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Assessing C-reactive protein/albumin ratio as a new predictor of Polycystic Ovary Syndrome: a case-control study

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Keywords:	polycystic ovary syndrome, inflammation, C-reactive protein, albumin, pathophysiology

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3 **1 Assessing C-reactive protein/albumin ratio as a new predictor of Polycystic Ovary**
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5 **2 Syndrome: a case-control study**
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9 Short title: CRP/Albumin as a predictor of PCOS
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Keywords: polycystic ovary syndrome, inflammation, pathophysiology, C-reactive protein, albumin

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3 35
4 36 **Abstract**

5
6 37 *Objective:* Polycystic ovary syndrome (PCOS) is an endocrine disorder affecting
7
8 38 approximately one in seven women who experience androgen excess, menstrual cycle
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10 39 irregularities, frequent anovulation, and a tendency for central obesity and insulin resistance.
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12 40 Chronic subclinical inflammation is now recognized as being common in the context of
13
14 41 PCOS, which led to the postulation that PCOS may fundamentally be an inflammatory
15
16 42 process. This study aimed to: 1) evaluate serum CRP/albumin ratio as a predictor of PCOS;
17
18 43 2) compare the relationship between CRP/albumin and PCOS to classical predictors of the
19
20 44 syndrome.

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22
23 45 *Design:* Case-control study.

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25 46 *Setting:* Adult obstetrics/gynaecology, endocrinology and outpatient clinics; university
26
27 47 hospital in Bahrain.

28
29 48 *Participants:* 200 premenopausal women with a diagnosis of PCOS, and 119 ethnically-
30
31 49 matched eumenorrheic premenopausal women.

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33 50 *Main Outcome Measures:* CRP/albumin ratio, anthropometric measures, insulin resistance,
34
35 51 androgen excess.

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37 52 *Results:* Independent of body mass index (BMI), receiver operating characteristic (ROC)
38
39 53 curve for CRP/albumin ratio as predictor of PCOS was 0.865 (95% CI: 0.824–0.905), which
40
41 54 was more sensitive than CRP alone. Binary regression analysis showed that CRP/albumin
42
43 55 ratio outperformed classical markers, free androgen index and insulin resistance, in predicting
44
45 56 PCOS for every BMI category.

46
47 57 *Conclusion:* CRP/albumin ratio, a marker for inflammation related to metabolic dysfunction,
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49 58 better predicts PCOS than either androgen excess or insulin resistance. Inflammation is
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51 59 known to be influenced by adiposity, but relative to controls, women with PCOS have higher
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3 60 levels of CRP/albumin irrespective of BMI. These findings support the view that
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5 61 inflammation plays a central role in the pathophysiology of PCOS.
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11 64 **Article Summary**

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13 65 *Strengths and limitations of this study*

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16 66 • This analysis addressed previous limitations of studies, namely small sample sizes,
17
18 67 heterogeneous populations, and confounding factors (such as BMI), that have
19
20 68 attempted to show PCOS is an inflammatory process
21
22 69 • The relationship between inflammation and PCOS was assessed using CRP/albumin
23
24 70 ratio, which may be a better marker for inflammation in the context of metabolic
25
26 71 dysfunction
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29 72 • Limitation: study used waist circumference as a substitute for visceral adiposity; gold
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31 73 standard is computed tomography (CT) or magnetic resonance imaging (MRI)
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75 Introduction

76 Polycystic ovary syndrome (PCOS) is the most common reproductive disorder, affecting 5 to
77 15 % of premenopausal women worldwide^{1,2}. The prevalence of PCOS is increasing¹,
78 which may partly be attributed to improved diagnosis, as well as an increase in environmental
79 factors that may predispose to the development of this complex metabolic condition. PCOS is
80 characterized by androgen excess, menstrual irregularities, ovulatory disturbances, and is
81 often associated with central obesity and insulin resistance³⁻⁵. As such, women with PCOS
82 are at an increased risk for a number of health issues, including infertility, cardiovascular
83 disease and diabetes^{3,6,7}.

84 Possibly related to the constellation of endocrine and metabolic dysfunction they experience,
85 women with PCOS are also found to have greater chronic subclinical inflammation⁸⁻¹¹,
86 which is often clinically assessed by measuring serum levels of C-reactive protein (CRP).
87 CRP is a liver-derived acute phase protein produced in response to IL-6 secreted from
88 activated cells such as macrophages and adipocytes^{12,13}. A meta-analysis of 31 studies
89 concluded that systemic CRP levels are 96% higher in women with PCOS compared to
90 control women¹⁴. Collectively, these findings have given rise to the speculation that
91 inflammation may play a pivotal role in the pathophysiology of PCOS⁸⁻¹⁰.

92 Elevated serum CRP levels are linked to several health risk factors experienced by women
93 with PCOS, particularly insulin resistance and heightened risk of type 2 diabetes^{7,15,16}.

94 Chronic inflammation also contributes to endothelial dysfunction, exacerbating the
95 development of atherosclerotic plaques, triggering the onset of cardiovascular disease (CVD)
96¹⁷. As such inflammation has been associated with both CVD and coronary artery disease in
97 women with PCOS¹⁰.

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3 98 In contrast to CRP, albumin is a negative acute phase response protein produced by the liver.
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5 99 Serum levels of albumin are reduced in individuals experiencing chronic inflammation¹⁸. In
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7 100 addition to its role as a binding molecule for sex steroids¹⁹, albumin also provides the
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9 101 majority of the total antioxidant capacity of normal plasma¹⁸. The ratio of serum CRP levels
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11 102 over serum albumin (CRP/albumin) was found to be strongly associated with more severe
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13 103 metabolic dysfunction in premenopausal women with induced alterations to their ovarian
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15 104 hormone status²⁰. CRP/albumin ratio was also found to be significantly higher in
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17 105 premenopausal women with PCOS relative to controls, and adversely predicted their bone
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19 106 quality²¹. Given the ability of CRP/albumin to simultaneously capture chronic inflammation
20
21 107 and metabolic dysfunction in premenopausal women, we hypothesized that CRP/albumin
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23 108 ratio would, in itself, serve as a strong predictor of PCOS in a cohort of similarly aged
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25 109 women. This case-control study investigated CRP/albumin ratio along with classical markers,
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27 110 androgen excess and insulin resistance, in their ability to predict PCOS in 319 premenopausal
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29 111 Bahraini Arab women.
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35 113 **Methods**36
37 114 *Study subjects*

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39 115 Women with PCOS (n = 200) were recruited from adult obstetrics/gynecology,
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41 116 endocrinology and outpatient clinics in Manama, Bahrain. Women without PCOS (n = 119)
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43 117 were ethnically-matched, eumenorrheic university employees and students, and healthy
44
45 118 volunteers representative of the Bahraini population. Women serving as controls were
46
47 119 examined in the follicular phase of their menstrual cycle, and had their testosterone levels
48
49 120 were within range. A diagnosis of PCOS was based on the 2003 Rotterdam Criteria, which
50
51 121 requires two of the three following criteria to be met: ultrasound evidence of polycystic
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53 122 ovarian morphology, anovulation, and hyperandrogenism²². Exclusion criteria included
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3 123 hyperprolactinemia, non-classical adrenal hyperplasia, androgen-producing tumors, 21-
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5 124 hydroxylase deficiency, Cushing's syndrome, and active thyroid disease. Additional
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7 125 exclusion criteria included extremes of body mass index (BMI; $<18 \text{ kg/m}^2$ or $>45 \text{ kg/m}^2$),
8
9 126 recent/present illness, and treatment affecting carbohydrate metabolism or hormonal levels,
10
11 127 for three months or longer before inclusion the study. Women using anti-hypertensive, oral
12
13 128 contraceptive, anti-inflammatory, and lipid-lowering drugs were also excluded. Demographic
14
15 129 information, along with detailed personal and family history of diabetes, hypertension,
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17 130 infertility, hypercholesterolemia, and ischemic heart disease were obtained from all
18
19 131 participants. This study was conducted in accord with the Helsinki II Declaration guidelines,
20
21 132 and all participants gave written informed consent to participate. Study approval was
22
23 133 obtained from the Bahraini Ministry of Health and Arabian Gulf University Research and
24
25 134 Ethics Committees (IRB number: 35-PI-01/15) and the Clinical Research Ethics Board of the
26
27 135 University of British Columbia (H16-02101).
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32 33 137 *Biochemical analysis*

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35 138 Peripheral venous fasting blood samples were obtained between 7:00 and 9:00 am following
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37 139 an overnight ($> 12 \text{ h}$) fast during the early follicular phase of the menstrual cycle (days 2 ± 5)
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39 140 for control women, or women with PCOS who did not present with menstrual irregularities,
40
41 141 or any day for women with PCOS with menstrual irregularities. Serum samples were
42
43 142 analyzed for sex hormone binding globulin (SHBG) by sandwich ELISA (R&D Systems,
44
45 143 Minneapolis, MN); assay sensitivity was 0.01 nmol/ml , and inter-assay and intra-assay
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47 144 precision (CV %) were 5.3% and 4.3%, respectively.
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51 145 Serum luteinizing hormone (LH), follicular stimulating hormone (FSH), thyroid stimulating
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53 146 hormone (TSH), testosterone, glucose (ADVIA Centaur, Bayer Vital, Fernwald, Germany),
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55 147 and insulin (IMMULITE 2000, DPC Biermann, Bad Nauheim, Germany), were measured by
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3 148 automated chemiluminescence immunoassays. Free (FT) and bioactive (BT) testosterone and
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5 149 free androgen index (FAI) were determined using Free & Bioavailable Testosterone
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7 150 Calculator (www.issam.ch/freetesto.htm). Concentrations of serum albumin were analyzed
8
9 151 by photospectrometry with albumin bromocresol purple assay on a COBAS c701 Chemistry
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11 152 Analyzer (Roche Diagnostics, Dubai, UAE). Insulin resistance (IR) was estimated by the
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13 153 homeostasis model assessment (HOMA-IR), defined as fasting serum insulin (IU/mL) ×
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15 154 fasting plasma glucose (mmol/L)/22.5.

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18 155 Measurement of plasma high sensitivity CRP levels was done by latex-enhanced
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20 156 nephelometry on a BN II Nephelometer (Dade Behring, Milan, Italy). Samples were assayed
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22 157 in duplicate in each analytical run; the lower limit of detection was 0.15 mg/L, and the assay
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24 158 range was 0.175–11.0 mg/L (initial dilution). Serial serum dilutions were made in measuring
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26 159 high CRP (>30 mg/L) levels. Percentile CRP values were estimated for comparison
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29 160 purposes.

31 32 161 *Statistical analysis*

33
34 162 The *core outcome set* of variables included assessment of CRP/albumin as a predictor of
35
36 163 PCOS while controlling for relevant factors, such as BMI and age, and to subsequently
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38 164 compare the strength of the relationship between CRP/albumin and PCOS with classical
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40 165 predictors of the syndrome, androgen excess and insulin resistance. Baseline characteristics
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42 166 were compared using the Mann-Whitney U test and the independent samples t-test for the
43
44 167 continuous variables, and the χ^2 test for categorical variables. Numerical variables are
45
46 168 presented as mean ± standard deviation (SD). Because distribution of CRP/albumin levels
47
48 169 was skewed to the right, correlations between CRP/albumin ratio and other continuous
49
50 170 variables were assessed using Spearman's rho. Univariate general linear models were applied
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52 171 to test independent associations between CRP/albumin ratio and other independent variables.
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3 172 The optimal cut-off level for the CRP/albumin ratio was determined by a receiver operating
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5 173 characteristic (ROC) curve analysis, and the areas under the curve (AUC) were measured and
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7 174 compared to assess the power of a model to identify patients who experienced metabolic
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9 175 disturbances. Cut-off values showing the greatest accuracy were determined using a
10
11 176 sensitivity/specificity versus criterion value plot. Quartiles of CRP and CRP/albumin ratio
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13 177 were calculated separately in PCOS and control groups; and since 25% quartile of CRP value
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15 178 in patient group was very close to standard value of normal level of CRP, we decided to use
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17 179 25 quartile values for CRP and CRP/albumin ratio in PCOS group as cut-off values for the
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19 180 two predictors respectively.

