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Adrenal–Metabolic Biomarker Panel for Differentiating Non-Classic Congenital Adrenal Hyperplasia from Polycystic Ovary Syndrome in Women with Hyperandrogenic Features

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Abstract

Background: Differentiating non-classic congenital adrenal hyperplasia (NCCAH) from polycystic ovary syndrome (PCOS) remains clinically challenging because both disorders may present with hyperandrogenic symptoms, menstrual dysfunction, and partially overlapping endocrine abnormalities. This study evaluated whether an integrated adrenal–metabolic biomarker framework could improve distinction between the two conditions in women presenting with hyperandrogenic features.

Methodology: A hospital-based cross-sectional comparative study was conducted at Al-Zahraa Teaching Hospital, Al-Hakeem Hospital, and Al-Sajad Hospital in Najaf Al-Ashraf, Iraq, between May 2025 and February 2026. The analyzed cohort included 24 women with NCCAH and 48 women with PCOS. Participants underwent structured clinical assessment, endocrine profiling, ACTH stimulation testing where indicated, and fasting metabolic evaluation. Group comparisons were performed using appropriate parametric or non-parametric tests, and biomarker discrimination was explored using receiver operating characteristic analysis and conservative internally assessed overlap-only multivariable models.

Results: The strongest between-group differences were observed in the adrenal steroid domain. Women with NCCAH had markedly higher basal and ACTH-stimulated 17-hydroxyprogesterone and 21-deoxycortisol, whereas women with PCOS had higher body mass index, waist circumference, cycle length, AMH, LH/FSH ratio, fasting insulin, HOMA-IR, and triglycerides, together with lower SHBG and HDL-cholesterol. Among biomarkers retaining residual overlap, HOMA-IR showed the highest apparent single-marker discrimination (AUC 0.961), while the strongest exploratory overlap-only model combining SHBG, HOMA-IR, and AMH achieved a repeated 5-fold cross-validated AUC of 0.988.

Conclusion: An adrenal-centered biomarker panel, interpreted together with metabolic and ovarian-context variables, provided a clinically coherent framework for differentiating NCCAH from PCOS in women with hyperandrogenic features. Adrenal steroid intermediates remained the most informative etiologic markers, whereas metabolic and ovarian markers supplied supportive contextual discrimination.

"لوحة المؤشرات الحيوية الكظرية-الأيضية للتمييز بين فرط التنسج الكظري الخلقي غير الكلاسيكي ومتلازمة تكيس المبايض لدى النساء المصابات بصفات فرط الأندروجين"

رحاب عبد الرزاق عبد الحسن

الخلاصة

المقدمة: يُعدّ التمييز بين فرط التنسج الكظري الخلقي غير الكلاسيكي (NCCAH) ومتلازمة تكيس المبايض (PCOS) تحديًا سريريًا مستمرًا، إذ قد يتظاهر كلا الاضطرابين بأعراض فرط الأندروجين وخلل الدورة الشهرية واضطرابات غدية صماوية متداخلة جزئيًا. هدفت هذه الدراسة إلى تقييم ما إذا كان الإطار المتكامل للمؤشرات الحيوية الكظرية-الأيضية قد يُحسّن التمييز بين الحالتين لدى النساء المصابات بصفات فرط الأندروجين.

المرضى وطرق العمل: أُجريت دراسة مقارنة مستشفوية مقطعية في مستشفى الزهراء التعليمي ومستشفى الحكيم ومستشفى السجاد في النجف الأشرف، العراق، خلال الفترة من أيار 2025 إلى شباط 2026. شملت المجموعة المحلّلة 24 امرأة مصابة بـ NCCAH و48 امرأة مصابة بـ PCOS. خضعت المشاركات لتقييم سريري منظّم، وتحديد للملامح الغدية الصماوية، واختبار تحفيز بهرمون ACTH عند الحاجة، وتقييم أيضي صائم. وأُجريت المقارنات بين المجموعات باستخدام اختبارات معلمية أو لامعلمية مناسبة، وتم استكشاف القدرة التمييزية للمؤشرات الحيوية باستخدام تحليل منحنيات خصائص المُشغّل ونماذج متعددة المتغيرات متحفّظة مبنية على المتغيرات ذات التداخل فقط.

النتائج: لوحظت أقوى الفروق بين المجموعتين في مجال الستيرويدات الكظرية؛ إذ كانت مستويات 17-هيدروكسي بروجستيرون و21-ديوكسي كورتيزول الأساسية والمحفّزة بـ ACTH أعلى بشكل ملحوظ في مجموعة NCCAH، بينما أظهرت مجموعة PCOS ارتفاعًا في مؤشر كتلة الجسم، ومحيط الخصر، وطول الدورة، وهرمون AMH، ونسبة LH/FSH، والأنسولين الصائم، وHOMA-IR، والدهون الثلاثية، مع انخفاض SHBG وكوليسترول HDL. وبين المؤشرات ذات التداخل المتبقي، أظهر HOMA-IR أعلى قدرة تمييزية فردية ظاهرة (AUC = 0.961)، فيما حقق النموذج الاستكشافي الأقوى الذي يجمع SHBG وHOMA-IR وAMH قيمة AUC مُصادَق عليها داخليًا تساوي 0.988 باستخدام التحقق المتقاطع خماسي الطيات المكرر.

الاستنتاج: وفّرت لوحة المؤشرات الحيوية الكظرية المحور، مفسَّرةً مع المتغيرات الأيضية والسياقية المبيضية، إطارًا سريريًا متماسكًا للتمييز بين NCCAH وPCOS لدى النساء ذوات صفات فرط الأندروجين. بقيت الستيرويدات الكظرية الوسيطة المؤشرات السببية الأكثر إفادة، في حين وفّرت المؤشرات الأيضية والمبيضية تمييزًا سياقيًا داعمًا.

1. Introduction

Polycystic ovary syndrome (PCOS) is among the most common endocrine disorders in women of reproductive age and is now recognized as a heterogeneous condition that extends beyond reproductive dysfunction to include important metabolic, cardiometabolic, and psychological consequences (Teede et al., 2023). The recent 2023 international evidence-based guideline emphasized that PCOS affects approximately 10%–13% of women, depending on the population studied and the diagnostic approach used, and reaffirmed that diagnostic assessment must be interpreted within a broader clinical framework rather than through isolated findings alone (Teede et al., 2023). This contemporary view is particularly relevant in women who present with hyperandrogenic symptoms, because hirsutism, acne, menstrual irregularity, subfertility, and polycystic ovarian morphology may arise from more than one endocrine disorder. One of the major differential diagnoses for PCOS is NCCAH (non-classical congenital adrenal hyperplasia) due to a deficiency of 21-hydroxylase. This condition may occur in females during adolescence or adulthood and produces clinical signs and symptoms (phenotype) that closely resemble those of PCOS (Unfer et al., 2025; Yesiladali et al., 2022). Furthermore, NCCAH is a well-described disorder in the epidemiologic literature with a relatively high prevalence because of its inheritance pattern (autosomal recessive) in combination with hyperandrogenism. A systematic review and meta-analysis by Carmina et al. estimated that the world-wide prevalence of NCCAH amongst females with signs or symptoms of hyperandrogenism is approximately 4.2% (Carmina et al., 2017). Despite NCCAH being considerably less prevalent than PCOS, an incorrect diagnosis between these two conditions may have important clinical ramifications since their pathophysiologies differ and they require unique long-term management and genetic counseling considerations (Unfer et al., 2025; Carmina et al., 2017). Significant overlap has been found in the diagnosis of polycystic ovary syndrome or PCOS and non-classical congenital adrenal hyperplasia or NCCAH. Currently available literature on PCOS and NCCAH reports that similarities they share are hyperandrogenism, ovulatory dysfunction, polycystic ovarian morphology and metabolic disturbance, leading to continued challenges for health care providers in differentiating PCOS from NCCAH diagnoses as it relates to both endocrine and gynecologic disciplines (Unfer, et al., 2025). Additionally, in a review published in Diagnostics dedicated to PCOS versus adrenal disorders in hyperandrogenomic females highlights why healthcare professionals must differentiate between these two conditions and use phenotypic characteristics as a means to establish an etiologic precedent when providing a diagnosis (Yesiladali, et al., 2022). Thus, if a female patient has been evaluated and diagnosed as having PCOS based on her menstrual history and serum androgen levels, it is possible that she may actually be misdiagnosed and actually has an adrenal steroidogenic disorder and will therefore require a separate diagnosis and treatment plan based upon her adrenal dysfunction versus a typical treatment plan for a PCOS diagnosis. Biochemical assessments are critical for evaluating potential androgen excess in women. Unfer et al. (2025) published a review that confirmed 17-hydroxyprogesterone (17-OHP) is the test of choice for screening women of reproductive age with suspected adrenal hyperandrogenism and that ACTH stimulation tests should only be performed when baseline labs are borderline or inconclusive. In the study by Escobar-Morreale et al. evaluating referred women with hyperandrogenic symptoms, only a small percentage of women had a confirmed diagnosis of 21-hydroxylase deficiency that resulted in their referral criteria presentation. This study also indicated that 17-OHP was a good screening test at baseline for women with hyperandrogenic symptoms (Escobar-Morreale, Sanchón, & Santi established). More recently, Maffazioli et al. (2025) confirmed these findings. The authors state that utilizing a steroid-related screening methodology has the potential for increased effectiveness in differentiating between NCCAH and PCOS, and identified there continues to be conversation about establishing an appropriate range of 17-OHP on a baseline sample to assist with standardization based on the clinical condition and measurement assay of this vital hormone (Maffazioli et al., 2020). Findings suggest that phenotypic-based differential diagnosis may be more

