplicate (ImmuChem; MP Biomedicals, Costa Mesa, CA). The mean intraassay and interassay coefficients of variation were less than 10% for all these assays, with the exception of the interassay coefficients of variation of the 11-deoxycortisol and 17-hydroxyprogesterone assays, which were 12.7 and 11.8%, respectively. The 11-deoxycortisol assay had a 2.5% cross reaction with 17-hydroxyprogesterone and 1.8% cross reaction with 17-hydroxypregnenolone. The assays used for other measurements have been reported earlier (2, 12). The free testosterone concentration was calculated from total testosterone and SHBG (13). Insulin resistance in the fasting state was measured by homeostasis model assessment (14).

Criteria for disease

The diagnostic categories of the women included in the present study were derived from the application of the 1990 National Institute of Child Health and Human Development conference criteria (5) because this study started long before the 2003 European Society of Human Reproduction and Embryology/American Society of Reproductive Medicine definition (6) and the 2006 Androgen Excess Society evidence-based definition (7) were published, and ovarian ultrasonography was not conducted routinely.

Women were diagnosed with PCOS when clinical and/or biochemical hyperandrogenism (total testosterone levels above 60 ng/dl, free testosterone levels above 1 ng/dl, androstenedione concentrations above 4.5 ng/ml, and/or dehydroepiandrosterone-sulfate levels above 3500 ng/ml) was present together with menstrual and/or ovulatory dysfunction (5). Those women presenting with clinical and biochemical hyperandrogenism but without menstrual and ovulatory dysfunction were diagnosed with idiopathic hyperandrogenism (15), and those presenting with hirsutism but without biochemical hyperandrogenism and without menstrual and ovulatory dysfunction were diagnosed as having idiopathic hirsutism (15, 16). Women presenting with normogonadotropic oligo- or amenorrhea but no evidence of clinical and biochemical hyperandrogenism were diagnosed with World Health Organization's class II anovulation (17). For all these diagnoses, specific etiologies were excluded. Cushing's syndrome, androgen secreting tumors, and drug-induced hirsutism were excluded by the absence of a typical clinical history, and hyperprolactinemia and congenital adrenal hyperplasia were excluded by basal serum prolactin levels less than 24 ng/ml, ACTH-stimulated 17-hydroxyprogesterone levels less than 10 ng/ml (18), and ACTH-stimulated 11-deoxycortisol levels less than 21 ng/ml [3-fold the 95th per-

centile (9) of a historical control group of 60 healthy women controls]. In women presenting with increased prolactin levels, macroprolactinemia was excluded as previously reported (19).

In those women presenting with ACTH-stimulated 17-hydroxyprogesterone levels more than or equal to 10 ng/ml, NCAH was confirmed by molecular genetic analysis of *CYP21* as described earlier (12, 20, 21). None of the 270 women presented with increased ACTH-stimulated 11-deoxycortisol levels, and, therefore, no molecular genetic analysis of mutations in *CYP11B1* was needed.

Statistical analysis

Continuous variables are expressed as means ± SD unless otherwise stated. The central tendencies of the groups of women were compared by the Kruskal-Wallis test, followed by Dunn's *post hoc* test.

For receiver operating characteristic (ROC) analysis of the performance of basal serum 17-hydroxyprogesterone levels, patients presenting NCAH confirmed at the molecular genetic level were considered affected, and the remaining women were considered nonaffected.

ROC curves are constructed by plotting the sensitivity (true-positive fraction) on the ordinate as a function of the complement of specificity (false-positive fraction), for all the possible cutoff values of the diagnostic test (22). Therefore, the more deviated toward the left-upper corner the curve is, the higher the sensitivity and the specificity the diagnostic test has for all possible cutoff values or, namely, the higher the adequacy of the diagnostic test for disease detection (23).

As opposed to accuracy, sensitivity and specificity are not dependent on the prevalence of the disease in the sample. Thus, ROC curve analysis provides a description of disease detectability, which is independent from both disease prevalence and decision threshold effects (23).