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23 181 Using the calculated cut-off values, regression analysis was performed to determine how well
24
25 182 each variable predicted PCOS. To fully explore the role of the CRP/albumin ratio as a
26
27 183 biomarker in the prediction of metabolic disturbances, CRP and CRP/albumin ratio were
28
29 184 additionally assessed as binary variables. Subjects were categorized into two groups based on
30
31 185 the cut-off values and their means (SD) for metabolic markers. Insulin resistance (HOMA-
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33 186 IR), free testosterone, total adiponectin, BMI and TSH were compared between subjects with
34
35 187 normal values, and those who had higher than normal values. P-values <0.05 were considered
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37 188 as statistically significant. All statistical analyses were performed using the IBM SPSS
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39 189 statistics software program version 22 (IBM, Armonk, NY).

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44 45 191 **Results**

46 47 192 *Study subjects*

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49 193 The sociodemographic, anthropometric, clinical, and biochemical characteristics of the 200
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51 194 women with PCOS and 119 controls women are summarized in **Table 1**. Relative to controls,
52
53 195 women with PCOS had fewer pregnancies and live births ($p<0.001$), were more likely to

196 have insulin resistance ($p<0.001$), and differed in education attainment with those with
 197 PCOS, having a higher number of high school and post-secondary graduates ($p=0.012$).

198 *Metabolic characteristics*

199 The proportion of women with a BMI greater than 30 kg/m^2 were higher in the PCOS cohort
 200 than controls ($p<0.001$), but the waist-to-hip ratio was not significantly different (**Table 1**).

201 Serum levels of adiponectin, an adipocyte-associated protein that tends to be inversely linked
 202 to visceral adiposity²³, was markedly lower in women with PCOS compared to controls.

203 Fasting plasma glucose, cholesterol, HDL, LDL and triglyceride levels did not differ
 204 significantly between women with and without PCOS. However, indices of insulin resistance
 205 and insulin sensitivity (HOMA-IR and QUICKI) indicated that women with PCOS had
 206 greater impaired regulation of insulin compared to controls (**Table 1**).

207 **Table 1: Demographic, clinical and hormonal characteristics of study population:**
 208 **women with polycystic ovary syndrome (PCOS) and controls.**

209 Data are presented as means (standard deviation).

	All (N=319)	PCOS (N=200)	Controls (N=119)	Mean difference	95 % CI of Mean Difference	
					lower	upper
Age (yrs)	27.9 (6.4)	28.4 (5.9)	27.2 (7.2)	1.24	-0.22	2.71
BMI (kg/m^2)	28 (5.9)	29 (6.3)	26.5 (5)	2.53	1.19	3.87
Waist/hip ratio	0.94 (0.09)	0.94 (0.09)	0.93 (0.09)	0.0067	-0.017	0.031
Menarche (yrs)	12.5 (1.4)	12.5 (1.5)	12.4 (1.2)	0.12	-0.21	0.45
HOMA-IR	3.2 (0.18)	3.8 (0.26)	2.1 (0.2)	1.67	0.94	2.4
QUICKI	0.6 (0.006)	0.57 (0.008)	0.65 (.01)	-0.078	-0.10	-0.05
Total adiponectin (ng/L)	33.8 (1.4)	28.4 (1.3)	44.6 (2.9)	-16.25	-21	-10

Albumin (g/L)	37.3 (0.46)	32.7 (0.46)	45 (0.35)	-12.22	-13.52	-10.93
CRP (mg/L)	11.1 (1.1)	15.5 (1.6)	3.6 (0.85)	11.89	7.61	16.17
CRP/Alb ratio	0.36 (0.04)	0.53 (0.06)	0.08 (0.02)	0.45	0.29	0.61
SHBG (nmol/L)	60.1 (1.5)	52.2 (1.4)	72 (2.8)	-19.72	-26.5	-13.93
Free testosterone index	0.025 (0.001)	0.029 (0.001)	0.017 (0.001)	0.011	0.007	0.015

210

211 BMI: Body Mass Index, HOMA.IR: Homeostatic Model Assessment for Insulin Resistance,

212 QUICKI: Quantitative insulin sensitivity check index, CRP: C-reactive protein, SHBG: Sex

213 hormone binding hormone.

214 *Reproductive hormone characteristics*

215 There were no statistically significant differences in plasma levels of estradiol, progesterone,

216 total testosterone, prolactin, FSH, LH and DHES between women with and without PCOS.

217 Free testosterone was higher and SHBG lower in women with PCOS (**Table 1**).218 *C-reactive protein (CRP)/albumin ratio as a predictor of polycystic ovary syndrome (PCOS)*219 *stratified by body mass index (BMI)*

220 Women with PCOS had markedly higher levels of CRP and lower levels of serum albumin

221 relative to controls ($p < 0.001$; **Table 1**). ROC curve analysis showed that the CRP/albumin

222 ratio had greater discriminatory power to differentiate between women with PCOS and

223 controls (AUC: 0.865, 95% CI: 0.824-0.905) compared to CRP alone (AUC: 0.820, 95% CI:

224 0.773-0.867); **Figure 1**. This greater efficacy of CRP/albumin ratio to discriminate between

225 cases and controls was also evident when taking into account the presence of insulin

226 resistance at every measure of sensitivity; for a sensitivity level of 75%, CRP/albumin ratio

227 had a specificity of 85% compared to 69% for CRP alone.

228 Spearman correlation analysis between CRP/albumin ratios and clinical and biochemical
 229 markers was subsequently performed. Variables found to be univariately linked to
 230 CRP/albumin values were included in a general linear model testing the relationship amongst
 231 PCOS diagnosis, BMI, and CRP/albumin levels. The model revealed that for any given BMI
 232 value, women with PCOS have markedly elevated CRP/albumin levels ($p < 0.001$, **Figure 2**).

233 Classical predictors of PCOS, free androgen index and insulin resistance, were compared to
 234 CRP/albumin ratio as predictors of PCOS in a binary regression analysis stratified by three
 235 BMI categories: $< 25 \text{ kg/m}^2$ [normal], $25 - 29.9 \text{ kg/m}^2$ [overweight], $> 30 \text{ kg/m}^2$ [obese];
 236 **Table 2.** A CRP/albumin ratio of ≥ 0.097 outperformed both insulin resistance and free
 237 androgen index in predicting PCOS for every BMI category (**Table 2**).

238

239 **Table 2: Summary of binary Regression Analysis for Variables Predicting PCOS with**
 240 **the Odds Ratio of each risk factor adjusted for other variables in the model**

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	Odds Ratio (95% Confidence Interval)		
	BMI < 25 (normal)	BMI 25-29.9 (overweight)	BMI \geq 30 (obese)
CRP/Albumin Ratio ¹			
< 0.097	1	1	1
≥ 0.097	11.21 (3.28-39.75)	19.32 (5.07-72.17)	34.5 (7.75-153.52)
Insulin Resistance ²			
No	1	1	1
Borderline	3.34 (0.645-17.33)	5.58 (0.907-34.41)	3.13 (0.53-18.48)
Yes	9.21 (1.63-51.93)	8.81 (1.75-44.31)	17.94 (1.81-177.61)
Free Androgen Index ³			
<3.95	1	1	1

≥ 3.95	2.28 (.536-9.75)	0.86 (.22-3.35)	3.79 (0.59-24.42)
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243 ¹Cut-off values were derived from the sensitivity and specificity analysis of the receiver

244 operating characteristic curve

245 ²Categories of insulin resistance based on normal values used for HOMA-IR

246 ³Cut-off values from normal laboratory reference ranges for free androgen index

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248 Discussion

249 This study demonstrated that CRP/albumin ratio is a better predictor of PCOS than both free
 250 androgens and insulin resistance in 319 ethnically-matched premenopausal women, and this
 251 relationship was independent of BMI. Despite being the most common reproductive disorder
 252 to affect women, the etiology of PCOS has remained elusive to date. In the absence of a
 253 definitive cure, treatment has focused on symptom management, and a goal to prevent the
 254 progression of serious health conditions, such as type 2 diabetes and CVD, for which women
 255 with PCOS are at heightened risk. Chronic low-grade inflammation has emerged as a
 256 common underlying state in women with PCOS, and a likely direct contributor to insulin
 257 resistance and heart disease risk. This has raised the question whether PCOS is fundamentally
 258 an inflammatory condition^{8, 10, 24}.

259 Small sample sizes, heterogeneous populations, and an inability to correct for confounding
 260 factors, such as BMI and use of oral contraceptives, both of which influence inflammatory
 261 markers such as CRP²¹, has in part hampered efforts in assigning inflammation as truly a
 262 defining feature of PCOS. This current analysis has overcome some of the main issues in
 263 assessing the independent relationship between PCOS and chronic low-grade inflammation.
 264 This was accomplished by accounting for many of the confounding variables and by using a
 265 more refined marker for inflammation, the CRP/albumin ratio, which may have greater

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3 266 specificity and sensitivity for inflammation associated with metabolic dysfunction. The
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5 267 CRP/albumin ratio was first found to be useful in assessing cardiometabolic and
6
7 268 inflammatory status following ovariectomy surgery²⁰. It was subsequently used to show the
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9 269 influence of chronic subclinical inflammation on bone quality in women with PCOS²¹.
10
11 270 Although CRP is used to predict cardiovascular risk and is associated with metabolic
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13 271 disorders associated with obesity and insulin resistance²⁵⁻²⁸, it has been criticized for being
14
15 272 too general and non-specific a marker for inflammation²⁹.
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18 273 When compared to CRP alone, we found that the CRP/albumin ratio had an improved ROC
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20 274 curve for predicting PCOS. Serum albumin, which is commonly measured to assess liver
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22 275 function and malnutrition, is not widely considered as an analyte of interest for PCOS.
23
24 276 However, this study showed for the first time that albumin is markedly reduced in women
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26 277 with PCOS relative to controls. This may, at least in part, be due to albumin being a negative
27
28 278 acute phase protein¹⁸. It is also possible that there is increased oxidation and glycation of
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30 279 albumin in women with PCOS - which can impact the structure, function and metabolism of
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32 280 the protein¹⁸. As one of the most abundant serum proteins, among albumin's many roles is
33
34 281 the transport of hormones¹⁹. Thus, reduced albumin levels can potentially contribute to
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36 282 higher free androgens in women with PCOS and exacerbation of disease phenotype.
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40 283 This analysis was limited by a lack of a more sensitive measure of visceral adiposity; the gold
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42 284 standard being imaging with computed tomography (CT) or magnetic resonance imaging
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44 285 (MRI)³⁰. Furthermore, the case-control design limited the ability to assess how CRP/albumin
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46 286 performs in predicting health outcomes in women with PCOS. Prospective studies are now
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48 287 needed to determine the use of CRP/albumin in predicting the progression of disorders linked
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50 288 to chronic inflammation and metabolic dysfunction that women with PCOS are at increased
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52 289 risk. These include not only cardiovascular disease and diabetes, but also depression³¹⁻³⁴.
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55 290 Importantly, CRP/albumin ratio may be particularly useful in assessing the effectiveness of

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3 291 new interventions targeting inflammation in women with PCOS as a novel approach to
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5 292 managing the condition and its long-term health consequences.
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8 293 *Conclusion:* CRP/albumin ratio, a marker for inflammation related to metabolic dysfunction,
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10 294 is a better predictor of PCOS than either androgen excess or insulin resistance. Inflammation
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12 295 is known to be influenced by adiposity, but relative to controls, women with PCOS have
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14 296 higher levels of CRP/albumin ratio irrespective of BMI. This supports the view that
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16 297 inflammation may play a central role in the pathophysiology of PCOS.
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19 298