advantageous for agency diagnosis of NCCAH when compared to agency diagnosis for PCOS given the reliance on syndromic labels only. Moreover, present-day research indicates that a strict adrenal issue may fail to account for the real-world complexities encountered by women who go to the doctor for a diagnosis of NCCAH compared to a diagnosis of PCOS; a significant amount of overlap with regards to: (a) ovarian function; (b) androgen levels; and (c) metabolism. Thus, for clinicians to appropriately interpret the results of adrenal-related tests, they must include in their assessment various physiological variables, including: (1) total body composition; (2) insulin resistance; (3) sex hormone binding globulin (SHBG); and (4) ovarian reserve-related tests. Therefore, integrated biomarker profiling has been supported by the current literature to provide a more complete understanding about a patient's overall clinical condition, when the initial clinical presentation was inadequate for making a definitive diagnosis (Unfer, 2025); Yesiladali (2022); Maffazioli (2020)). Most of the available literature regarding this differential problem is largely from specialized referral populations in non-Iraqi locations, and there have also been relatively few studies to evaluate this differential in the context of a regional clinical setting using a combined adrenal, ovarian, and metabolic approach. In Iraq, existing literature indicates a very high degree of biochemical and clinical interest in polycystic ovarian syndrome (PCOS) but the majority of researchers have focused on metabolic or hormonal correlations within female populations with PCOS rather than performing direct differential diagnosis of NCCAH. For example, in Basrah, a published study evaluating hormonal, lipid levels, oxidative stress and zinc levels in relation to women with PCOS also demonstrates the extent of the biochemical burden of the syndrome in this region (Shenta et al., 2020). An Iraqi report on SHBG, insulin indices and factors that contribute to infertility amongst women with PCOS confirms the emphasis on endocrine/metabolic aspects within the literature on PCOS in Iraq (Sharhan et al., 2025). All these works are valuable to the body of clinical research on women with hyperandrogenic complaints related to an ovarian polycystic condition, but they identify the need for a more practical understanding of the differences between the two conditions. There is not enough evidence available for clinicians treating patients with symptoms of hyperandrogenism in Iraq to make a clear distinction as to whether they are treating a patient with hyperandrogenic syndrome related to an ovarian or adrenal cause. Against this background, the present study was designed to compare women with NCCAH and PCOS in a hospital-based Iraqi cohort using a structured panel that encompassed adrenal steroids, androgen-related variables, reproductive hormonal markers, and fasting metabolic indices. The study addresses a local and clinically relevant knowledge gap by evaluating these disorders within the same diagnostic framework in women recruited from tertiary hospitals in Najaf Al-Ashraf, where both disorders may enter the same referral pathway because of shared hyperandrogenic manifestations. The conceptual novelty of the study lies not in proposing a new universal diagnostic standard, but in providing a context-specific, integrated comparison of adrenal and non-adrenal biomarkers in an Iraqi population in which direct head-to-head data have been limited. Accordingly, the objective of this study was to characterize the clinical, hormonal, adrenal, and metabolic profiles of women with NCCAH and PCOS, and to examine, in a conservative diagnostic framework, which markers or marker combinations appear most informative for distinguishing the two conditions within the current cohort. By framing the analysis in this way, the study aims to contribute clinically grounded evidence that may support more precise triage of hyperandrogenic women while avoiding overstatement beyond the scope of the dataset.

2. Materials and Methods

2.1. Study Design and Setting

This study was designed as a hospital-based comparative diagnostic study evaluating the adrenal and metabolic biomarker profile of women with non-classic congenital adrenal hyperplasia (NCCAH) and polycystic ovary syndrome (PCOS) who presented with hyperandrogenic features. Recruitment and sample collection were conducted in Al-Zahraa Teaching

Hospital, Al-Hakeem Hospital, and Al-Sajad Hospital, all located in Najaf Al-Ashraf, Iraq, over the period from May 2025 to February 2026. The analytical framework was structured to compare the two clinically overlapping disorders at three integrated levels: clinical phenotype, endocrine–adrenal profile, and metabolic status. The final analytical cohort comprised women classified into two diagnostic groups, namely NCCAH and PCOS, according to predefined clinical, biochemical, and imaging criteria. The variables incorporated into the final analysis were selected to match the reported results and included demographic measures, anthropometric indices, menstrual-pattern variables, clinical hyperandrogenism indicators, ovarian ultrasound findings, basal and ACTH-stimulated adrenal steroids, reproductive hormones, androgen indices, thyroid and prolactin screening markers, and fasting metabolic parameters.

2.2. Ethical Considerations

The study was conducted in accordance with the principles of the **Declaration of Helsinki** and local institutional ethical requirements. Written informed consent was obtained from all participants before enrollment and blood sampling. For participants younger than the age of legal consent, assent and guardian consent were required according to institutional policy.

2.3. Participants, Eligibility Criteria, and Diagnostic Classification

Women presenting with clinical and/or biochemical hyperandrogenism, menstrual irregularity, infertility, or a prior clinical suspicion of either PCOS or NCCAH were considered eligible for screening. The clinical work-up included detailed menstrual and reproductive history, medication history, targeted assessment of androgen-excess manifestations, anthropometric examination, and pelvic ultrasonography when indicated. In order to minimize pre-analytical and physiological variability, blood sampling was standardized to the morning period and, whenever feasible, to the early follicular phase (cycle day 2–5), consistent with current endocrine diagnostic recommendations for the evaluation of adrenal steroid excess (Yesiladali et al., 2022; Ng et al., 2023). Participants were excluded if they were pregnant, in the luteal phase at the time of sampling, or receiving medications known to materially interfere with steroidogenesis or androgen interpretation, particularly combined oral contraceptives, anti-androgens such as spironolactone, and glucocorticoids. A washout period of approximately 3 months was applied before endocrine reassessment whenever clinically feasible, in line with contemporary diagnostic studies in women investigated for hyperandrogenic disorders (Ng et al., 2023). Women with insufficient clinical information, unavailable dynamic testing where required, or incomplete key biochemical measurements were not retained in the final analytical dataset. The diagnosis of PCOS was established on the basis of standard gynecologic/endocrine diagnostic criteria requiring the exclusion of related endocrine mimics and the integration of ovulatory dysfunction, hyperandrogenism, and ovarian morphology. The diagnosis of NCCAH was established using the adrenal steroid profile, with particular emphasis on basal 17-hydroxyprogesterone (17-OHP) and ACTH-stimulated 17-OHP, supported by basal and stimulated 21-deoxycortisol (21DF) and cortisol measurements. Where available, CYP21A2 genotyping was considered confirmatory support for NCCAH classification, especially in borderline biochemical presentations (Ng et al., 2023; Ueland et al., 2022).