The area under the ROC curve (AURC) represents the probability of correctly distinguishing between affected and nonaffected subjects (24). Therefore, the perfect diagnostic test, not having false-positive or false-negative results, would have an AURC of one, and, on the contrary, tests with AURC less than or equal to 0.5 would not discriminate between affected and nonaffected individuals. The Wilcoxon statistic, W, is used to test the null hypothesis that the diagnostic test cannot be used to discriminate between affected and nonaffected subjects (*i.e.* the probability equals 0.5), and is also used to predict the statistical properties of the AURC (24).

$P < 0.05$ was considered statistically significant for all analyses.

Results

Prevalence of NCAH and other diagnoses

Six of the 270 consecutive patients had 21-hydroxylase deficiency because their ACTH-stimulated levels were more than or equal to 10 ng/ml and had mutations in both alleles of *CYP21*. Their genotypes and clinical and hormonal variables are summarized in Table 1. Five of these six women carried severe *CYP21* mutations in one allele requiring genetic counseling, highlighting the importance of reaching the diagnosis of NCAH among hyperandrogenic women.

Of note, although several other hyperandrogenic women presented with increased serum basal 17-hydroxyprogesterone concentrations (≥2 ng /ml), only the women finally diagnosed with NCAH by molecular genetic analyses presented with ACTH-stimulated 17-hydroxyprogesterone levels more than or equal to 10 ng/ml. These analyses were also conducted in a hyperandrogenic woman presenting with basal and ACTH-stimulated 17-hydroxyprogesterone levels of 3.7 and 9.2 ng/ml, respectively, but both of her *CYP21* alleles were wild type showing no mutations. No other patient presented with ACTH-stimulated 17-hydroxyprogesterone levels more than or equal to 9 ng/ml.

TABLE 1. Clinical variables, androgen and adrenal steroid profiles, and *CYP21* genotypes of the women diagnosed with NCAH

Patient	Age (yr)	BMI (kg/m^2)	Hirsutism score	Virilization	Ovulatory dysfunction	Total T (ng/dl)	Free T (ng/dl)	DHEAS (ng/ml)	A (ng/ml)	Basal 17OHP (ng/ml)	ACTH-stimulated 17OHP (ng/ml)	*CYP21* genotype
1	28	27.8	18	Mild	Amenorrhea	142	3.9	3500	11.5	45.6	62.3	V281 L/deletion
2	13	20.2	13	No	Oligomenorrhea	66	0.8	1940	4.7	6.5	28.4	V281 L/V281 L
3	43	28.7	11	No	Oligomenorrhea	77	1.6	3010	2.8	2.1	40.9	V281 L/R356W
4	25	17.7	9	No	Oligomenorrhea	81	1.1	3160	5.2	1.7	11.7	V281 L/deletion
5	20	20.6	10	Mild	Reduced luteal P4	140	3.6	2360	7.1	7.7	47.7	V281 L + I$_2$ splice/V281 L
6	20	32.4	19	No	Oligomenorrhea	93	2.5	4250	10.5	4.9	27.8	P453S/conversion

To convert to Systeme International units, multiply total testosterone (T) by 0.03467 (result in nmol/liter), free testosterone by 34.67 (result in pmol/liter), dehydroepiandrosterone-sulfate (DHEAS) by 0.002714 (result in μmol/liter), androstenedione (A) by 3.49 (result in nmol/liter), and 17-hydroxyprogesterone (17OHP) by 3.026 (result in nmol/liter). BMI, Body mass index; P4, progesterone.

Regarding 11β-hydroxylase deficiency, no women had increased ACTH-simulated 11-deoxycortisol levels. Therefore, the overall NCAH prevalence coincided with that of 21-hydroxylase deficiency: six of 270 cases, for 2.2% prevalence with 95% confidence limits of 0.5–3.9%.