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26

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30
31 304 their support and time, this work would not be possible.
32
33

34 305 **Competing Interests Statement**

35
36 306 SK is Director of Scientific Innovation at Qu Biologics Inc., a clinical-stage biotechnology
37
38 307 company. All other authors have no conflict of interest to declare.
39

40 308 **Author Contributions**

41
42 309 SK designed the study, interpreted the data and wrote the first draft of the manuscript. AG
43
44 310 performed the statistical analysis and helped interpret the analysis. SS and WA collected all
45
46 311 the data, performed the biochemical analysis and managed the clinical operations of the
47
48 312 study. AJ assisted with literature review and manuscript preparation. All authors reviewed
49
50 313 and approved the final manuscript.
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4 412 **Figure Legends**

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6 413 **Figure 1.** Receiver Operating Characteristic (ROC) curve plotting the true positive rate

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8 414 against the false positive rate for CRP/Albumin (green line) and CRP (blue line) in

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10 415 differentiating women with and without PCOS. The area under the curve (AUC) for

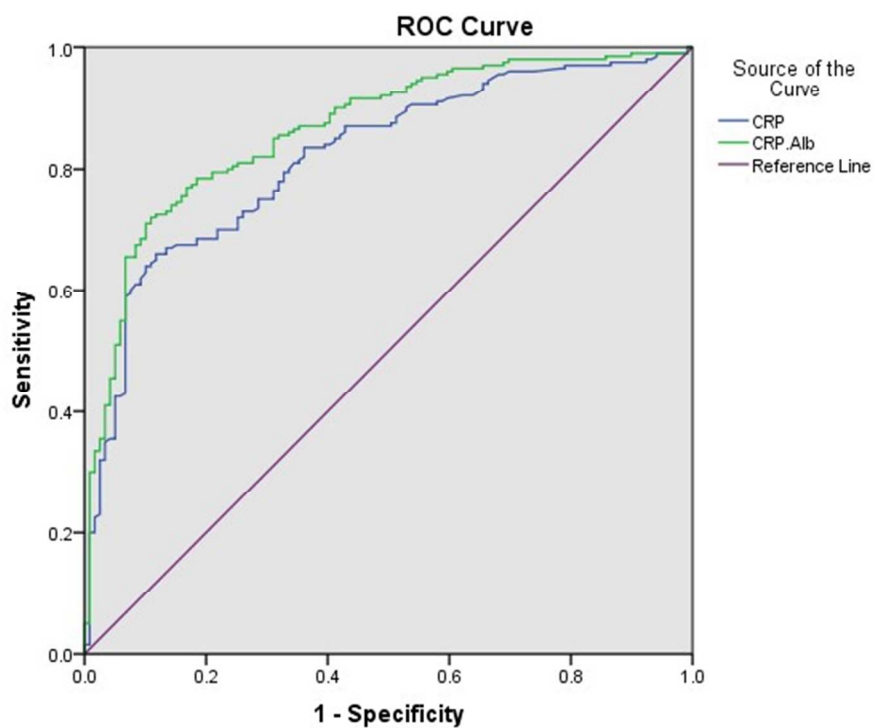
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12 416 CRP/Albumin: 0.865, 95% CI: 0.824-0.905; for CRP: 0.820, 95% CI: 0.773-0.867.

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17 418 **Figure 2.** Scatter plot analysis showing the age-adjusted CRP/albumin values by body mass

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19 419 index (BMI) in women with PCOS and controls.

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33 Figure 1. Receiver Operating Characteristic (ROC) curve plotting the true positive rate against the false
34 positive rate for CRP/Albumin (green line) and CRP (blue line) in differentiating women with and without
35 PCOS. The area under the curve (AUC) for CRP/Albumin: 0.865, 95% CI: 0.824-0.905; for CRP: 0.820, 95%
36 CI: 0.773-0.867.

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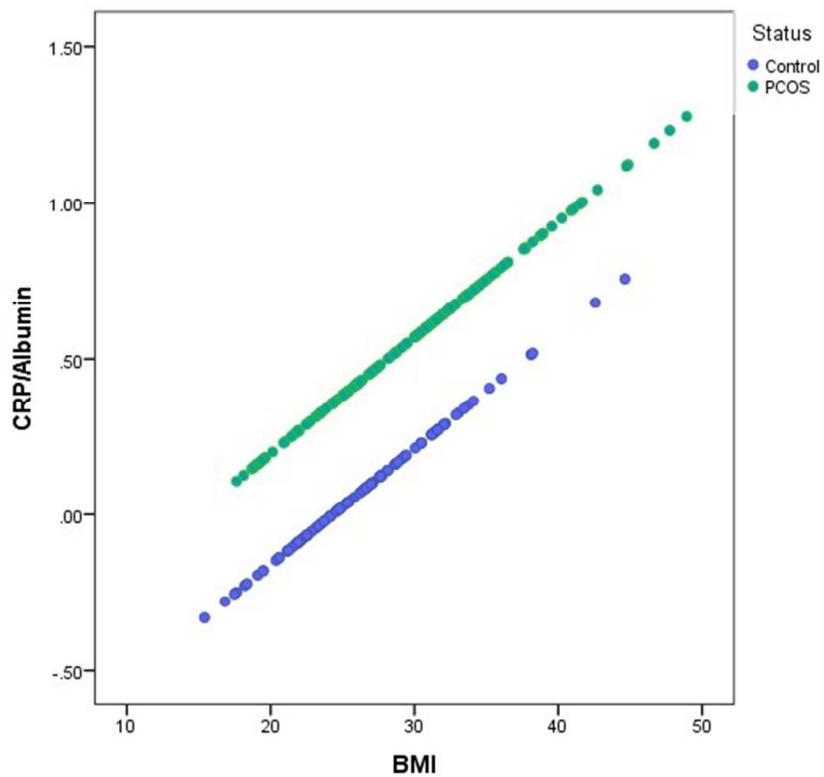


Figure 2. Scatter plot analysis showing the age-adjusted CRP/albumin values by body mass index (BMI) in women with PCOS and controls.

166x133mm (96 x 96 DPI)

BMJ Open

Assessing C-reactive protein/albumin ratio as a new predictor of Polycystic Ovary Syndrome: a case-control study of women from Bahraini medical clinics

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Keywords:	polycystic ovary syndrome, inflammation, C-reactive protein, albumin, pathophysiology

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3 **1 Assessing C-reactive protein/albumin ratio as a new predictor of Polycystic Ovary**
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5 **2 Syndrome: a case-control study of women from Bahraini medical clinics**
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8 3
9 4 Short title: CRP/Albumin as a predictor of PCOS
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12 7 Shirin Kalyan ^{1*}, Azita Goshtesabi ¹, Sameh Sarray ^{2,3}, Angela Joannou ¹, Wassim Almawi ³
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38 31 Keywords: polycystic ovary syndrome, inflammation, pathophysiology, C-reactive protein,
39 32 albumin
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4 36 **Abstract**

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6 37 *Objective:* Polycystic ovary syndrome (PCOS) is an endocrine disorder affecting
7
8 38 approximately one in seven women who experience androgen excess, menstrual cycle
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10 39 irregularities, frequent anovulation, and a tendency for central obesity and insulin resistance.
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12 40 Chronic subclinical inflammation is now recognized as being common in the context of
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14 41 PCOS, which led to the postulation that PCOS may fundamentally be an inflammatory
15
16 42 process. This study aimed to: 1) evaluate serum CRP/albumin ratio as a predictor of PCOS;
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18 43 2) compare the relationship between CRP/albumin and PCOS to variables classically
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20 44 associated with the syndrome.

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23 45 *Design:* Case-control study.

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25 46 *Setting:* Adult obstetrics/gynaecology, endocrinology and outpatient clinics; university
26
27 47 hospital in Bahrain.

28
29 48 *Participants:* 200 premenopausal women with a diagnosis of PCOS, and 119 ethnically-
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31 49 matched eumenorrheic premenopausal women.

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33 50 *Main Outcome Measures:* CRP/albumin ratio, anthropometric measures, insulin resistance,
34
35 51 androgen excess.

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37 52 *Results:* Independent of body mass index (BMI), receiver operating characteristic (ROC)
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39 53 curve for CRP/albumin ratio as predictor of PCOS was 0.865 (95% CI: 0.824–0.905), which
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41 54 was more sensitive than CRP alone. Binary regression analysis showed that CRP/albumin
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43 55 ratio outperformed classical correlates, free androgen index and insulin resistance, in
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45 56 predicting PCOS for every BMI category.

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47 57 *Conclusion:* CRP/albumin ratio, a marker for inflammation related to metabolic dysfunction,
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49 58 better predicts PCOS than either androgen excess or insulin resistance. Inflammation is
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51 59 known to be influenced by adiposity, but relative to controls, women with PCOS have higher
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3 60 levels of CRP/albumin irrespective of BMI. These findings support the view that
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5 61 inflammation plays a central role in the pathophysiology of PCOS.

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10 11 64 **Article Summary**

12 13 65 *Strengths and limitations of this study*

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16 66 • This analysis addressed previous limitations of studies, namely small sample sizes,
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18 67 heterogeneous populations, and confounding factors (such as BMI), that have
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20 68 attempted to show PCOS is an inflammatory process
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22 69 • The relationship between inflammation and PCOS was assessed using CRP/albumin
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24 70 ratio, which may be a better marker for inflammation in the context of metabolic
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26 71 dysfunction
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29 72 • Limitation: study used waist circumference as a substitute for visceral adiposity; gold
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31 73 standard is computed tomography (CT) or magnetic resonance imaging (MRI)

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75 **Introduction**

76 Polycystic ovary syndrome (PCOS) is the most common reproductive disorder, affecting 5 to
77 15 % of premenopausal women worldwide, [1, 2] and the prevalence of PCOS appears to be
78 increasing. [1] This rise may partly be attributed to improved diagnosis as well as to an
79 increase in environmental factors that predispose to the development of this complex
80 metabolic condition. PCOS is characterized by androgen excess, menstrual irregularities,
81 ovulatory disturbances, and is often associated with central obesity and insulin resistance. [3-
82 5] As such, women with PCOS are at an increased risk for a number of health issues,
83 including infertility, cardiovascular disease and diabetes. [3, 6, 7]
84 Possibly related to the constellation of endocrine and metabolic dysfunction they experience,
85 women with PCOS are also found to have greater chronic subclinical inflammation, [8-11]
86 which is often clinically assessed by measuring serum levels of C-reactive protein (CRP).
87 CRP is a liver-derived acute phase protein produced in response to IL-6 secreted from
88 activated cells such as macrophages and adipocytes. [12, 13] A meta-analysis of 31 studies
89 concluded that systemic CRP levels are 96% higher in women with PCOS compared to
90 control women. [14] Collectively, these findings have given rise to the speculation that
91 inflammation may play a pivotal role in the pathophysiology of PCOS. [8-10]
92 Elevated serum CRP levels are linked to several health risk factors experienced by women
93 with PCOS, particularly insulin resistance and heightened risk of type 2 diabetes. [7, 15, 16]
94 Chronic inflammation also contributes to endothelial dysfunction, exacerbating the
95 development of atherosclerotic plaques, triggering the onset of cardiovascular disease (CVD).
96 [17] As such inflammation has been associated with both CVD and coronary artery disease in
97 women with PCOS. [10]