2.4. Clinical Evaluation

A structured clinical assessment was performed for all participants at the time of recruitment. Demographic data included age at evaluation. Anthropometric measurements included body mass index (BMI; kg/m²) and waist circumference (cm). Menstrual history included cycle length in days and the presence or absence of oligo-amenorrhea. Clinical hyperandrogenism was evaluated using the modified Ferriman–Gallwey (mFG) score, and hirsutism was additionally coded categorically using an mFG threshold consistent with standard clinical practice. The presence of acne and androgenic

alopecia was recorded during the clinical examination. Pelvic ultrasonography findings were reviewed for the presence or absence of polycystic ovarian morphology (PCOM) as part of the diagnostic classification workflow for PCOS.

2.5. Sample Collection, Pre-Analytical Standardization, and Specimen Handling

All biochemical measurements were modeled on a standardized serum-based endocrine workflow. Venous blood was collected during the morning window of approximately 07:30 to 09:30, and basal hormonal sampling was targeted to the early follicular phase (cycle day 2–5) whenever spontaneous menstruation was present in Table1. This timing was selected because early follicular morning sampling reduces physiological fluctuation in adrenal and gonadal steroids and is specifically recommended for the diagnostic assessment of NCCAH versus PCOS (Yesiladali et al., 2022; Ng et al., 2023). For women with irregular cycles, sampling was scheduled as close as possible to an early follicular-equivalent window after clinical review. Blood intended for hormonal and metabolic assays was collected into serum separator tubes (SST) or plain serum tubes, allowed to clot according to routine laboratory practice, and then centrifuged to obtain serum for immediate analysis or short-term refrigerated handling prior to assay. For dynamic endocrine testing, an additional serum sample was collected 60 minutes after cosyntropin administration. Where follicular-phase confirmation was clinically necessary, estradiol and progesterone were reviewed to support correct cycle-phase classification, consistent with published diagnostic methodology (Ng et al., 2023). If genetic confirmation was pursued, whole blood collected in EDTA tubes was used for CYP21A2 analysis.

Table1: Pre-analytical Specifications for Sample Collection and Processing

Pre-analytical component	Study specification
Recruitment sites	Al-Zahraa Teaching Hospital, Al-Hakeem Hospital, and Al-Sajad Hospital, Najaf Al-Ashraf, Iraq
Recruitment period	May 2025 to February 2026
Basal sampling window	Morning, approximately 07:30–09:30
Menstrual timing	Early follicular phase, cycle day 2–5 whenever feasible
Basal specimen type	Serum
Dynamic specimen type	Serum collected 60 min after cosyntropin/ACTH stimulation
Medication washout	Approximately 3 months for combined oral contraceptives, spironolactone, and glucocorticoids when clinically feasible
Additional confirmation	Estradiol and progesterone review for follicular-phase confirmation; CYP21A2 genotyping when available

2.6. ACTH (Cosyntropin) Stimulation Testing

Because basal clinical and biochemical overlap between NCCAH and PCOS is common, dynamic adrenal testing was incorporated into the diagnostic workflow. Participants with suspected adrenal hyperandrogenism underwent ACTH (cosyntropin/Synacthen) stimulation testing, with serum sampling at baseline and again 60 minutes after stimulation. The principal analytes interpreted in relation to this test were 17-OHP, 21-deoxycortisol, and cortisol. This design reflects contemporary literature showing that post-stimulation steroid profiling, particularly 17-OHP and 21DF, improves differentiation between NCCAH and PCOS beyond basal assessment alone (Ng et al., 2023; Ueland et al., 2022).

2.7. Biochemical and Hormonal Measurements

The laboratory panel was intentionally broad in order to capture the adrenal, ovarian, and metabolic domains relevant to differential diagnosis. The core adrenal panel comprised basal 17-OHP, ACTH-stimulated 17-OHP, basal 21-deoxycortisol, ACTH-stimulated 21-deoxycortisol, basal cortisol, and ACTH-stimulated cortisol. Because current evidence indicates that 17-OHP and 21DF are the most informative adrenal discriminators in women undergoing evaluation for NCCAH, these analytes were treated as the primary adrenal biomarkers (Ng et al., 2023; Ueland et al., 2022). Given the recognized analytical advantages of mass spectrometry for adrenal steroids, basal and stimulated 17-OHP, 21DF, and cortisol were specified as being measured using a validated liquid chromatography–tandem mass spectrometry (LC-MS/MS) workflow whenever available, in line with modern diagnostic studies (Ng et al., 2023; Ueland et al., 2022).

The ovarian and androgen-related panel included total testosterone, sex hormone-binding globulin (SHBG), free androgen index (FAI), dehydroepiandrosterone sulfate (DHEA-S), androstenedione, anti-Müllerian hormone (AMH), luteinizing hormone (LH), follicle-stimulating hormone (FSH), LH/FSH ratio, prolactin, and thyroid-stimulating hormone (TSH). Fasting metabolic assessment included fasting glucose, fasting insulin, homeostatic model assessment for insulin resistance (HOMA-IR), triglycerides, and high-density lipoprotein cholesterol (HDL-C), see Table2.

Table2: Laboratory Analytes, Specimen Types, Analytical Methods, and Reporting Units

Analyte group	Variable(s)	Specimen	Method/platform basis	Reporting unit
Adrenal steroid core panel	Basal 17-OHP, ACTH-stimulated 17-OHP, basal 21DF, ACTH-stimulated 21DF, basal cortisol, ACTH-stimulated cortisol	Serum	Validated LC-MS/MS adrenal steroid profiling workflow aligned with current NCCAH diagnostic literature (Ng et al., 2023; Ueland et al., 2022)	nmol/L
Androgen status	Total testosterone	Serum/plasma	Roche Elecsys Testosterone II ECLIA on cobas e immunoassay analyzers (Roche Diagnostics, 2024a)	nmol/L
Binding protein	SHBG	Serum/plasma	Roche Elecsys SHBG ECLIA on cobas e immunoassay analyzers (Roche Diagnostics, 2024b)	nmol/L
Adrenal androgen	DHEA-S	Serum/plasma	Roche Elecsys DHEA-S ECLIA on cobas e immunoassay analyzers (Roche Diagnostics, 2024c)	µmol/L
Ovarian reserve/supportive marker	AMH	Serum/plasma	Roche Elecsys AMH Plus immunoassay on cobas e immunoassay analyzers (Roche Diagnostics, 2024d)	ng/mL
Metabolic hormone	Fasting insulin	Serum/plasma	Roche Elecsys Insulin ECLIA on Elecsys/cobas e immunoassay analyzers (Roche Diagnostics, 2024e)	µU/mL
Additional reproductive hormones	LH, FSH, prolactin, TSH	Serum/plasma	Quantified using validated automated immunoassays per routine clinical laboratory procedures and manufacturer instructions	mIU/mL (LH, FSH); ng/mL (prolactin); mIU/L (TSH)
Routine metabolic chemistry	Fasting glucose, triglycerides, HDL-C	Serum	Standard automated clinical chemistry methods used in the participating hospital laboratories	mg/dL

Analyte group	Variable(s)	Specimen	Method/platform basis	Reporting unit
Supportive androgenic steroid	Androstenedione	Serum/plasma	Standard laboratory steroid assay workflow	nmol/L

The verified Roche assay information used in this section was taken from the corresponding manufacturer product pages. Elecsys Testosterone II is described by Roche as an immunoassay for the quantitative determination of testosterone in human serum and plasma and is intended for use on cobas e immunoassay analyzers (Roche Diagnostics, 2024a). Elecsys SHBG is an ECLIA assay for the quantitative determination of SHBG in human serum and plasma (Roche Diagnostics, 2024b). Elecsys DHEA-S is an ECLIA assay for the quantitative determination of dehydroepiandrosterone sulfate in human serum and plasma (Roche Diagnostics, 2024c). Elecsys AMH Plus is indicated for quantitative determination of AMH in human serum and plasma (Roche Diagnostics, 2024d), and Elecsys Insulin is intended for quantitative determination of human insulin in human serum and plasma (Roche Diagnostics, 2024e).

2.8. Derived Variables

The free androgen index (FAI) was calculated as:

FAI (%) = 100 × total testosterone (nmol/L) / SHBG (nmol/L).

HOMA-IR was calculated using the standard formula:

HOMA-IR = fasting insulin (μU/mL) × fasting glucose (mmol/L) / 22.5.