Ordered from more to less prevalent, other diagnoses were: PCOS (171 cases, 63.3% prevalence, 95% confidence limits 57.6–69.0%); idiopathic hyperandrogenism (61 cases, 22.6% prevalence, 95% confidence limits 17.6–27.6%); idiopathic hirsutism (24 cases, 8.9% prevalence, confidence limits 5.5–12.3%); World Health Organization's class II anovulation (six cases, 2.2% prevalence, 95% confidence limits 0.5–3.9%); and hyperprolactinemia (two cases, 0.7% prevalence, 95% confidence limits 0.0–1.8%). No women studied here had a clinical history suggesting Cushing's syndrome, androgen secreting tumors, or drug-induced hirsutism.

Comparison of the clinical and biochemical characteristics of the women diagnosed with NCAH with those diagnosed with other androgen excess phenotypes

As a group, the clinical and biochemical characteristics of the six NCAH patients were almost indistinguishable from those of the women presenting with PCOS because the hirsutism score and androgen levels were not different between both groups, and all the NCAH and PCOS patients had evidence of ovulatory dysfunction (Tables 1 and 2). However, two of the NCAH patients had very high testosterone concentrations, accompanied by mild virilization and defeminization, which was not found in any other women studied here (Table 1). The hirsutism scores of NCAH women were similar to those of women presenting with idiopathic hyperandrogenism and idiopathic hirsutism, yet NCAH women and women with idiopathic hyperandrogenism had increased androgen levels compared with women with idiopathic hirsutism (Table 2).

Specifically, NCAH patients presented with increased basal and ACTH-stimulated adrenal steroids, including 11-deoxycortisol, 17-hydroxyprogesterone, and androstenedione, compared with the other androgen excess phenotypes and with women with World Health Organization's class II anovulation (Table 2). On the contrary, no differences were found in fasting glucose and insulin concentrations, and in insulin resistance as measured by homeostasis model assessment.

ROC curve analysis of the performance of basal serum 17-hydroxyprogesterone levels and serum androgen concentrations for the screening of 21-hydroxylase-deficient NCAH among women presenting with hyperandrogenic signs and symptoms

Present guidelines recommend the use of basal serum 17-hydroxyprogesterone levels for the screening of NCAH among hy-

TABLE 2. Comparison of clinical and biochemical characteristics among women with NCAH and other groups of women presenting with hyperandrogenic symptoms