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3 98 In contrast to CRP, albumin is a negative acute phase response protein produced by the liver.
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5 99 Serum levels of albumin are reduced in individuals experiencing chronic inflammation. [18]
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7 100 In addition to its role as a binding molecule for sex steroids, [19] albumin also provides the
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9 101 majority of the total antioxidant capacity of normal plasma. [18] The ratio of serum CRP
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11 102 levels over serum albumin (CRP/albumin) was found to be strongly associated with more
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13 103 severe metabolic dysfunction in premenopausal women with induced alterations to their
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15 104 ovarian hormone status. [20] CRP/albumin ratio was also found to be significantly higher in
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17 105 premenopausal women with PCOS relative to controls, and adversely predicted their bone
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19 106 quality. [21] Given the ability of CRP/albumin to simultaneously capture chronic
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21 107 inflammation and metabolic dysfunction in premenopausal women, we hypothesized that
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23 108 CRP/albumin ratio would, in itself, serve as a strong predictor of PCOS in a cohort of
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25 109 similarly aged women. This case-control study investigated CRP/albumin ratio along with
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27 110 classical markers, androgen excess and insulin resistance, in their ability to predict PCOS in
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29 111 319 premenopausal Bahraini Arab women.
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35 113 **Methods**37 114 *Patient and public involvement*

39 115 The development of the research question and the study's setting was influenced by the wish
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41 116 many women in Bahrain have to bear children. Infertility is a consequence of PCOS, [22] and
42
43 117 the combination of both can impact women's health-related quality of life. [23] Patients were
44
45 118 not involved in the design of the study or the recruitment. Study participants will not be re-
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47 119 contacted by the study investigators; however, the results of this study will be disseminated to
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49 120 the Bahraini community through press releases of the open access publication.
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52 121 *Study subjects*

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3 122 Women with PCOS (n = 200) were recruited from adult obstetrics/gynecology,
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5 123 endocrinology and outpatient clinics in Manama, Bahrain. Women without PCOS (n = 119)
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7 124 were ethnically-matched, eumenorrheic university employees and students, and healthy
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9 125 volunteers representative of the Bahraini population. The sample size was based on the
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11 126 ability to detect differences between cases and controls with 10% precision (two-tailed t-test
12
13 127 with $\alpha=0.05$) and 80% power, taking into account the estimated prevalence of PCOS in
14
15 128 Bahrain of 7.5-8.0% (unpublished data; Bahraini Ministry of Health) and Z-value for 95% CI
16
17 129 (confidence interval). Women serving as controls were examined in the follicular phase of
18
19 130 their menstrual cycle and had their testosterone levels were within range. A diagnosis of
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21 131 PCOS was based on the 2003 Rotterdam Criteria, which requires two of the three following
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23 132 criteria to be met: ultrasound evidence of polycystic ovarian morphology, anovulation, and
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25 133 hyperandrogenism. [24] Exclusion criteria included hyperprolactinemia, non-classical adrenal
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27 134 hyperplasia, androgen-producing tumors, 21-hydroxylase deficiency, Cushing's syndrome,
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29 135 and active thyroid disease. Additional exclusion criteria included extremes of body mass
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31 136 index (BMI; $<18 \text{ kg/m}^2$ or $>45 \text{ kg/m}^2$), recent/present illness, and treatment affecting
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33 137 carbohydrate metabolism or hormonal levels, for three months or longer before inclusion the
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35 138 study. Women using anti-hypertensive, oral contraceptive, anti-inflammatory, and lipid-
36
37 139 lowering drugs were also excluded. Demographic information, along with detailed personal
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39 140 and family history of diabetes, hypertension, infertility, hypercholesterolemia, and ischemic
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41 141 heart disease were obtained from all participants. This study was conducted in accord with
42
43 142 the Helsinki II Declaration guidelines, and all participants gave written informed consent to
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45 143 participate. Study approval was obtained from the Bahraini Ministry of Health and Arabian
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47 144 Gulf University Research and Ethics Committees (IRB number: 35-PI-01/15) and the Clinical
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49 145 Research Ethics Board of the University of British Columbia (H16-02101).
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147 *Biochemical analysis*

148 Peripheral venous fasting blood samples were obtained between 7:00 and 9:00 am following
149 an overnight (> 12 h) fast during the early follicular phase of the menstrual cycle (days 2 ± 5)
150 for control women, or women with PCOS who did not present with menstrual irregularities,
151 or any day for women with PCOS with menstrual irregularities. Serum samples were
152 analyzed for sex hormone binding globulin (SHBG) by sandwich ELISA (R&D Systems,
153 Minneapolis, MN); assay sensitivity was 0.01 nmol/ml, and inter-assay and intra-assay
154 precision (CV %) were 5.3% and 4.3%, respectively.

155 Serum luteinizing hormone (LH), follicular stimulating hormone (FSH), thyroid stimulating
156 hormone (TSH), testosterone, glucose (ADVIA Centaur, Bayer Vital, Fernwald, Germany),
157 and insulin (IMMULITE 2000, DPC Biermann, Bad Nauheim, Germany), were measured by
158 automated chemiluminescence immunoassays. Free (FT) and bioactive (BT) testosterone and
159 free androgen index (FAI) were determined using Free & Bioavailable Testosterone
160 Calculator (www.issam.ch/freetesto.htm). Concentrations of serum albumin were analyzed
161 by photospectrometry with albumin bromocresol purple assay on a COBAS c701 Chemistry
162 Analyzer (Roche Diagnostics, Dubai, UAE). Insulin resistance (IR) was estimated by the
163 homeostasis model assessment (HOMA-IR), defined as fasting serum insulin (IU/mL) \times
164 fasting plasma glucose (mmol/L)/22.5. HOMA-IR values were characterized as Normal
165 (insulin-sensitive) if <2.40; Borderline if between 2.40-3.50, and High (insulin-resistant) if >
166 3.50.

167

168 Measurement of plasma high sensitivity CRP levels was done by latex-enhanced
169 nephelometry on a BN II Nephelometer (Dade Behring, Milan, Italy). Samples were assayed
170 in duplicate in each analytical run; the lower limit of detection was 0.15 mg/L, and the assay

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3 171 range was 0.175–11.0 mg/L (initial dilution). Serial serum dilutions were made in measuring
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5 172 high CRP (>30 mg/L) levels. Percentile CRP values were estimated for comparison
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7 173 purposes.
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10 174 *Statistical analysis*

11
12 175 The *core outcome set* of variables included assessment of CRP/albumin as a predictor of
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14 176 PCOS while controlling for relevant factors, such as BMI and age, and to subsequently
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16 177 compare the strength of the relationship between CRP/albumin and PCOS with variables
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18 178 known to strongly link to the syndrome, namely androgen excess and insulin resistance. The
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20 179 Shapiro-Wilk test was used to evaluate the distribution of the variables, and many variables
21
22 180 for the PCOS cohort were flagged as being non-normally distributed. However, upon testing
23
24 181 for skewness, we found the values for asymmetry and kurtosis fell between -2 to +2 for all.
25
26 182 Thus, we used parametric tests, as suggested, [25] given the sample sizes were >30. We
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28 183 reassessed the validity of our analysis by running the Mann–Whitney U test in addition to
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30 184 student t test for variables we had detected as being non-normal and confirmed that the
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32 185 results were similar. Baseline characteristics were compared using the Mann-Whitney U test
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34 186 and the independent samples t-test for the continuous variables, and the χ^2 test for categorical
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36 187 variables. Numerical variables are presented as mean \pm standard deviation (SD). Because
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38 188 distribution of CRP/albumin levels was skewed to the right, correlations between
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40 189 CRP/albumin ratio and other continuous variables were assessed using Spearman's rho.
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42 190 Univariate general linear models were applied to test independent associations between
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44 191 CRP/albumin ratio and other independent variables.
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49 192 The optimal cut-off level for the CRP/albumin ratio was determined by a receiver operating
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51 193 characteristic (ROC) curve analysis, and the areas under the curve (AUC) were measured and
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53 194 compared to assess the power of a model to identify patients who experienced metabolic
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55 195 disturbances. Cut-off values showing the greatest accuracy were determined using a
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3 196 sensitivity/specificity versus criterion value plot. Quartiles (i.e. 0-25%, 26-50%, 51-75%, and
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5 197 76-100%) of CRP and CRP/albumin ratio were calculated separately in PCOS and control
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7 198 groups. Because the 25% quartile of CRP value in the PCOS cohort was similar to the
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9 199 standard normal values of CRP, we used the 25% quartile of CRP and CRP/albumin ratio as
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11 200 the cut-off point for both predictors..

14 201 Using the calculated cut-off values, regression analysis was performed to determine how well
15
16 202 each variable predicted PCOS. To fully explore the role of the CRP/albumin ratio as a
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18 203 biomarker in the prediction of metabolic disturbances, CRP and CRP/albumin ratio were
19
20 204 additionally assessed as binary variables. Subjects were categorized into two groups based on
21
22 205 the cut-off values and their means (SD) for metabolic markers. Insulin resistance (HOMA-
23
24 206 IR), free testosterone, total adiponectin, BMI and TSH were compared between subjects with
25
26 207 normal values, and those who had higher than normal values. P-values <0.05 were considered
27
28 208 as statistically significant. All statistical analyses were performed using the IBM SPSS
29
30 209 statistics software program version 22 (IBM, Armonk, NY).

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36 211 **Results**

38 212 *Study subjects*

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41 213 The sociodemographic, anthropometric, clinical, and biochemical characteristics of the 200
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43 214 women with PCOS and 119 controls women are summarized in **Table 1**. Relative to controls,
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45 215 women with PCOS had fewer pregnancies and live births ($p<0.001$), were more likely to
46
47 216 have insulin resistance ($p<0.001$), and differed in education attainment with those with
48
49 217 PCOS, having a higher number of high school and post-secondary graduates ($p=0.012$).

51 52 218 *Metabolic characteristics*

219 The proportion of women with a BMI greater than 30 kg/m² were higher in the PCOS cohort
 220 than controls (p<0.001), but the waist-to-hip ratio was not significantly different (**Table 1**).
 221 Serum levels of adiponectin, an adipocyte-associated protein that tends to be inversely linked
 222 to visceral adiposity, [26] was markedly lower in women with PCOS compared to controls.
 223 Fasting plasma glucose, cholesterol, HDL, LDL and triglyceride levels did not differ
 224 significantly between women with and without PCOS. However, indices of insulin resistance
 225 and insulin sensitivity (HOMA-IR and QUICKI) indicated that women with PCOS had
 226 greater impaired regulation of insulin compared to controls (**Table 1**).

227 **Table 1: Demographic, clinical and hormonal characteristics of study population:**
 228 **women with polycystic ovary syndrome (PCOS) and controls.**

229 Data are presented as means (standard deviation).

	All (N=319)	PCOS (N=200)	Controls (N=119)	Mean difference	95 % CI of Mean Difference	
					lower	upper
Age (yrs)	27.9 (6.4)	28.4 (5.9)	27.2 (7.2)	1.24	-0.22	2.71
BMI (kg/m ²)	28 (5.9)	29 (6.3)	26.5 (5)	2.53	1.19	3.87
Waist/hip ratio	0.94 (0.09)	0.94 (0.09)	0.93 (0.09)	0.0067	-0.017	0.031
Menarche (yrs)	12.5 (1.4)	12.5 (1.5)	12.4 (1.2)	0.12	-0.21	0.45
HOMA-IR	3.2 (0.18)	3.8 (0.26)	2.1 (0.2)	1.67	0.94	2.4
QUICKI	0.6 (0.006)	0.57 (0.008)	0.65 (.01)	-0.078	-0.10	-0.05
Total adiponectin (ng/L)	33.8 (1.4)	28.4 (1.3)	44.6 (2.9)	-16.25	-21	-10
Albumin (g/L)	37.3 (0.46)	32.7 (0.46)	45 (0.35)	-12.22	-13.52	-10.93
CRP (mg/L)	11.1 (1.1)	15.5 (1.6)	3.6 (0.85)	11.89	7.61	16.17
CRP/Albumin ratio	0.36 (0.04)	0.53 (0.06)	0.08 (0.02)	0.45	0.29	0.61

SHBG (nmol/L)	60.1 (1.5)	52.2 (1.4)	72 (2.8)	-19.72	-26.5	-13.93
Free testosterone index	0.025 (0.001)	0.029 (0.001)	0.017 (0.001)	0.011	0.007	0.015

230

231 BMI: Body Mass Index, HOMA.IR: Homeostatic Model Assessment for Insulin Resistance,

232 QUICKI: Quantitative insulin sensitivity check index, CRP: C-reactive protein, SHBG: Sex

233 hormone binding hormone.