The LH/FSH ratio was calculated by dividing serum LH by serum FSH. Binary clinical indicators, including oligo-amenorrhea, hirsutism, acne, alopecia, and PCOM, were coded as present or absent according to the final clinical assessment. Hirsutism was operationalized from the mFG score using a clinically accepted cut-off for categorical analysis.

2.9. Data Structure and Outcome Definition

The primary analytic objective was to distinguish women with NCCAH from those with PCOS using adrenal, androgenic, reproductive, and metabolic biomarkers. For diagnostic performance analyses, NCCAH was treated as the positive reference class. Basal steroid measurements represented morning follicular-phase serum values, while stimulated steroid measurements represented serum values obtained 60 minutes after cosyntropin stimulation. The final variable set included age, BMI, waist circumference, cycle characteristics, mFG score, categorical androgen-excess manifestations, ultrasound morphology, adrenal steroid variables, androgen profile, reproductive hormones, and metabolic markers.

2.10. Statistical Analysis

Continuous variables with approximate normal distribution were summarized as mean ± standard deviation (SD) and compared between groups using Welch's t test. Continuous variables showing skewed distributions, particularly endocrine and metabolic laboratory variables, were summarized as median and interquartile range (IQR) and compared using the Mann–Whitney U test. Categorical variables were presented as number/total (%) and compared using the chi-square test or Fisher's exact test, as appropriate according to expected cell counts. Single-marker diagnostic performance was assessed by receiver operating characteristic (ROC) curve analysis. For each biomarker, the area under the ROC curve (AUC) was calculated, and the internally derived optimal cutoff was selected using the maximum Youden index. Sensitivity and specificity were then estimated at that internally selected threshold. To quantify uncertainty in single-marker AUC estimates, 95% confidence intervals (CIs) were obtained using nonparametric bootstrap resampling with 2000 replicates.

Exploratory multivariable diagnostic modeling was performed using logistic regression after predictor standardization. The reported conservative multivariable models were restricted to predictors retaining residual between-group overlap in order to reduce the risk of overstatement caused by complete or near-complete separation. Model performance was summarized by apparent AUC, internal cutoff, apparent sensitivity, apparent specificity, and Brier score. Internal validation was conducted using repeated stratified 5-fold cross-validation with 100 repetitions. All statistical tests were two-sided, and a p value < 0.05 was considered statistically significant.

3. Results

3.1. Baseline Demographic and Clinical Characteristics

The cohort comprised 24 women with NCCAH and 48 women with PCOS. Baseline comparisons showed that the PCOS group had a more adverse anthropometric and menstrual profile, with significantly higher body mass index and waist circumference, longer cycle length, more frequent oligo-/amenorrhea, and a higher prevalence of polycystic ovarian morphology. By contrast, age, modified Ferriman–Gallwey score, hirsutism, acne, and alopecia did not differ significantly between groups, supporting the clinical observation that overt hyperandrogenic presentation alone does not reliably distinguish the two disorders in routine assessment, the presented in Table3.

Table3: Baseline Demographic and Clinical Characteristics of The Study Cohort.

Variable	NCCAH (n=24)	PCOS (n=48)	P value
Age, years	25.09 ± 3.77	26.07 ± 3.94	0.311
Body mass index, kg/m²	25.70 ± 3.90	31.87 ± 4.76	<0.001
Waist circumference, cm	87.80 ± 5.60	99.10 ± 9.39	<0.001
Cycle length, days	32.00 (29.00–49.50)	49.00 (35.00–62.00)	0.005
Modified Ferriman–Gallwey score	14.00 (10.75–15.00)	13.00 (11.00–15.00)	1.000
Oligo-/amenorrhea: Yes	10/24 (41.7%)	37/48 (77.1%)	0.004
Oligo-/amenorrhea: No	14/24 (58.3%)	11/48 (22.9%)	
Hirsutism: Yes	24/24 (100.0%)	48/48 (100.0%)	1.000
Hirsutism: No	0/24 (0.0%)	0/48 (0.0%)	
Acne: Yes	14/24 (58.3%)	25/48 (52.1%)	0.802
Acne: No	10/24 (41.7%)	23/48 (47.9%)	
Alopecia: Yes	3/24 (12.5%)	15/48 (31.2%)	0.147
Alopecia: No	21/24 (87.5%)	33/48 (68.8%)	
Polycystic ovarian morphology: Yes	9/24 (37.5%)	38/48 (79.2%)	0.001
Polycystic ovarian morphology: No	15/24 (62.5%)	10/48 (20.8%)	

Age, body mass index, and waist circumference are presented as mean ± SD and were compared using Welch's t test. Cycle length and modified Ferriman–Gallwey scores are presented as median (IQR) and were compared using the Mann–Whitney U test. Categorical variables are presented as n/N (%) and were compared using the chi-square test or Fisher's exact test, as appropriate.

3.2. Endocrine, Adrenal, and Metabolic Laboratory Profile

The biochemical comparisons showed that the clearest between-group differences were centered on adrenal steroid precursors, with markedly higher basal and ACTH-stimulated 17-hydroxyprogesterone and 21-deoxycortisol in NCCAH, whereas basal and stimulated cortisol concentrations were similar between the two groups Table4. In contrast, the PCOS group demonstrated a stronger ovarian-metabolic profile, characterized by higher total testosterone, lower SHBG, higher free androgen index, higher AMH, higher LH and LH/FSH ratio, and a more adverse fasting glycemic and lipid pattern. DHEA-S and prolactin did not differ significantly, whereas androstenedione was higher in NCCAH.

Table4: Baseline Hormonal and Metabolic Laboratory Profile of The Study Cohort.

Analyte	NCCAH (n=24)	PCOS (n=48)	P value
Basal 17-hydroxyprogesterone, nmol/L	7.13 (6.17–9.03)	1.81 (1.26–2.31)	<0.001
ACTH-stimulated 17-hydroxyprogesterone at 60 min, nmol/L	46.16 (31.28–49.91)	9.00 (7.51–13.36)	<0.001
Basal 21-deoxycortisol, nmol/L	1.08 (0.83–1.46)	0.17 (0.12–0.24)	<0.001
ACTH-stimulated 21-deoxycortisol at 60 min, nmol/L	24.93 (17.60–30.61)	3.97 (2.53–4.88)	<0.001
Basal cortisol, nmol/L	338.85 (266.88–382.32)	337.60 (279.62–383.77)	0.914
ACTH-stimulated cortisol at 60 min, nmol/L	585.45 (512.10–689.97)	640.85 (528.80–701.30)	0.507
Total testosterone, nmol/L	2.05 (1.77–2.11)	2.34 (2.12–2.56)	<0.001
SHBG, nmol/L	43.05 (36.07–45.40)	24.20 (17.80–31.27)	<0.001
Free androgen index, %	4.81 (3.78–5.82)	10.02 (7.58–12.42)	<0.001
DHEA-S, µmol/L	8.71 (7.28–10.12)	7.83 (6.27–9.09)	0.137
Androstenedione, nmol/L	12.30 (11.40–13.70)	10.64 (9.37–12.27)	0.002
Fasting glucose, mg/dL	88.65 (86.30–92.25)	94.45 (91.12–98.60)	<0.001
Fasting insulin, µU/mL	10.05 (6.88–11.20)	18.10 (15.35–20.45)	<0.001
HOMA-IR	2.16 (1.56–2.40)	4.26 (3.68–4.84)	<0.001
Triglycerides, mg/dL	88.45 (76.12–117.40)	156.10 (135.68–171.68)	<0.001
HDL-cholesterol, mg/dL	56.00 (51.30–64.00)	43.05 (39.17–47.52)	<0.001
AMH, ng/mL	3.81 (2.79–4.58)	6.75 (5.97–8.35)	<0.001
LH, mIU/mL	6.05 (5.00–6.86)	9.07 (8.14–10.13)	<0.001
FSH, mIU/mL	6.22 (5.84–6.91)	5.55 (4.87–6.35)	0.006
LH/FSH ratio	0.98 (0.80–1.09)	1.62 (1.41–1.90)	<0.001
Prolactin, ng/mL	11.46 (8.76–13.62)	12.50 (9.75–15.75)	0.150
TSH, mIU/L	1.42 (1.23–1.70)	2.08 (1.67–2.65)	<0.001
All laboratory variables are presented as median (IQR) and were compared using the Mann–Whitney U test as a conservative approach for potentially skewed endocrine and metabolic measurements. Basal steroid measurements			

Analyte	NCCAH (n=24)	PCOS (n=48)	P value
represent morning serum samples collected during the early follicular phase whenever feasible. ACTH-stimulated steroid measurements represent serum samples obtained 60 min after cosyntropin stimulation.			