	NCAH (n = 6)	PCOS (n = 171)	Idiopathic hyperandrogenism (n = 61)	Idiopathic hirsutism (n = 24)	WHO-II anovulation (n = 6)	Hyperprolactinemia (n = 2)
Age (yr)	25 ± 10	25 ± 6	24 ± 6	27 ± 8	30 ± 9	32 ± 10
Body mass index (kg/m^2)	24.6 ± 5.9	29.2 ± 7.6	28.1 ± 7.3	25.2 ± 5.3	30.0 ± 8.9	26.2 ± 12.5
Waist-to hip ratio	0.82 ± 0.05	0.78 ± 0.08	0.76 ± 0.06	0.75 ± 0.06	0.85 ± 0.12	0.75 ± 0.07
Hirsutism score	13 ± 4	11 ± 6	13 ± 6	13 ± 4	4 ± 2[a]	5 ± 1
Ovulatory dysfunction (%)[b]	100	100	0	0	100	100
Total testosterone (ng/dl)	100 ± 33	63 ± 25	68 ± 19	43 ± 10[c]	38 ± 7[c]	43 ± 12
Free testosterone (ng/dl)	2.3 ± 1.3	1.3 ± 0.6	1.3 ± 0.4	0.7 ± 0.2[c]	0.6 ± 0.2[d]	0.7 ± 0.2
SHBG (μg/dl)	277 ± 181	294 ± 181	281 ± 136	429 ± 207	363 ± 176	341± 18
DHEAS (ng/ml)	3125 ± 830	2382 ± 1070	3031 ± 1382	1933 ± 794	1916 ± 889	957 ± 76
Basal F (μg/dl)	13 ± 6	15 ± 5	16 ± 6	14 ± 6	13 ± 5	14 ± 3
ACTH-stimulated F (μg/dl)	23 ± 6	27 ± 6	29 ± 7	29 ± 7	26 ± 8	25 ± 7
Basal S (ng/ml)	4.4 ± 2.4	2.2 ± 1.1	2.7 ± 1.6	1.8 ± 0.7[a]	1.4 ± 0.5[a]	1.4 ± 0.2
ACTH-stimulated S (ng/ml)	7.5 ± 1.9	3.8 ± 1.6[d]	4.1 ± 1.9[a]	3.7 ± 1.3[a]	2.4 ± 1.1[c]	4.0 ± 0.9
Basal 17OHP (ng/ml)	11.4 ± 17	1.0 ± 0.6[d]	1.1 ± 0.7[d]	0.7 ± 0.3[c]	0.6 ± 0.3[c]	1.1 ± 1.0
ACTH-stimulated 17OHP (ng/ml)	36.5 ± 17.7	2.6 ± 1.2[c]	2.7 ± 1.2[d]	2.5 ± 1.1[d]	2.4 ± 1.3[d]	3.0 ± 1.0
Basal A (ng/ml)	7.0 ± 3.4	3.8 ± 1.3	4.0 ± 1.3	3.0 ± 0.8[d]	2.5 ± 0.5[c]	2.8 ± 0.4
ACTH-stimulated A (ng/ml)	8.9 ± 3.2	4.8 ± 1.6[a]	5.0 ± 1.4	4.0 ± 1.0[c]	3.0 ± 0.5[c]	3.3 ± 0.9
Fasting glucose (mg/dl)	95 ± 19	90 ± 9	86 ± 8	86 ± 8	121 ± 55	87 ± 2.8
Fasting insulin (μU/ml)	14 ± 8	14 ± 10	11 ± 5	10 ± 5	10 ± 5	5 ± 4
HOMA-IR	3.5 ± 3.1	3.3 ± 2.6	2.4 ± 1.2	2.0 ± 1.0	3.3 ± 2.4	1.1 ± 0.9

Results are means ± SD unless otherwise stated. The central tendencies of the groups were compared by the Kruskal-Wallis test, and the differences between the NCAH and the other groups were further identified by the Dunn test. To convert to Systeme International units, multiply total testosterone by 0.03467 (result in nmol/liter), free testosterone by 34.67 (result in pmol/liter), SHBG by 0.111 (result in nmol/liter), dehydroepiandrosterone-sulfate (DHEAS) by 0.002714 (result in μmol/liter), cortisol (F) by 27.59 (result in nmol/liter), 11-deoxycortisol (S) by 2.89 (result in nmol/liter), 17-hydroxyprogesterone (17OHP) by 3.026 (result in nmol/liter), androstenedione (A) by 3.49 (result in nmol/liter), glucose by 0.555 (result in mmol/liter), and insulin by 6.945 (result in pmol/liter). HOMA-IR, Insulin resistance according to homeostasis model assessment; WHO-II, World Health Organization's class II.

[a] $P < 0.05$ compared with the NCAH group.

[b] No statistics were applied here.

[c] $P < 0.001$ compared with the NCAH group.

[d] $P < 0.01$ compared with the NCAH group.

perandrogenic women (6). In those women presenting with increased basal serum 17-hydroxyprogesterone levels, ACTH-stimulated 17-hydroxyprogesterone levels more than or equal to 10 ng/ml, followed by molecular genetic analysis of *CYP21*, if available, must be used to confirm the diagnosis (18).

When adequately measured during the morning and during the follicular phase of the menstrual cycle, the recommended cutoff value for basal serum 17-hydroxyprogesterone levels varies between 2 and 3 ng/ml (25, 26).