234 *Reproductive hormone characteristics*

235 There were no statistically significant differences in plasma levels of estradiol, progesterone,

236 total testosterone, prolactin, FSH, LH and DHES between women with and without PCOS.

237 Free testosterone was higher and SHBG lower in women with PCOS (**Table 1**).238 *C-reactive protein (CRP)/albumin ratio as a predictor of polycystic ovary syndrome (PCOS)*239 *stratified by body mass index (BMI)*

240 Women with PCOS had markedly higher levels of CRP and lower levels of serum albumin

241 relative to controls ($p < 0.001$; **Table 1**). ROC curve analysis showed that the CRP/albumin

242 ratio had greater discriminatory power to differentiate between women with PCOS and

243 controls (AUC: 0.865, 95% CI: 0.824-0.905) compared to CRP alone (AUC: 0.820, 95% CI:

244 0.773-0.867); **Figure 1**. This greater efficacy of CRP/albumin ratio to discriminate between

245 cases and controls was also evident when taking into account the presence of insulin

246 resistance at every measure of sensitivity; for a sensitivity level of 75%, CRP/albumin ratio

247 had a specificity of 85% compared to 69% for CRP alone.

248 Spearman correlation analysis between CRP/albumin ratios and clinical and biochemical

249 markers was subsequently performed. Variables found to be univariately linked to

250 CRP/albumin values were included in a general linear model testing the relationship amongst

251 PCOS diagnosis, BMI, and CRP/albumin levels. The model revealed that for any given BMI
 252 value, women with PCOS have markedly elevated CRP/albumin levels ($p < 0.001$, **Figure 2**).

253 Variables that are known to strongly associate with PCOS, namely free androgen index and
 254 insulin resistance, were compared to CRP/albumin ratio as predictors of PCOS in a binary
 255 regression analysis stratified by three BMI categories: $< 25 \text{ kg/m}^2$ [normal], $25 - 30 \text{ kg/m}^2$
 256 [overweight], $> 30 \text{ kg/m}^2$ [obese]; **Table 2**. A CRP/albumin ratio of ≥ 0.097 outperformed
 257 both insulin resistance and free androgen index in predicting PCOS for every BMI category
 258 (**Table 2**).

260 **Table 2: Summary of binary Regression Analysis for Variables Predicting PCOS with**
 261 **the Odds Ratio of each risk factor adjusted for other variables in the model**

	Odds Ratio (95% Confidence Interval)		
	BMI < 25 (normal)	BMI 25-30 (overweight)	BMI \geq 30 (obese)
CRP/Albumin Ratio ¹			
< 0.097	1	1	1
≥ 0.097	11.21 (3.28-39.75)	19.32 (5.07-72.17)	34.5 (7.75-153.52)
Insulin Resistance ²			
No	1	1	1
Borderline	3.34 (0.645-17.33)	5.58 (0.907-34.41)	3.13 (0.53-18.48)
Yes	9.21 (1.63-51.93)	8.81 (1.75-44.31)	17.94 (1.81-177.61)
Free Androgen Index ³			
< 3.95	1	1	1
≥ 3.95	2.28 (.536-9.75)	0.86 (.22-3.35)	3.79 (0.59-24.42)

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3 264 ¹Cut-off values were derived from the sensitivity and specificity analysis of the receiver
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5 265 operating characteristic curve

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7 266 ²Categories of insulin resistance based on normal values used for HOMA-IR

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9 267 ³Cut-off values from normal laboratory reference ranges for free androgen index

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12 13 269 **Discussion**

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15 270 This study demonstrated that CRP/albumin ratio is a better predictor of PCOS than both free
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17 271 androgens and insulin resistance in 319 ethnically-matched premenopausal women, and this
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19 272 relationship was independent of BMI. Despite being the most common reproductive disorder
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21 273 to affect women, the etiology of PCOS has remained elusive to date. In the absence of a
22
23 274 definitive cure, treatment has focused on symptom management, and a goal to prevent the
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25 275 progression of serious health conditions, such as type 2 diabetes and CVD, for which women
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27 276 with PCOS are at heightened risk. Chronic low-grade inflammation has emerged as a
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29 277 common underlying state in women with PCOS, and a likely direct contributor to insulin
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31 278 resistance and heart disease risk. This has raised the question whether PCOS is fundamentally
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33 279 an inflammatory condition. [8, 10, 27]

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38 280 Small sample sizes, heterogeneous populations, and an inability to correct for confounding
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40 281 factors, such as BMI and use of oral contraceptives, both of which influence inflammatory
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42 282 markers such as CRP, [21] has in part hampered efforts in assigning inflammation as truly a
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44 283 defining feature of PCOS. This current analysis has overcome some of the main issues in
45
46 284 assessing the independent relationship between PCOS and chronic low-grade inflammation.
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48 285 This was accomplished by accounting for many of the confounding variables and by using a
49
50 286 more refined marker for inflammation, the CRP/albumin ratio, which may have greater
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52 287 specificity and sensitivity for inflammation associated with metabolic dysfunction. The
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54 288 CRP/albumin ratio was first found to be useful in assessing cardiometabolic and

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3 289 inflammatory status following ovariectomy surgery. [20] It was subsequently used to show
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5 290 the influence of chronic subclinical inflammation on bone quality in women with PCOS. [21]
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7 291 Although CRP is used to predict cardiovascular risk and is associated with metabolic
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9 292 disorders associated with obesity and insulin resistance, [28-31] it has been criticized for
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11 293 being too general and non-specific a marker for inflammation. [32]
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14 294 When compared to CRP alone, we found that the CRP/albumin ratio had an improved ROC
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16 295 curve for predicting PCOS. Serum albumin, which is commonly measured to assess liver
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18 296 function and malnutrition, is not widely considered as an analyte of interest for PCOS.
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20 297 However, this study showed for the first time that albumin is markedly reduced in women
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22 298 with PCOS relative to controls. This may, at least in part, be due to albumin being a negative
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24 299 acute phase protein. [18] It is also possible that there is increased oxidation and glycation of
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26 300 albumin in women with PCOS - which can impact the structure, function and metabolism of
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28 301 the protein. [18] As one of the most abundant serum proteins, among albumin's many roles is
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30 302 the transport of hormones. [19] Thus, reduced albumin levels can potentially contribute to
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32 303 higher free androgens in women with PCOS and exacerbation of disease phenotype.
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36 304 This analysis was limited by a lack of a more sensitive measure of visceral adiposity; the gold
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38 305 standard being imaging with computed tomography (CT) or magnetic resonance imaging
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40 306 (MRI). [33] Furthermore, the case-control design limited the ability to assess how
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42 307 CRP/albumin performs in predicting health outcomes in women with PCOS. Prospective
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44 308 studies are now needed to determine the use of CRP/albumin in predicting the progression of
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46 309 disorders linked to chronic inflammation and metabolic dysfunction that women with PCOS
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48 310 are at increased risk. These include not only cardiovascular disease and diabetes, but also
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50 311 depression. [34-37] Importantly, CRP/albumin ratio may be particularly useful in assessing
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52 312 the effectiveness of new interventions targeting inflammation in women with PCOS as a
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54 313 novel approach to managing the condition and its long-term health consequences.
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3 314 *Conclusion:* CRP/albumin ratio, a marker for inflammation related to metabolic dysfunction,
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5 315 is a better predictor of PCOS than either androgen excess or insulin resistance. Inflammation
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7 316 is known to be influenced by adiposity, but relative to controls, women with PCOS have
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9 317 higher levels of CRP/albumin ratio irrespective of BMI. This supports the view that
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11 318 inflammation may play a central role in the pathophysiology of PCOS.
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14 319

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17
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19
20 322 Coastal Health Research Institute in support of Dr. Shirin Kalyan.

21 22 323 **Data Sharing Statement**

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24 324 Due to subject confidentiality, the complete data cannot be made publicly available.
25
26 325 However, researchers who would like controlled access to the data are welcome to contact
27
28 326 Dr. Wassim Y. Almawi at: wassim.almawi@outlook.com.

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32
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34
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36 37 330 **Competing Interests Statement**

38
39 331 SK is Director of Scientific Innovation at Qu Biologics Inc., a clinical-stage biotechnology
40
41 332 company. All other authors have no conflict of interest to declare.

42 43 44 333 **Author Contributions**

45
46 334 SK designed the study, interpreted the data and wrote the first draft of the manuscript. AG
47
48 335 performed the statistical analysis and helped interpret the analysis. SS and WA collected all
49
50 336 the data, performed the biochemical analysis and managed the clinical operations of the
51
52 337 study. AJ assisted with literature review and manuscript preparation. All authors reviewed
53
54 338 and approved the final manuscript.
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47 443 10.1097/AOG.0b013e318202b0a4.
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3 446 **Figure Legends**

4 447

5 448 **Figure 1.** Receiver Operating Characteristic (ROC) curve plotting the true positive rate

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7 449 against the false positive rate for CRP/Albumin (green line) and CRP (blue line) in

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9 450 differentiating women with and without PCOS. The area under the curve (AUC) for

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11 451 CRP/Albumin: 0.865, 95% CI: 0.824-0.905; for CRP: 0.820, 95% CI: 0.773-0.867.

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15 452 **Figure 2.** Linear regression analysis of adjusted CRP/albumin values by body mass index

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17 453 (BMI) in women with PCOS and controls.