To visualize the distributional contrast in the adrenal domain, the selected adrenal markers are shown graphically in Fig.1, which illustrates the marked upward shift of 17-hydroxyprogesterone and 21-deoxycortisol in NCCAH relative to PCOS.

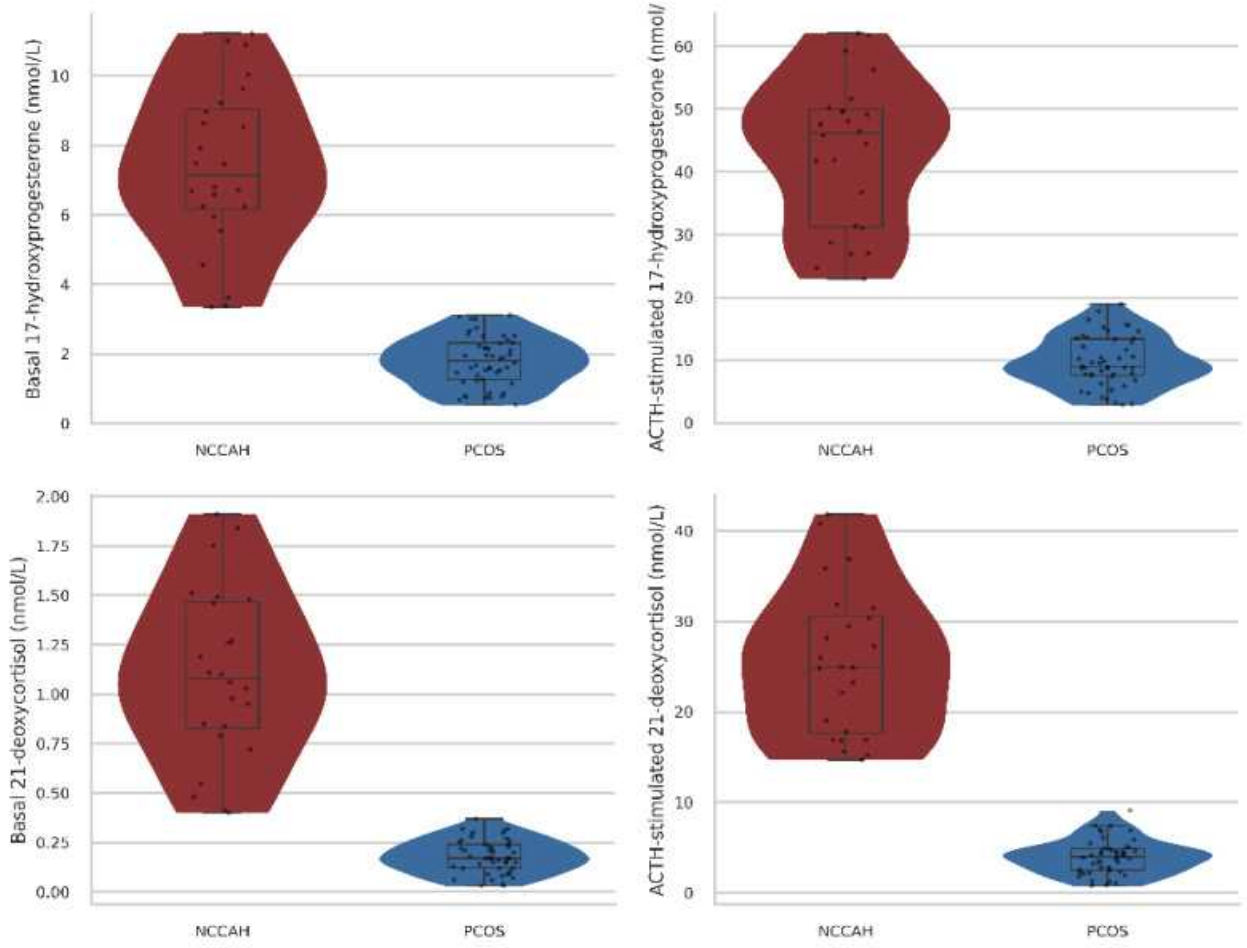

Figure1: Distribution of Selected Adrenal Biomarkers in The Study Cohort.

3.3. Apparent Discrimination of Individual Biomarkers

When NCCAH was treated as the positive reference class, the strongest apparent single-marker discrimination was observed for basal and ACTH-stimulated 17-hydroxyprogesterone and 21-deoxycortisol, all of which showed apparent complete between-group nonoverlap within the current dataset. Among markers with residual overlap, HOMA-IR showed the highest apparent discrimination, followed by AMH, LH/FSH ratio, free androgen index, and SHBG. Because cutoff

points were selected internally, the values shown in Table5 should be interpreted as within-dataset thresholds rather than externally validated clinical decision limits.

Table5: Diagnostic Performance of Individual Biomarkers Within the Study Cohort.

Biomarker	Direction for NCCAH	Internal cutoff	Apparent AUC	95% CI	Sensitivity	Specificity	Nonoverlap
ACTH-stimulated 17-hydroxyprogesterone	≥ cutoff	22.98	1.000	1.000–1.000	100.0%	100.0%	Yes
ACTH-stimulated 21-deoxycortisol	≥ cutoff	14.70	1.000	1.000–1.000	100.0%	100.0%	Yes
Basal 17-hydroxyprogesterone	≥ cutoff	3.34	1.000	1.000–1.000	100.0%	100.0%	Yes
Basal 21-deoxycortisol	≥ cutoff	0.40	1.000	1.000–1.000	100.0%	100.0%	Yes
HOMA-IR	≤ cutoff	3.15	0.961	0.915–0.992	95.8%	85.4%	No
AMH	≤ cutoff	5.95	0.944	0.885–0.983	100.0%	77.1%	No
LH/FSH ratio	≤ cutoff	1.30	0.942	0.877–0.988	91.7%	89.6%	No
Free androgen index	≤ cutoff	6.42	0.915	0.849–0.973	91.7%	83.3%	No
SHBG	≥ cutoff	32.40	0.895	0.808–0.961	91.7%	83.3%	No

To avoid visually overstating discrimination through multiple nonoverlap adrenal curves, Fig.2 focuses on markers that retained residual overlap and, on the overlap, -only multivariable model with the strongest internally assessed performance.

3.4. Exploratory Overlap-Only Multivariable Models

Exploratory multivariable modeling was intentionally restricted to biomarkers that retained residual overlap between groups. Under this conservative approach, the strongest internally assessed model was Model A (SHBG, HOMA-IR, and AMH), with an apparent AUC of 0.993 and a repeated 5-fold cross-validated AUC of 0.988 Fig.2. A closely related model combining SHBG, HOMA-IR, and LH/FSH ratio also showed high internal performance. Adrenal-containing logistic models were not emphasized because the marked nonoverlap of adrenal markers in the current dataset would produce unstable or trivially perfect model behavior, as indicated in Table6.