Because we routinely measured ACTH-stimulated serum 17-hydroxyprogesterone levels regardless of the basal concentration of this steroid, thereby detecting all the NCAH patients in our series of consecutive hyperandrogenic patients (a diagnosis that was confirmed later by molecular genetic analysis of *CYP21*), our design allows the use of ROC analysis to establish the overall diagnostic performance of basal serum 17-hydroxyprogesterone measurement, and to select the optimal cutoff value for this test using the ImmuChem direct RIA assay.

The AURC of basal serum 17-hydroxyprogesterone levels for the diagnosis of 21-hydroxylase-deficient NCAH was 0.971 (Fig. 1, 95% confidence interval 0.934–1.008), meaning that in our experience, a single basal serum 17-hydroxyprogesterone measurement performed in the morning and during the follicular phase of the menstrual cycle had a 97.1% chance of correctly identifying women with NCAH among women presenting with hyperandrogenic complaints.

Furthermore, according to ROC analysis, the most appropriate basal serum 17-hydroxyprogesterone cutoff value for the screening of 21-hydroxylase-deficient NCAH was 1.7 ng/ml (100% sensitivity, 88.6% specificity).

On the contrary, the proposed cutoff values of 2 ng/ml (83.3% sensitivity, 93.6% specificity) or 3 ng/ml (66.7% sensitivity, 98.9% specificity) were less appropriate because they lacked the sensitivity needed for an optimal screening test. Accordingly, the application of a 2 ng/ml cutoff to basal 17-hydroxyprogesterone concentrations would have missed one of the six NCAH patients in our series, and the 3 ng/ml cutoff would have missed two of them.

Finally, ROC analysis showed that the screening of NCAH among hyperandrogenic women should not rely on serum androgen concentrations. The AURC of serum total testosterone, free testosterone, androstenedione, and dehydroepiandrosterone-sulfate levels for the diagnosis of 21-hydroxylase-deficient NCAH were 0.859 (95% confidence interval 0.759–0.960; $P = 0.003$), 0.754 (95% confidence interval 0.529–0.979; $P = 0.033$), 0.815 (95% confidence interval 0.591–1.038; $P = 0.008$), and 0.707 (95% confidence interval 0.541–0.872; $P = 0.084$), respectively, yet the highest possible specificity of the cutoff values of these hormones showing 100% sensitivity was unacceptably low, even for a screening test (65.2% for total testosterone, 25.9% for free testosterone, 21.3% for androstenedione, and 36.9% for dehydroepiandrosterone-sulfate; Fig. 2).

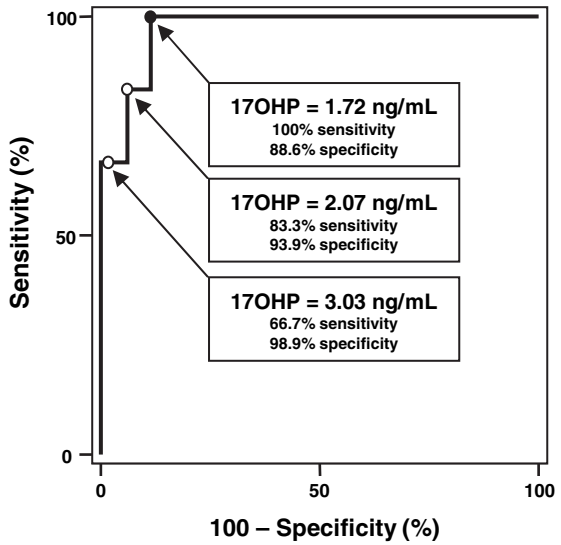

FIG. 1. ROC curve analysis of the performance of basal serum 17-hydroxyprogesterone (17OHP) levels for the screening of 21-hydroxylase-deficient NCAH among women presenting with hyperandrogenic signs and symptoms. ROC curves are constructed by plotting the sensitivity (true-positive fraction) on the ordinate as a function of the complement of specificity (false-positive fraction), for all the possible cutoff values of the diagnostic test (22). Therefore, the more deviated toward the *left upper corner* the curve is, the higher the sensitivity and specificity the diagnostic test has for all possible cutoff values or, namely, the higher the adequacy of the diagnostic test for disease detection (23). The *black dot* is the proposed 1.72 ng/ml cutoff value for the screening of NCAH using basal 17-hydroxyprogesterone levels. The *white dots* correspond to the 2 and 3 ng/ml cutoff values previously suggested by others (25, 26). To convert to Systeme International units, multiply 17-hydroxyprogesterone by 3.026 (result in nmol/liter).