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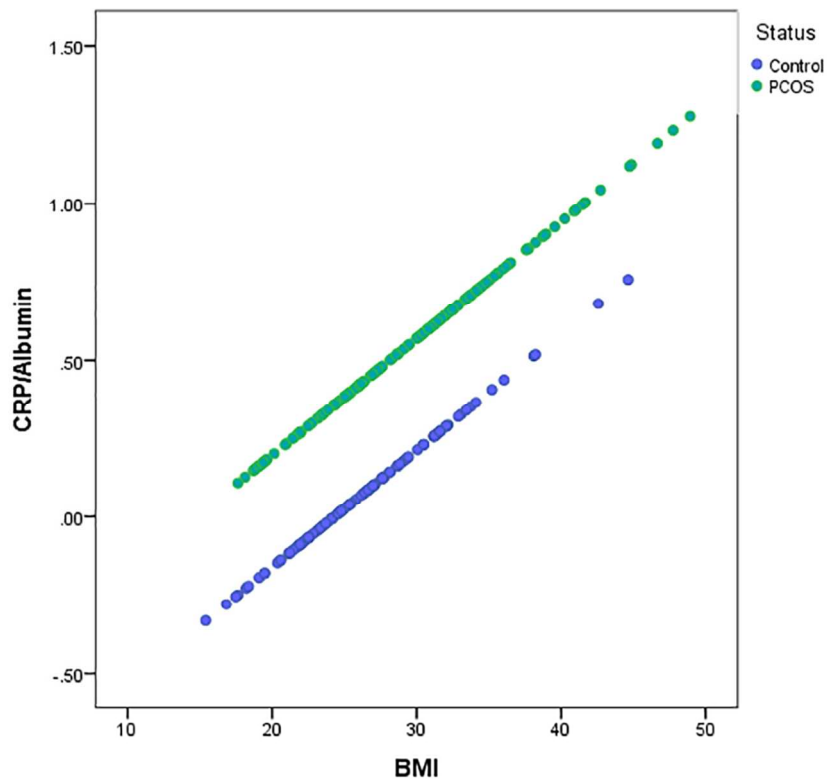
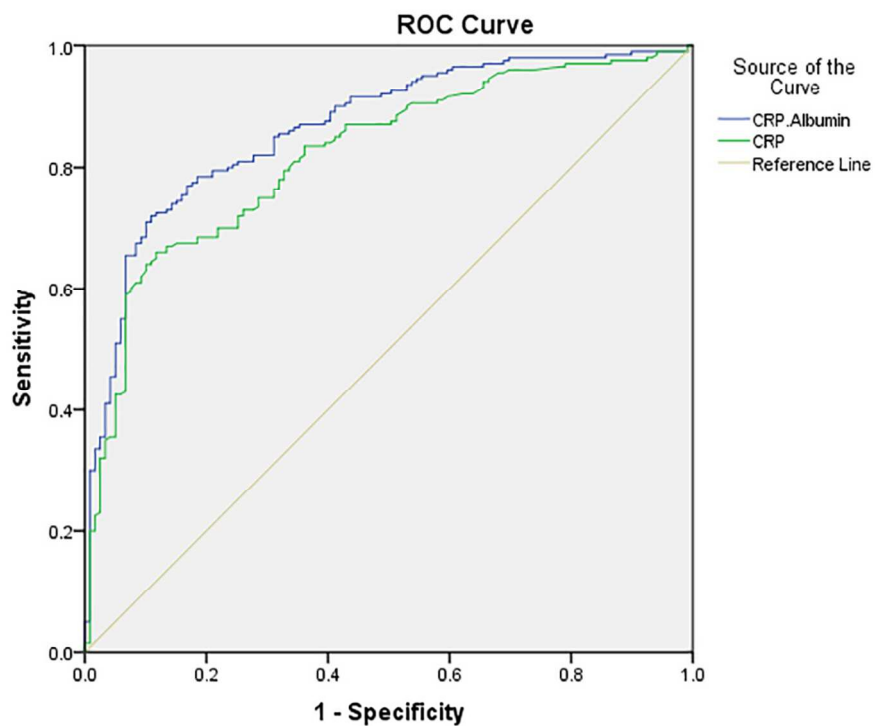


Figure 2. Linear regression analysis of adjusted CRP/albumin values by body mass index (BMI) in women with PCOS and controls. A univariate generalized linear model was computed investigating the relationship between CRP/albumin and BMI, adjusting for the variables found to associate with CRP/albumin (insulin, free testosterone, progesterone and adiponectin) plus age stratified by PCOS diagnosis.

101x81mm (300 x 300 DPI)



Diagonal segments are produced by ties.

33 Figure 1. Receiver Operating Characteristic (ROC) curve plotting the true positive rate against the false
34 positive rate for CRP/Albumin (green line) and CRP (blue line) in differentiating women with and without
35 PCOS. The area under the curve (AUC) for CRP/Albumin: 0.865, 95% CI: 0.824-0.905; for CRP: 0.820, 95%
36 CI: 0.773-0.867.

101x81mm (300 x 300 DPI)

STROBE Statement

Checklist of items that should be included in reports of observational studies

Section/Topic	Item No	Recommendation	Reported on Page No
Title and abstract	1	(a) Indicate the study's design with a commonly used term in the title or the abstract	1
		(b) Provide in the abstract an informative and balanced summary of what was done and what was found	2,3
Introduction			
Background/rationale	2	Explain the scientific background and rationale for the investigation being reported	4-5
Objectives	3	State specific objectives, including any prespecified hypotheses	5
Methods			
Study design	4	Present key elements of study design early in the paper	5
Setting	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection	5-6
Participants	6	(a) <i>Cohort study</i> —Give the eligibility criteria, and the sources and methods of selection of participants. Describe methods of follow-up	5-6
		<i>Case-control study</i> —Give the eligibility criteria, and the sources and methods of case ascertainment and control selection. Give the rationale for the choice of cases and controls	
		<i>Cross-sectional study</i> —Give the eligibility criteria, and the sources and methods of selection of participants	
Variables	7	(b) <i>Cohort study</i> —For matched studies, give matching criteria and number of exposed and unexposed	5-7
		<i>Case-control study</i> —For matched studies, give matching criteria and the number of controls per case	
Data sources/measurement	8*	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable	5-7
Bias	9	For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group	6,7
Study size	10	Describe any efforts to address potential sources of bias	5,6
Quantitative variables	11	Explain how the study size was arrived at	5
Statistical methods	12	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why	7,8
		(a) Describe all statistical methods, including those used to control for confounding	7,8
		(b) Describe any methods used to examine subgroups and interactions	-
		(c) Explain how missing data were addressed	-
		(d) <i>Cohort study</i> —If applicable, explain how loss to follow-up was addressed	5
<i>Case-control study</i> —If applicable, explain how matching of cases and controls was addressed			
		<i>Cross-sectional study</i> —If applicable, describe analytical methods taking account of sampling strategy	
		(e) Describe any sensitivity analyses	-

Section/Topic	Item No	Recommendation	Reported on Page No
Results			
Participants	13*	(a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed	9
		(b) Give reasons for non-participation at each stage	-
		(c) Consider use of a flow diagram	-
Descriptive data	14*	(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders	Table 1
		(b) Indicate number of participants with missing data for each variable of interest	Table 1
		(c) <i>Cohort study</i> —Summarise follow-up time (eg, average and total amount)	
Outcome data	15*	<i>Cohort study</i> —Report numbers of outcome events or summary measures over time	
		<i>Case-control study</i> —Report numbers in each exposure category, or summary measures of exposure	9, Table 1
		<i>Cross-sectional study</i> —Report numbers of outcome events or summary measures	
Main results	16	(a) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included	9-11
		(b) Report category boundaries when continuous variables were categorized	Table 2, 11
		(c) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period	-
Other analyses	17	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses	Figures 1-2, Table 2
Discussion			
Key results	18	Summarise key results with reference to study objectives	12-13
Limitations	19	Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias	13-14
Interpretation	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence	14
Generalisability	21	Discuss the generalisability (external validity) of the study results	13, 14
Other Information			
Funding	22	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based	14

*Give information separately for cases and controls in case-control studies and, if applicable, for exposed and unexposed groups in cohort and cross-sectional studies.

Note: An Explanation and Elaboration article discusses each checklist item and gives methodological background and published examples of transparent reporting. The STROBE checklist is best used in conjunction with this article (freely available on the Web sites of PLoS Medicine at <http://www.plosmedicine.org/>, Annals of Internal Medicine at <http://www.annals.org/>, and Epidemiology at <http://www.epidem.com/>). Information on the STROBE Initiative is available at www.strobe-statement.org.

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Assessing C-reactive protein/albumin ratio as a new biomarker for Polycystic Ovary Syndrome: a case-control study of women from Bahraini medical clinics

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3 **1 Assessing C-reactive protein/albumin ratio as a new biomarker for Polycystic Ovary**
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5 **2 Syndrome: a case-control study of women from Bahraini medical clinics**
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8 3
9 4 Short title: CRP/Albumin as a biomarker of PCOS
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11 6

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4 37 **Abstract**

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6 38 *Objective:* Polycystic ovary syndrome (PCOS) is an endocrine disorder affecting
7
8 39 approximately one in seven women who experience androgen excess, menstrual cycle
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10 40 irregularities, frequent anovulation, and a tendency for central obesity and insulin resistance.
11
12 41 Chronic subclinical inflammation is now recognized as being common in the context of
13
14 42 PCOS, which led to the postulation that PCOS may fundamentally be an inflammatory
15
16 43 process. This study aimed to: 1) evaluate serum CRP/albumin ratio as a potential predictive
17
18 44 biomarker for PCOS; 2) compare the relationship between CRP/albumin and PCOS to
19
20 45 variables classically associated with the syndrome.
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22

23 46 *Design:* Case-control study.
24

25 47 *Setting:* Adult obstetrics/gynaecology, endocrinology and outpatient clinics; university
26
27 48 hospital in Bahrain.
28

29 49 *Participants:* 200 premenopausal women with a diagnosis of PCOS, and 119 ethnically-
30
31 50 matched eumenorrheic premenopausal women.
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33

34 51 *Main Outcome Measures:* CRP/albumin ratio, anthropometric measures, insulin resistance,
35
36 52 androgen excess.
37

38 53 *Results:* Independent of body mass index (BMI), receiver operating characteristic (ROC)
39
40 54 curve for CRP/albumin ratio as a selective biomarker for PCOS was 0.865 (95% CI: 0.824–
41
42 55 0.905), which was more sensitive than CRP alone. Binary regression analysis showed that
43
44 56 CRP/albumin ratio outperformed classical correlates, free androgen index and insulin
45
46 57 resistance, in predicting PCOS for every BMI category.
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49 58 *Conclusion:* CRP/albumin ratio, a marker for inflammation related to metabolic dysfunction,
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51 59 was found to have a stronger association with PCOS than either androgen excess or insulin
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53 60 resistance. Inflammation is known to be influenced by adiposity, but relative to controls,
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3 61 women with PCOS have higher levels of CRP/albumin irrespective of BMI. These findings
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5 62 support the view that inflammation plays a central role in the pathophysiology of PCOS.
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11 65 **Article Summary**

12 66 *Strengths and limitations of this study*

- 13 67 • This analysis addressed previous limitations of studies, namely small sample sizes,
14 68 heterogeneous populations, and confounding factors (such as BMI), that have
15 69 attempted to show PCOS is an inflammatory process
- 16 70 • The relationship between inflammation and PCOS was assessed using CRP/albumin
17 71 ratio, which may be a better marker for inflammation in the context of metabolic
18 72 dysfunction
- 19 73 • Limitation: study used waist circumference as a substitute for visceral adiposity; gold
20 74 standard is computed tomography (CT) or magnetic resonance imaging (MRI)
21 75

76 **Introduction**

77 Polycystic ovary syndrome (PCOS) is the most common reproductive disorder, affecting 5 to
78 15 % of premenopausal women worldwide, [1, 2] and the prevalence of PCOS appears to be
79 increasing. [1] This rise may partly be attributed to improved diagnosis as well as to an
80 increase in environmental factors that predispose to the development of this complex
81 metabolic condition. PCOS is characterized by androgen excess, menstrual irregularities,
82 ovulatory disturbances, and is often associated with central obesity and insulin resistance. [3-
83 5] As such, women with PCOS are at an increased risk for a number of health issues,
84 including infertility, cardiovascular disease and diabetes. [3, 6, 7]

85 Possibly related to the constellation of endocrine and metabolic dysfunction they experience,
86 women with PCOS are also found to have greater chronic subclinical inflammation, [8-11]
87 which is often clinically assessed by measuring serum levels of C-reactive protein (CRP).
88 CRP is a liver-derived acute phase protein produced in response to IL-6 secreted from
89 activated cells such as macrophages and adipocytes. [12, 13] A meta-analysis of 31 studies
90 concluded that systemic CRP levels are 96% higher in women with PCOS compared to
91 control women. [14] Collectively, these findings have given rise to the speculation that
92 inflammation may play a pivotal role in the pathophysiology of PCOS. [8-10]

93 Elevated serum CRP levels are linked to several health risk factors experienced by women
94 with PCOS, particularly insulin resistance and heightened risk of type 2 diabetes. [7, 15, 16]
95 Chronic inflammation also contributes to endothelial dysfunction, exacerbating the
96 development of atherosclerotic plaques, triggering the onset of cardiovascular disease (CVD).
97 [17] As such inflammation has been associated with both CVD and coronary artery disease in
98 women with PCOS. [10]