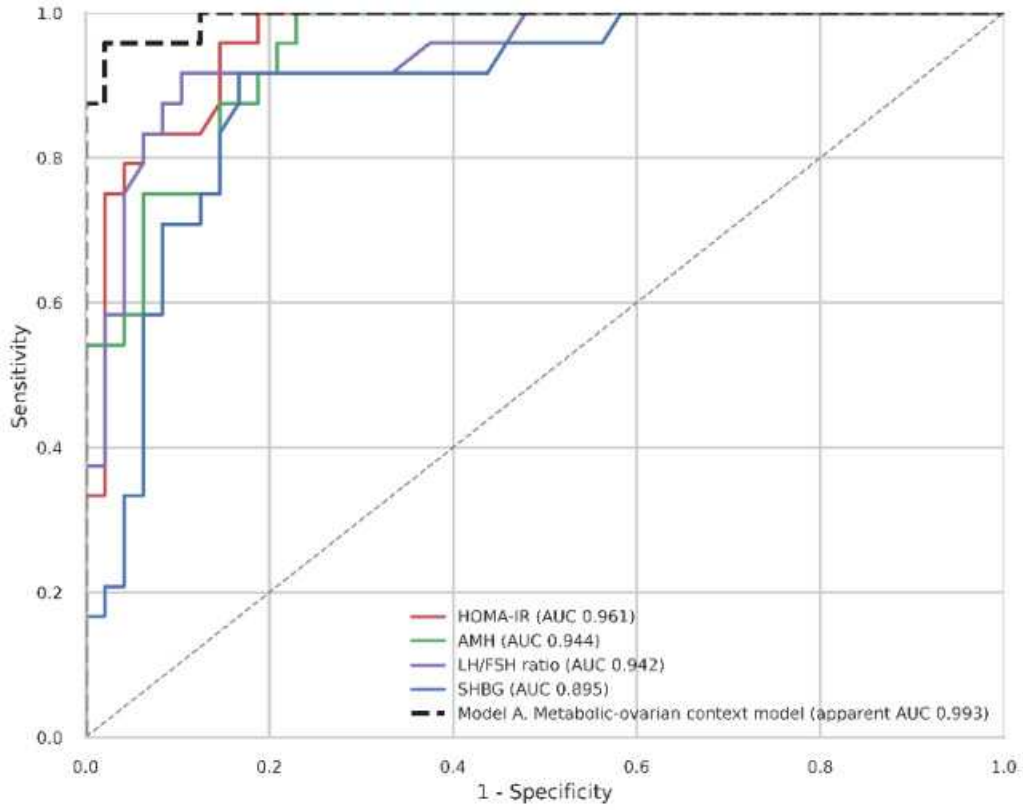

Figure2: Receiver Operating Characteristic Curves for Overlapping Biomarkers and An Overlap-Only Multivariable Model.

Table4: Conservative Summary of Overlap-Only Multivariable Models.

Model	Predictors	Apparent AUC	95% CI	5-fold CV AUC	CV interval	Brier	Sens/Spec
Model A. Metabolic-ovarian context	SHBG, HOMA-IR, AMH	0.993	0.976–1.000	0.988	0.983–0.991	0.038	95.8% / 97.9%
Model B. Metabolic-androgen context	SHBG, HOMA-IR, LH/FSH ratio	0.987	0.959–1.000	0.983	0.977–0.986	0.040	95.8% / 97.9%
Adrenal-containing logistic models	Basal 17-OHP and/or basal 21DF	Not emphasized	Not reported	Not reported	Not reported	N/R	N/R

To reduce overstatement related to separation, multivariable summaries were restricted to models based on biomarkers with residual between-group overlap. Apparent AUC values were estimated in the full dataset, whereas internal validation was summarized using repeated stratified 5-fold cross-validation with 100 repetitions. These models should be interpreted as exploratory and internally assessed.

The relationship between binding protein status, androgenic context, and insulin resistance is shown in Fig.3, which demonstrates inverse within-group associations of SHBG with both free androgen index and HOMA-IR, with the metabolic relationship appearing more pronounced in PCOS.

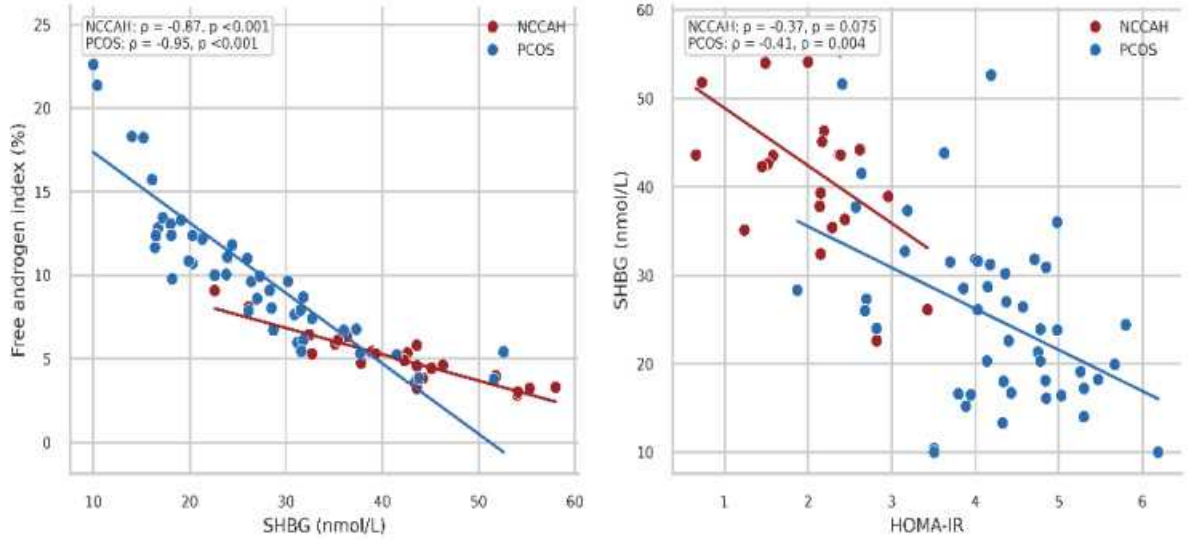

Figure3: Group-Specific Associations of SHBG With Free Androgen Index and Insulin Resistance Markers.

For a compact comparison of the internally estimated discrimination metrics across overlapping markers and models, **Fig.4** presents a forest plot of AUC estimates and their uncertainty intervals.

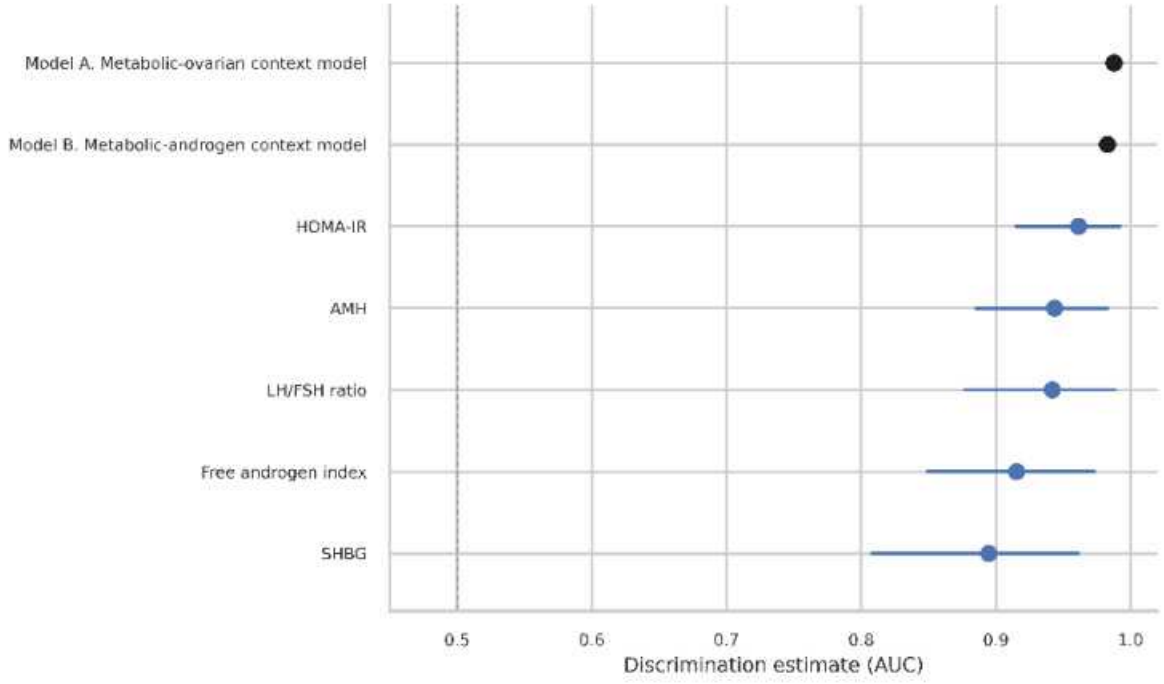

Figure4: Forest Plot of Discrimination Estimates For Selected Overlapping Biomarkers and Overlap-Only