Discussion

The prevalence of NCAH among female Spaniards presenting with hyperandrogenic complaints has not been established earlier. Our present unbiased study demonstrates a 2.2% prevalence (0.5–3.9% 95% confidence limits) of this disorder among unselected women complaining of hyperandrogenic signs and symptoms from Madrid, Spain.

This figure is coincident with the overall 2% prevalence of NCAH in hyperandrogenic patients reported worldwide (7), with different studies reporting prevalences from as low as 0.6% to as high as 8.4% (27–40). Most of these studies evaluated only 21-hydroxylase-deficient NCAH, yet the highest prevalence of NCAH among hyperandrogenic women reported to date consisted in nonclassical 11β-hydroxylase deficiency in seven of 83 (8.4%) Turkish patients (36).

Much smaller prevalences of 11β-hydroxylase deficiency in hyperandrogenic women have been reported in Italy (30) and the United States (32), and, therefore, the high prevalence of this type of NCAH reported in Turkey might be related to ethnic factors, given that 11β-hydroxylase deficiency is prevalent in the Middle East, especially among Jewish immigrants from Morocco (9). Of note, ACTH-stimulated 11-deoxycortisol levels excluded 11β-hydroxylase deficiency in the 270 patients in our series.

Our present results also confirm that NCAH patients are almost clinically and biochemically indistinguishable from PCOS patients, and that only increased 17-hydroxyprogesterone and 11-deoxycortisol levels were different among these groups of patients. Elevated 11-deoxycortisol concentrations have been reported in patients with classical 21-hydroxylase and in patients