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3 99 In contrast to CRP, albumin is a negative acute phase response protein produced by the liver.
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5 100 Serum levels of albumin are reduced in individuals experiencing chronic inflammation. [18]
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7 101 In addition to its role as a binding molecule for sex steroids, [19] albumin also provides the
8
9 102 majority of the total antioxidant capacity of normal plasma. [18] The ratio of serum CRP
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11 103 levels over serum albumin (CRP/albumin) was found to be strongly associated with more
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13 104 severe metabolic dysfunction in premenopausal women with induced alterations to their
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15 105 ovarian hormone status. [20] CRP/albumin ratio was also found to be significantly higher in
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17 106 premenopausal women with PCOS relative to controls, and adversely predicted their bone
18
19 107 quality. [21] Given the ability of CRP/albumin to simultaneously capture chronic
20
21 108 inflammation and metabolic dysfunction in premenopausal women, we hypothesized that
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23 109 CRP/albumin ratio may serve as a strong predictor of PCOS in a cohort of similarly aged
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25 110 women. This case-control study investigated CRP/albumin ratio along with classical markers,
26
27 111 androgen excess and insulin resistance, in their association with PCOS in 319 premenopausal
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29 112 Bahraini Arab women.
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35 114 **Methods**36
37 115 *Patient and public involvement*

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39 116 The development of the research question and the study's setting was influenced by the wish
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41 117 many women in Bahrain have to bear children. Infertility is a consequence of PCOS, [22] and
42
43 118 the combination of both can impact women's health-related quality of life. [23] Patients were
44
45 119 not involved in the design of the study or the recruitment. Study participants will not be re-
46
47 120 contacted by the study investigators; however, the results of this study will be disseminated to
48
49 121 the Bahraini community through press releases of the open access publication.
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53 122 *Study subjects*
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3 123 Women with PCOS (n = 200) were recruited from adult obstetrics/gynecology,
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5 124 endocrinology and outpatient clinics in Manama, Bahrain. Women without PCOS (n = 119)
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7 125 were ethnically-matched, eumenorrheic university employees and students, and healthy
8
9 126 volunteers representative of the Bahraini population. The sample size was based on the
10
11 127 ability to detect differences between cases and controls with 10% precision (two-tailed t-test
12
13 128 with $\alpha=0.05$) and 80% power, taking into account the estimated prevalence of PCOS in
14
15 129 Bahrain of 7.5-8.0% (Bahraini Ministry of Health;
16
17 130 www.moh.gov.bh/Content/Files/Publications/statistics/HS2015) and Z-value for 95% CI
18
19 131 (confidence interval). Women serving as controls were examined in the follicular phase of
20
21 132 their menstrual cycle and had their testosterone levels were within range. A diagnosis of
22
23 133 PCOS was based on the 2003 Rotterdam Criteria, which requires two of the three following
24
25 134 criteria to be met: ultrasound evidence of polycystic ovarian morphology, anovulation, and
26
27 135 hyperandrogenism. [24] Exclusion criteria included hyperprolactinemia, non-classical adrenal
28
29 136 hyperplasia, androgen-producing tumors, 21-hydroxylase deficiency, Cushing's syndrome,
30
31 137 and active thyroid disease (overt, central and subclinical hypothyroidism and
32
33 138 hyperthyroidism). Additional exclusion criteria included extremes of body mass index (BMI;
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35 139 $<18 \text{ kg/m}^2$ or $>45 \text{ kg/m}^2$), recent/present illness, and treatment affecting carbohydrate
36
37 140 metabolism or hormonal levels, for three months or longer before inclusion the study.
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39 141 Women using anti-hypertensive, oral contraceptive, anti-inflammatory, and lipid-lowering
40
41 142 drugs were also excluded. Demographic information, along with detailed personal and family
42
43 143 history of diabetes, hypertension, infertility, hypercholesterolemia, and ischemic heart disease
44
45 144 were obtained from all participants. This study was conducted in accord with the Helsinki II
46
47 145 Declaration guidelines, and all participants gave written informed consent to participate.
48
49 146 Study approval was obtained from the Bahraini Ministry of Health and Arabian Gulf
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3 147 University Research and Ethics Committees (IRB number: 35-PI-01/15) and the Clinical
4
5 148 Research Ethics Board of the University of British Columbia (H16-02101).
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7 149
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9 150 *Biochemical analysis*
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11 151 Peripheral venous fasting blood samples were obtained between 7:00 and 9:00 am following
12
13 152 an overnight (> 12 h) fast during the early follicular phase of the menstrual cycle (days 2 ± 5)
14
15 153 for control women, or women with PCOS who did not present with menstrual irregularities,
16
17 154 or any day for women with PCOS with menstrual irregularities. Serum samples were
18
19 155 analyzed for sex hormone binding globulin (SHBG) by sandwich ELISA (R&D Systems,
20
21 156 Minneapolis, MN); assay sensitivity was 0.01 nmol/ml, and inter-assay and intra-assay
22
23 157 precision (CV %) were 5.3% and 4.3%, respectively. Samples were tested in duplicates for
24
25 158 adiponectin levels (Cat. No. DRP300) by sandwich enzyme-linked immunosorbent assay
26
27 159 (R&D Systems); assay sensitivity was 0.891 ng/ml, and inter-assay and intra-assay precision
28
29 160 (CV%) were 6.5% and 3.5%, respectively.
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33 161 Serum luteinizing hormone (LH), follicular stimulating hormone (FSH), thyroid stimulating
34
35 162 hormone (TSH), testosterone, glucose (ADVIA Centaur, Bayer Vital, Fernwald, Germany),
36
37 163 and insulin (IMMULITE 2000, DPC Biermann, Bad Nauheim, Germany), were measured by
38
39 164 automated chemiluminescence immunoassays. Free (FT) and bioactive (BT) testosterone and
40
41 165 free androgen index (FAI) were determined using Free & Bioavailable Testosterone
42
43 166 Calculator (www.issam.ch/freetesto.htm). Progesterone and estradiol serum levels were
44
45 167 quantitated by radioimmunoassay, with comparable CV% ($< 5\%$), while DHEA-S levels
46
47 168 were measured by solid-phase competitive immunoassay (Immulite; Siemens); inter- and
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49 169 intra-assay CV were 9.4 and 7.0%, respectively.
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3 171 Concentrations of serum albumin were analyzed by photospectrometry with albumin
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5 172 bromocresol purple assay on a COBAS c701 Chemistry Analyzer (Roche Diagnostics, Dubai,
6
7 173 UAE). Insulin resistance (IR) was estimated by the homeostasis model assessment (HOMA-
8
9 174 IR), defined as fasting serum insulin (IU/mL) \times fasting plasma glucose
10
11 175 (mmol/L)/22.5. HOMA-IR values were characterized as Normal (insulin-sensitive) if <2.40 ;
12
13 176 Borderline if between 2.40-3.50, and High (insulin-resistant) if > 3.50 .

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19 178 Measurement of plasma high sensitivity CRP levels was done by latex-enhanced
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21 179 nephelometry on a BN II Nephelometer (Dade Behring, Milan, Italy). Samples were assayed
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23 180 in duplicate in each analytical run; the lower limit of detection was 0.15 mg/L, and the assay
24
25 181 range was 0.175–11.0 mg/L (initial dilution). Serial serum dilutions were made in measuring
26
27 182 high CRP (>30 mg/L) levels. Percentile CRP values were estimated for comparison
28
29 183 purposes.

30 31 32 33 184 *Statistical analysis*

34
35 185 The *core outcome set* of variables included assessment of CRP/albumin as a predictor of
36
37 186 PCOS while controlling for relevant factors, such as BMI and age, and to subsequently
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39 187 compare the strength of the relationship between CRP/albumin and PCOS with variables
40
41 188 known to strongly link to the syndrome, namely androgen excess and insulin resistance. The
42
43 189 Shapiro-Wilk test was used to evaluate the distribution of the variables, and many variables
44
45 190 for the PCOS cohort were flagged as being non-normally distributed. However, upon testing
46
47 191 for skewness, we found the values for asymmetry and kurtosis fell between -2 to +2 for all.
48
49 192 Thus, we used parametric tests, as suggested, [25] given the sample sizes were >30 . We
50
51 193 reassessed the validity of our analysis by running the Mann–Whitney U test in addition to
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53 194 student t test for variables we had detected as being non-normal and confirmed that the
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3 195 results were similar. Baseline characteristics were compared using the Mann-Whitney U test
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5 196 and the independent samples t-test for the continuous variables, and the χ^2 test for categorical
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7 197 variables. Numerical variables are presented as mean \pm standard deviation (SD). Because
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9 198 distribution of CRP/albumin levels was skewed to the right, correlations between
10
11 199 CRP/albumin ratio and other continuous variables were assessed using Spearman's rho.
12
13 200 Univariate general linear models were applied to test independent associations between
14
15 201 CRP/albumin ratio and other independent variables.
16
17
18 202 The optimal cut-off level for the CRP/albumin ratio was determined by a receiver operating
19
20 203 characteristic (ROC) curve analysis, and the areas under the curve (AUC) were measured and
21
22 204 compared to assess the power of a model to identify patients who experienced metabolic
23
24 205 disturbances. Cut-off values showing the greatest accuracy were determined using a
25
26 206 sensitivity/specificity versus criterion value plot. Quartiles (i.e. 0-25%, 26-50%, 51-75%, and
27
28 207 76-100%) of CRP and CRP/albumin ratio were calculated separately in PCOS and control
29
30 208 groups. Because the 25% quartile of CRP value in the PCOS cohort was similar to the
31
32 209 standard normal values of CRP, we used the 25% quartile of CRP and CRP/albumin ratio as
33
34 210 the cut-off point for both predictors.
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37
38 211 Using the calculated cut-off values, regression analysis was performed to determine how well
39
40 212 each variable predicted PCOS. To fully explore the role of the CRP/albumin ratio as a
41
42 213 biomarker in the prediction of metabolic disturbances, CRP and CRP/albumin ratio were
43
44 214 additionally assessed as binary variables. Subjects were categorized into two groups based on
45
46 215 the cut-off values and their means (SD) for metabolic markers. Insulin resistance (HOMA-
47
48 216 IR), free testosterone, total adiponectin, BMI and TSH were compared between subjects with
49
50 217 normal values, and those who had higher than normal values. P-values <0.05 were considered
51
52 218 as statistically significant. All statistical analyses were performed using the IBM SPSS
53
54 219 statistics software program version 22 (IBM, Armonk, NY).
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220

221 **Results**222 *Study subjects*

223 The sociodemographic, anthropometric, clinical, and biochemical characteristics of the 200
 224 women with PCOS and 119 controls women are summarized in **Table 1**. Relative to controls,
 225 women with PCOS had fewer pregnancies and live births ($p<0.001$), were more likely to
 226 have insulin resistance ($p<0.001$), and differed in education attainment with those with
 227 PCOS, having a higher number of high school and post-secondary graduates ($p=0.012$).

228 *Metabolic characteristics*

229 The proportion of women with a BMI greater than 30 kg/m^2 were higher in the PCOS cohort
 230 than controls ($p<0.001$), but the waist-to-hip ratio was not significantly different (**Table 1**).
 231 Serum levels of adiponectin, an adipocyte-associated protein that tends to be inversely linked
 232 to visceral adiposity, [26] was markedly lower in women with PCOS compared to controls.
 233 Fasting plasma glucose, cholesterol, HDL, LDL and triglyceride levels did not differ
 234 significantly between women with and without PCOS. However, indices of insulin resistance
 235 and insulin sensitivity (HOMA-IR and QUICKI) indicated that women with PCOS had
 236 greater impaired regulation of insulin compared to controls (**Table 1**).