4. Discussion

In this study of women presenting with hyperandrogenic features, the most important finding was that the clearest separation between NCCAH and PCOS occurred at the level of adrenal steroid intermediates, whereas the broader

metabolic and ovarian-context profile was more strongly aligned with PCOS. Within the present cohort, basal and ACTH-stimulated 17-hydroxyprogesterone and 21-deoxycortisol were markedly higher in NCCAH, while women classified as PCOS had higher body mass index, larger waist circumference, longer cycle intervals, more frequent oligo-/amenorrhea, a higher prevalence of polycystic ovarian morphology, higher total testosterone, lower SHBG, higher free androgen index, higher AMH, higher LH and LH/FSH ratio, and a more adverse glyco-metabolic and lipid profile. At the same time, some features that often motivate initial clinical referral—particularly hirsutism, acne, and overall hyperandrogenic presentation—showed substantial overlap between groups. Taken together, these findings support a two-level interpretation: first, clinical phenotype alone is insufficient for etiologic discrimination between NCCAH and PCOS in hyperandrogenic women; second, targeted adrenal steroid testing remains central when the differential diagnosis includes NCCAH. This broad pattern is consistent with the systematic review by Papadakis and colleagues, who emphasized that PCOS and nonclassic congenital adrenal hyperplasia share many clinical features, including hirsutism, menstrual dysfunction, obesity, insulin resistance, dyslipidaemia, polycystic ovarian morphology, and subfertility, making clinical separation difficult in routine practice (Papadakis et al., 2019). Our own data reproduce that overlap clearly. All women in both groups were hirsute, the modified Ferriman–Gallwey score did not differ, acne was common in both groups, and alopecia was numerically but not significantly more frequent in PCOS. These observations argue against reliance on surface phenotype alone, particularly in referral populations enriched for androgen excess. They also help explain why women with NCCAH are frequently initially evaluated within a PCOS framework before biochemical clarification is undertaken (Papadakis et al., 2019). The clinical and ovarian-context differences in the present cohort, however, still favored a more classic PCOS profile in the PCOS group. Cycle length was longer, oligo-/amenorrhea was more frequent, polycystic ovarian morphology was much more prevalent, LH was higher, FSH was modestly lower, and the LH/FSH ratio was clearly shifted upward in PCOS. This directional pattern is in line with the study by Pall et al. (2010), who reported that women with PCOS had a higher frequency of oligomenorrhea or amenorrhea than women with 21-hydroxylase-deficient NCCAH, together with a higher prevalence of polycystic ovaries and a greater likelihood of LH/FSH ratio >2. Therefore, although menstrual dysfunction and ovarian morphology are not sufficient to exclude NCCAH, they remain informative in describing the dominant reproductive phenotype once biochemical classification has been established. In practical terms, our results suggest that NCCAH in a hyperandrogenic clinic population may remain clinically visible as androgen excess, yet be less dominated by the ovarian dysfunction signature that typifies PCOS. The present findings also align with the available Iraqi PCOS literature, which, although mostly designed as PCOS-versus-control comparisons rather than NCCAH differential-diagnosis studies, provides an important local benchmark. Abdalqader and Hussein (2020) reported in Iraqi women that hirsutism, acne, elevated LH, and increased free testosterone were significantly more frequent in PCOS, supporting the view that ovarian-pattern hyperandrogenism and gonadotropin disturbance are prominent in local PCOS cohorts. Alsaadi and Mohamad (2019) similarly found that BMI, LH, FSH, and testosterone differed significantly between Iraqi women with PCOS and controls, and that hirsutism and alopecia were common clinical manifestations, with obesity strengthening this phenotype. In the same direction, Al-Lami, Al-Tu'ma and Al-Safi (2020) observed that Iraqi women with PCOS had significantly higher AMH, insulin, insulin resistance, LH, total testosterone, and fasting blood glucose than controls, while FSH did not differ significantly. Collectively, these Iraqi data make the current PCOS profile biologically and regionally plausible: our PCOS group displayed the same clustering of ovarian dysfunction, androgen excess, and metabolic disturbance described in local studies, even though the present work addressed a different clinical question, namely discrimination from NCCAH rather than comparison with healthy controls. One of the most important messages of the current study concerns the adrenal steroid axis. Basal and stimulated 17-hydroxyprogesterone, as well as basal and

stimulated 21-deoxycortisol, were substantially higher in NCCAH than in PCOS, whereas basal and stimulated cortisol concentrations were not materially different between groups. This indicates that, in our cohort, the discriminatory information arose primarily from steroid precursor accumulation, not from gross differences in cortisol output. That interpretation is strongly supported by the recent study of Ng et al. (2023), who evaluated women with androgen excess and showed that both 17-hydroxyprogesterone and 21-deoxycortisol, especially after ACTH stimulation, had high diagnostic utility for NCCAH. In that real-world study, optimal thresholds were 3.0 nmol/L for basal 17-hydroxyprogesterone, 20.7 nmol/L for stimulated 17-hydroxyprogesterone, 0.31 nmol/L for basal 21-deoxycortisol, and 13.3 nmol/L for stimulated 21-deoxycortisol, with stimulated 21-deoxycortisol achieving perfect sensitivity and specificity in their evaluated subset (Ng et al., 2023). Notably, the internally derived cutoffs in our dataset for these same markers were directionally similar, which increases the face validity of the present biomarker pattern. However, this similarity must be interpreted very cautiously because our thresholds were selected internally and within the present cohort, whereas the thresholds reported by Ng et al. were derived from a real diagnostic population (Ng et al., 2023). Equally important is what should not be concluded from our adrenal biomarker results. In Table3, basal and stimulated 17-hydroxyprogesterone and 21-deoxycortisol showed apparent complete between-group nonoverlap with apparent AUC values of 1.00. In a manuscript for a high-quality journal, such findings should be described as within-dataset apparent separation, not as proof of universally perfect clinical performance. This restraint is necessary for two reasons. First, the current cohort is relatively modest, so the observed nonoverlap may not fully capture the biological variability encountered in broader clinical practice. Second, even in real cohorts, small overlaps and borderline values can occur. Pall et al. (2010) reported that basal follicular-phase 17-hydroxyprogesterone >2 ng/mL was found in 87% of women with NCCAH, but also in 25% of lean women with PCOS, 20% of obese women with PCOS, and 7% of controls. This observation is particularly relevant because it shows that basal 17-hydroxyprogesterone alone can be informative without being absolutely disease-exclusive, reinforcing the need for careful timing, standardized testing conditions, and confirmatory dynamic assessment when indicated. Thus, our data support the strong utility of adrenal biomarkers, but they do not justify claiming perfect external discrimination. The current androgen profile further refines the distinction between the two disorders. In our cohort, total testosterone and free androgen index were higher in PCOS, while SHBG was lower, consistent with a more pronounced ovarian-androgen plus metabolic phenotype in the PCOS group. By contrast, androstenedione was higher in NCCAH, while DHEA-S did not differ significantly. This mixed pattern is clinically plausible. Pall et al. (2010) observed that mean androstenedione and DHEAS levels were highest in NCCAH, whereas the current cohort identified a clearer difference for androstenedione than for DHEA-S. The lack of a statistically significant DHEA-S difference in our data should therefore not be interpreted as evidence against adrenal involvement; rather, it suggests that not all adrenal-related androgens carry equal discriminatory value in every cohort. The stronger separation of 17-hydroxyprogesterone and 21-deoxycortisol compared with DHEA-S in the present analysis is fully compatible with the literature emphasizing steroid intermediates rather than nonspecific adrenal androgens as the most direct biochemical markers of 21-hydroxylase-related disease (Ng et al., 2023; Papadakis et al., 2019). Our AMH and gonadotropin findings also deserve attention. AMH was clearly higher in PCOS than NCCAH, and LH was higher while FSH was slightly lower in PCOS, producing a higher LH/FSH ratio. These results fit well with the ovarian-context dominance of the PCOS phenotype in the present cohort. They are also concordant with Iraqi evidence. Saleh, Ibraheem and Ameen (2015) demonstrated the relevance of AMH in Iraqi women with PCOS evaluated under a structured early-cycle protocol and, importantly, documented that congenital adrenal hyperplasia and other competing hyperandrogenic disorders were excluded in that PCOS framework. Al-Lami, Al-Tu'ma and Al-Safi (2020) likewise reported higher AMH and LH in Iraqi women with PCOS, whereas FSH was not significantly different. Accordingly, the