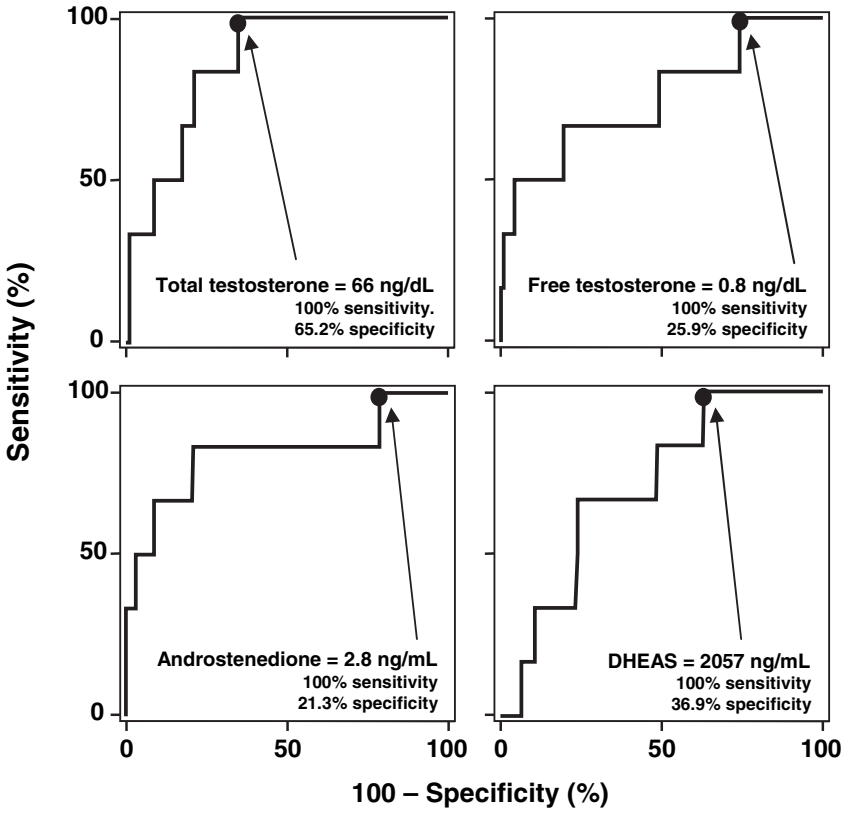

FIG. 2. ROC curve analysis of the performance of serum androgen levels for the screening of 21-hydroxylase-deficient NCAH among women presenting with hyperandrogenic signs and symptoms. The *black dots* are the cutoff values with the highest specificity for 100% sensitivity. *Left upper panel*, Total testosterone. *Right upper panel*, Free testosterone. *Left lower panel*, Androstenedione. *Right lower panel*, Dehydroepiandrosterone-sulfate (DHEAS). To convert to Systeme International units, multiply total testosterone by 0.03467 (result in nmol/liter), free testosterone by 34.67 (result in pmol/liter), androstenedione by 3.49 (result in nmol/liter), and dehydroepiandrosterone-sulfate by 0.002714 (result in μmol/liter).

with PCOS, suggesting that androgens may suppress 11-hydroxylase activity (41). Yet also, the cross reaction of the 11-deoxycortisol assay used here with the high 17-hydroxyprogesterone levels observed in these patients might have also contributed to these increased 11-deoxycortisol levels.

Compared with earlier studies, our present approach has the strengths of having evaluated simultaneously the prevalences of the two congenital adrenal hyperplasias that cause hyperandrogenism, of having measured basal and ACTH-stimulated steroid levels in every women, that the cases found were confirmed at the molecular genetic level by *CYP21* genotyping, and that we carefully avoided any selection bias that might have artificially increased NCAH prevalence.

The results of the *CYP21* genotyping performed in our study highlight the importance of excluding NCAH in women complaining of symptoms of androgen excess. Although it can be argued that the prevalence is small, and that the presence of NCAH does not influence greatly the management of these women [oral contraceptives and antiandrogens are possibly the most useful drugs for hyperandrogenism in NCAH (18)], five of the six NCAH patients in our series carried a severe *CYP21* allele, and required genetic counseling because of the unquestionable risk of delivering a child with classical congenital adrenal hyperplasia (42). In addition, the treatment of choice for infertility in the adult woman with NCAH is glucocorticoid therapy, which usually restores ovulation. Therefore, every effort must be made to diagnose NCAH among women complaining of hyperandrogenic symptoms.

Finally, because we measured both basal and ACTH-stimulated 17-hydroxyprogesterone levels in all the patients, we have been able to provide an accurate estimation of the performance of basal circulating 17-hydroxyprogesterone concentrations for the screening of NCAH among hyperandrogenic patients. A single early morning measurement of this steroid performed during the follicular phase of the menstrual cycle has a striking 0.97 chance of classifying correctly a hyperandrogenic patient as having or not having NCAH. However, our results suggest that at least in our country, and using the ImmuChem 17-hydroxyprogesterone RIA assay, the cutoff value should be lowered from the recommended 2 ng/ml value to 1.7 ng/ml, to reach 100% sensitivity for this diagnostic procedure. On the contrary, serum androgen levels should not be used as an indication for the screening of NCAH, given their poor diagnostic performance.

In summary, NCAH is relatively rare among unselected women presenting with hyperandrogenic complaints, yet its routine exclusion is very important to detect carriers of *CYP21* severe alleles that require genetic counseling. A single early morning basal serum 17-hydroxyprogesterone measurement is an extremely accurate screening method that permits restricting the use of ACTH stimulation to those women presenting with increased basal 17-hydroxyprogesterone concentrations. However, the cutoff value of this test must be carefully established at each laboratory, or, if this is not possible, the cutoff value should be lowered to approximately 1.7 ng/ml from the currently recommended 2 ng/ml upper limit of the normal range.

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