current finding of higher AMH in PCOS does not challenge the presence of hyperandrogenism in NCCAH; instead, it suggests that AMH may reflect the ovarian follicular milieu and ovarian dysfunction burden more strongly than the adrenal etiology of androgen excess. This interpretation is also consistent with the lower prevalence of polycystic ovarian morphology in NCCAH reported by Pall et al. (2010). The metabolic results in this study were among the most clinically relevant non-adrenal findings. Compared with NCCAH, the PCOS group had higher body mass index, higher waist circumference, higher fasting glucose, higher fasting insulin, higher HOMA-IR, and higher triglycerides, together with lower HDL-cholesterol. This constellation indicates a markedly less favorable cardiometabolic profile in the PCOS group. The direction of these findings is strongly supported by Iraqi and international literature. In Iraq, Al-Lami, Al-Tu'ma and Al-Safi (2020) reported higher fasting blood glucose, insulin, and insulin resistance in women with PCOS, while Alsaadi and Mohamad (2019) showed a clear excess of overweight and obesity in Iraqi PCOS populations. Internationally, Piróg et al. (2024) found that both NCCAH and PCOS could display metabolic abnormalities relative to controls, but that PCOS showed the more adverse metabolic profile, including higher triglycerides, fasting insulin, and HOMA-IR, whereas NCCAH had lower insulin and HOMA-IR than PCOS. Pall et al. (2010) similarly showed that metabolic dysfunction was greatest in obese women with PCOS, while women with NCCAH and lean women with PCOS did not differ materially in metabolic dysfunction. Our results fit squarely within this framework, and suggest that, in women presenting with androgen excess, metabolic context may provide supportive—but not etiologically definitive—information pointing toward PCOS.

These metabolic findings have important mechanistic and clinical implications. Mechanistically, the current data are consistent with the view that the PCOS phenotype in many women is characterized not only by androgen excess but also by a stronger association with adiposity, insulin resistance, and dyslipidaemia, whereas NCCAH may be more specifically driven by an adrenal steroidogenic abnormality that can overlap phenotypically with PCOS without reproducing the full ovarian-metabolic syndrome in every case (Papadakis et al., 2019; Piróg et al., 2024). Clinically, this means that a hyperandrogenic woman with obesity, longer cycles, marked insulin resistance, high AMH, and polycystic ovarian morphology may still require adrenal testing if NCCAH is part of the differential diagnosis, but the pre-test clinical impression may reasonably lean toward PCOS. Conversely, when adrenal markers are elevated despite a less pronounced metabolic phenotype, NCCAH becomes more plausible. The key point is that metabolic severity should guide risk stratification and management, but should not replace etiologic endocrine testing. The apparent within-dataset discrimination analyses reinforce this interpretation. Among markers with residual overlap, HOMA-IR, AMH, LH/FSH ratio, free androgen index, and SHBG all showed strong apparent discriminatory capacity, and the exploratory overlap-only model combining SHBG, HOMA-IR, and AMH yielded excellent internally cross-validated performance. Yet these variables should be understood as contextual classifiers of phenotype, not substitutes for etiologic adrenal assessment. Papadakis et al. (2019) underscored that the overlap between PCOS and NCCAH is substantial across clinical and metabolic domains, and the current findings extend that principle: even if an overlap-only model performs well inside one dataset, such performance does not eliminate the need to identify the underlying disorder correctly. In other words, ovarian-metabolic markers may help frame probability, but adrenal biomarkers remain decisive for confirming NCCAH. A further clinically important point is that the present data argue for a structured diagnostic pathway rather than single-stage labeling of all hyperandrogenic women as PCOS. Iraqi PCOS studies have generally applied Rotterdam-based diagnostic frameworks while explicitly excluding congenital adrenal hyperplasia and other secondary causes of hyperandrogenism (Saleh, Ibraheem & Ameen, 2015). That approach is methodologically sound and remains clinically important. Our findings strengthen that principle by showing how markedly the adrenal steroid profile can diverge even when the presenting symptoms appear similar. The practical implication is that NCCAH should remain part of the routine differential

diagnosis in women with hyperandrogenism, especially when biochemical evaluation is available and particularly in settings where referral populations are enriched for menstrual irregularity and hirsutism (Ng et al., 2023; Papadakis et al., 2019; Pall et al., 2010). The present study has several strengths. It examined both basal and ACTH-stimulated adrenal steroids, including 21-deoxycortisol, which is increasingly recognized as a highly informative NCCAH biomarker (Ng et al., 2023). It also integrated adrenal, ovarian, and metabolic domains in the same analytical framework, allowing a clinically useful comparison between etiologic markers and phenotypic context markers. In addition, the discussion can be grounded against both local Iraqi studies and international literature, which strengthens the regional interpretability of the PCOS phenotype observed here (Abdalqader & Hussein, 2020; Saleh, Ibraheem & Ameen, 2015; Al-Lami, Al-Tu'ma & Al-Safi, 2020; Alsaadi & Mohamad, 2019). At the same time, the study has important limitations, and these limitations should be stated explicitly to avoid overclaiming. Most importantly, the present study was conducted in a relatively modest hospital-based cohort, and therefore the observed degree of between-group separation—especially for adrenal steroids—cannot be assumed to reproduce the full variability of routine clinical practice. The sample size was also modest, particularly for NCCAH, which increases the instability of internally optimized cutoffs and amplifies the possibility of nonoverlap by chance or by construction. For the same reason, the multivariable models should be regarded as exploratory and internally assessed only, not as externally validated diagnostic tools. In addition, the Iraqi comparator literature available for this discussion consists largely of PCOS-versus-control studies rather than direct NCCAH-versus-PCOS head-to-head analyses, which limits the precision of regional comparison on the NCCAH side (Abdalqader & Hussein, 2020; Saleh, Ibraheem & Ameen, 2015; Al-Lami, Al-Tu'ma & Al-Safi, 2020; Alsaadi & Mohamad, 2019). Finally, because our study was not designed to address treatment response, reproductive outcomes, or longitudinal cardiometabolic trajectories, the present findings should be interpreted as contributing to diagnostic differentiation, not long-term prognosis. These limitations naturally define the most important future directions. Prospective real-world studies in Iraqi and broader Middle Eastern populations are needed to validate basal and ACTH-stimulated 17-hydroxyprogesterone and 21-deoxycortisol thresholds under standardized follicular-phase conditions, ideally with clearly adjudicated NCCAH and PCOS diagnoses. Larger studies should also evaluate whether overlap-only markers such as HOMA-IR, AMH, SHBG, and LH/FSH ratio add incremental value once adrenal steroids are already known, rather than being interpreted in isolation. Because the current data and recent literature both suggest a heavier metabolic burden in PCOS than NCCAH (Piróg et al., 2024; Pall et al., 2010), future work should also examine whether combined endocrine-metabolic phenotyping can improve clinical triage, cost-effective testing strategies, and personalized follow-up without sacrificing diagnostic accuracy. Most importantly, external validation in real cohorts is essential before any internally derived threshold or multivariable model from the present analysis is considered for clinical adoption.

Conclusion

In this hospital-based cohort of women presenting with hyperandrogenic features, the most informative distinction between NCCAH and PCOS was observed in the adrenal steroid profile, particularly 17-hydroxyprogesterone and 21-deoxycortisol, whereas the PCOS phenotype was characterized more strongly by menstrual dysfunction, ovarian-pattern hormonal disturbance, insulin resistance, and a less favorable lipid profile. These findings support the practical value of an adrenal-centered, context-enriched biomarker framework for differential evaluation in women with androgen excess, while also indicating that exploratory multivariable models based on overlapping non-adrenal markers should be interpreted as supportive rather than definitive.

Declarations

Ethics approval and consent to participate

The study was conducted in accordance with the Declaration of Helsinki and local institutional ethical requirements. Written informed consent was obtained from all participants before enrollment and blood sampling.

Consent for publication

Not applicable.

Funding

No specific external funding was declared for this work.

Conflicts of Interest

The author declares no conflict of interest.

Authors' contributions

The author contributed to study conception and design, participant recruitment, data acquisition, laboratory coordination, statistical analysis, interpretation of the findings, drafting of the manuscript, critical revision of the article, and approval of the final submitted version.

Data availability

The data that support the findings of this study are available from the corresponding author on reasonable request, subject to institutional and ethical restrictions.

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