237 **Table 1: Demographic, clinical and hormonal characteristics of study population:**
 238 **women with polycystic ovary syndrome (PCOS) and controls.**

239 Data are presented as means (standard deviation).

	All (N=319)	PCOS (N=200)	Controls (N=119)	Mean difference	95 % CI of Mean Difference	p values

					lower	upper	
Age (yrs)	27.9 (6.4)	28.4 (5.9)	27.2 (7.2)	1.24	-0.22	2.71	0.09
BMI (kg/m ²)	28 (5.9)	29 (6.3)	26.5 (5)	2.53	1.19	3.87	0.000
Waist/hip ratio	0.94 (0.09)	0.94 (0.09)	0.93 (0.09)	0.0067	-0.017	0.031	0.58
Menarche (yrs)	12.5 (1.4)	12.5 (1.5)	12.4 (1.2)	0.12	-0.21	0.45	0.47
HOMA ₁ IR	3.2 (0.18)	3.8 (0.26)	2.1 (0.2)	1.67	0.94	2.4	0.000
QUICKI	0.6 (0.006)	0.57 (0.008)	0.65 (.01)	-0.078	-0.10	-0.05	0.000
Total adiponectin (ng/L)	33.8 (1.4)	28.4 (1.3)	44.6 (2.9)	-16.25	-21	-10	0.000
Albumin (g/L)	37.3 (0.46)	32.7 (0.46)	45 (0.35)	-12.22	-13.52	-10.93	0.000
CRP (mg/L)	11.1 (1.1)	15.5 (1.6)	3.6 (0.85)	11.89	7.61	16.17	0.000
CRP/Albumin ratio	0.36 (0.04)	0.53 (0.06)	0.08 (0.02)	0.45	0.29	0.61	0.000
SHBG (nmol/L)	60.1 (1.5)	52.2 (1.4)	72 (2.8)	-19.72	-26.5	-13.93	0.000
DHEAS (nmol/L)	6.2 (0.28)	6.2 (0.29)	6.3 (0.95)	-0.18	-0.174	1.36	0.81
Total testosterone (nmol/L)	1.7 (0.06)	1.8 (0.07)	1.5 (0.1)	0.23	-0.02	0.49	0.08
Bioavailable testosterone (nmol/L)	0.49 (0.02)	0.52 (0.03)	0.42 (0.03)	0.098	0.015	0.18	0.02
Free Androgen Index	3.4 (0.16)	4 (0.22)	2.4 (0.16)	1.57	0.93	2.2	0.000
Free testosterone	0.025 (0.001)	0.029 (0.001)	0.017 (0.001)	0.011	0.007	0.015	0.000

index							
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240 BMI: Body Mass Index, HOMA.IR: Homeostatic Model Assessment for Insulin Resistance,

241 QUICKI: Quantitative insulin sensitivity check index, CRP: C-reactive protein, SHBG: Sex

242 hormone binding hormone.

243 *Reproductive hormone characteristics*

244 There were no statistically significant differences in plasma levels of estradiol, progesterone,

245 total testosterone, prolactin, FSH, LH and DHES between women with and without PCOS.

246 Free testosterone was higher and SHBG lower in women with PCOS (**Table 1**).

247 *C-reactive protein (CRP)/albumin ratio as a predictor of polycystic ovary syndrome (PCOS)*

248 *stratified by body mass index (BMI)*

249 Women with PCOS had markedly higher levels of CRP and lower levels of serum albumin

250 relative to controls ($p < 0.001$; **Table 1**). ROC curve analysis showed that the CRP/albumin

251 ratio had greater discriminatory power to differentiate between women with PCOS and

252 controls (AUC: 0.865, 95% CI: 0.824-0.905) compared to CRP alone (AUC: 0.820, 95% CI:

253 0.773-0.867); **Figure 1**. This greater efficacy of CRP/albumin ratio to discriminate between

254 cases and controls was also evident when taking into account the presence of insulin

255 resistance at every measure of sensitivity; for a sensitivity level of 75%, CRP/albumin ratio

256 had a specificity of 85% compared to 69% for CRP alone.

257 Spearman correlation analysis between CRP/albumin ratios and clinical and biochemical

258 markers was subsequently performed. Variables found to be univariately linked to

259 CRP/albumin values were included in a general linear model testing the relationship amongst

260 PCOS diagnosis, BMI, and CRP/albumin levels. The model revealed that for any given BMI

261 value, women with PCOS have markedly elevated CRP/albumin levels ($p < 0.001$, **Figure 2**).

262 Variables that are known to strongly associate with PCOS, namely free androgen index and
 263 insulin resistance, were compared to CRP/albumin ratio as predictors of PCOS in a binary
 264 regression analysis stratified by three BMI categories: $<25 \text{ kg/m}^2$ [normal], $25 - 30 \text{ kg/m}^2$
 265 [overweight], $>30 \text{ kg/m}^2$ [obese]); **Table 2.** A CRP/albumin ratio of ≥ 0.097 outperformed
 266 both insulin resistance and free androgen index in predicting PCOS for every BMI category
 267 (**Table 2**).

268

269 **Table 2: Summary of binary Regression Analysis for Variables Predicting PCOS with**
 270 **the Odds Ratio of each risk factor adjusted for other variables in the model**

271

	Odds Ratio (95% Confidence Interval)		
	BMI < 25 (normal)	BMI 25-30 (overweight)	BMI \geq 30 (obese)
CRP/Albumin Ratio ¹			
< 0.097	1	1	1
≥ 0.097	11.21 (3.28-39.75)	19.32 (5.07-72.17)	34.5 (7.75-153.52)
Insulin Resistance ²			
No	1	1	1
Borderline	3.34 (0.645-17.33)	5.58 (0.907-34.41)	3.13 (0.53-18.48)
Yes	9.21 (1.63-51.93)	8.81 (1.75-44.31)	17.94 (1.81-177.61)
Free Androgen Index ³			
<3.95	1	1	1
≥ 3.95	2.28 (.536-9.75)	0.86 (.22-3.35)	3.79 (0.59-24.42)

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273 ¹Cut-off values were derived from the sensitivity and specificity analysis of the receiver
 274 operating characteristic curve

275 ²Categories of insulin resistance based on normal values used for HOMA-IR

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3 276 ³Cut-off values from normal laboratory reference ranges for free androgen index
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7 278 **Discussion**
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9 279 This study demonstrated that CRP/albumin ratio is a stronger correlate of PCOS than both
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11 280 free androgens and insulin resistance in 319 ethnically-matched premenopausal women, and
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13 281 this relationship was independent of BMI. Despite being the most common reproductive
14
15 282 disorder to affect women, the etiology of PCOS has remained elusive to date. In the absence
16
17 283 of a definitive cure, treatment has focused on symptom management, and a goal to prevent
18
19 284 the progression of serious health conditions, such as type 2 diabetes and CVD, for which
20
21 285 women with PCOS are at heightened risk. Chronic low-grade inflammation has emerged as a
22
23 286 common underlying state in women with PCOS, and a likely direct contributor to insulin
24
25 287 resistance and heart disease risk. This has raised the question whether PCOS is fundamentally
26
27 288 an inflammatory condition. [8, 10, 27]
28
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30
31 289 Small sample sizes, heterogeneous populations, and an inability to correct for confounding
32
33 290 factors, such as BMI and use of oral contraceptives, both of which influence inflammatory
34
35 291 markers such as CRP, [21] has in part hampered efforts in assigning inflammation as truly a
36
37 292 defining feature of PCOS. This current analysis has overcome some of the main issues in
38
39 293 assessing the independent relationship between PCOS and chronic low-grade inflammation.
40
41 294 This was accomplished by accounting for many of the confounding variables and by using a
42
43 295 more refined marker for inflammation, the CRP/albumin ratio, which may have greater
44
45 296 specificity and sensitivity for inflammation associated with metabolic dysfunction. The
46
47 297 CRP/albumin ratio was first found to be useful in assessing cardiometabolic and
48
49 298 inflammatory status following ovariectomy surgery. [20] It was subsequently used to show
50
51 299 the influence of chronic subclinical inflammation on bone quality in women with PCOS. [21]
52
53 300 Although CRP is used to predict cardiovascular risk and is associated with metabolic
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3 301 disorders associated with obesity and insulin resistance, [28-31] it has been criticized for
4
5 302 being too general and non-specific a marker for inflammation. [32]
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7
8 303 When compared to CRP alone, we found that the CRP/albumin ratio has an improved ROC
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10 304 curve for predicting PCOS. Serum albumin, which is commonly measured to assess liver
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12 305 function and malnutrition, is not widely considered as an analyte of interest for PCOS.
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14 306 However, this study showed for the first time that albumin is markedly reduced in women
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16 307 with PCOS relative to controls. This may, at least in part, be due to albumin being a negative
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18 308 acute phase protein. [18] It is also possible that there is increased oxidation and glycation of
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20 309 albumin in women with PCOS, which can impact the structure, function and metabolism of
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22 310 the protein. [18] Albumin is one of the most abundant serum proteins, and among its many
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24 311 roles is the transport of hormones. [19] Thus, reduced albumin levels can potentially
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26 312 contribute to higher free androgens in women with PCOS and exacerbation of disease
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28 313 phenotype.
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32 314 This analysis was limited by a lack of a more sensitive measure of visceral adiposity; the gold
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34 315 standard is imaging with computed tomography (CT) or magnetic resonance imaging (MRI).
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36 316 [33] Furthermore, the case-control design limited the ability to assess how CRP/albumin
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38 317 performs in predicting health outcomes in women with PCOS. Prospective studies are now
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40 318 needed to determine the use of CRP/albumin in predicting the progression of disorders linked
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42 319 to chronic inflammation and metabolic dysfunction that women with PCOS are at increased
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44 320 risk. These include not only cardiovascular disease and diabetes, but also depression. [34-37]
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46 321 Importantly, CRP/albumin ratio may be particularly useful in assessing the effectiveness of
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48 322 new interventions targeting inflammation in women with PCOS as a novel approach to
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50 323 managing the condition and its long-term health consequences.
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3 324 *Conclusion:* CRP/albumin ratio, a marker for inflammation related to metabolic dysfunction,
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5 325 was found to have a stronger association with PCOS than either androgen excess or insulin
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7 326 resistance. Inflammation is known to be influenced by adiposity, but relative to controls,
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9 327 women with PCOS have higher levels of CRP/albumin ratio irrespective of BMI. This
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11 328 supports the view that inflammation may play a central role in the pathophysiology of PCOS.
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14 329

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17
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19
20 332 Coastal Health Research Institute in support of Dr. Shirin Kalyan.

21 22 333 **Data Sharing Statement**

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24 334 Due to subject confidentiality, the complete data cannot be made publicly available.
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26 335 However, researchers who would like controlled access to the data are welcome to contact
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28 336 Dr. Wassim Y. Almawi at: wassim.almawi@outlook.com.

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32
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34
35 339 their support and time, this work would not be possible.

36 37 340 **Competing Interests Statement**

38
39 341 SK is Director of Scientific Innovation at Qu Biologics Inc., a clinical-stage biotechnology
40
41 342 company. All other authors have no conflict of interest to declare.

42 43 44 343 **Author Contributions**

45
46 344 SK designed the study, interpreted the data and wrote the first draft of the manuscript. AG
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48 345 performed the statistical analysis and helped interpret the analysis. SS and WA collected all
49
50 346 the data, performed the biochemical analysis and managed the clinical operations of the
51
52 347 study. AJ assisted with literature review and manuscript preparation. All authors reviewed
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54 348 and approved the final manuscript.
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3 456 **Figure Legends**

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5 458 **Figure 1.** Receiver Operating Characteristic (ROC) curve plotting the true positive rate

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7 459 against the false positive rate for CRP/Albumin (green line) and CRP (blue line) in

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9 460 differentiating women with and without PCOS. The area under the curve (AUC) for

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11 461 CRP/Albumin: 0.865, 95% CI: 0.824-0.905; for CRP: 0.820, 95% CI: 0.773-0.867.

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15 462 **Figure 2.** Linear regression analysis of adjusted CRP/albumin values by body mass index

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17 463 (BMI) in women with PCOS and controls. A univariate generalized linear model was

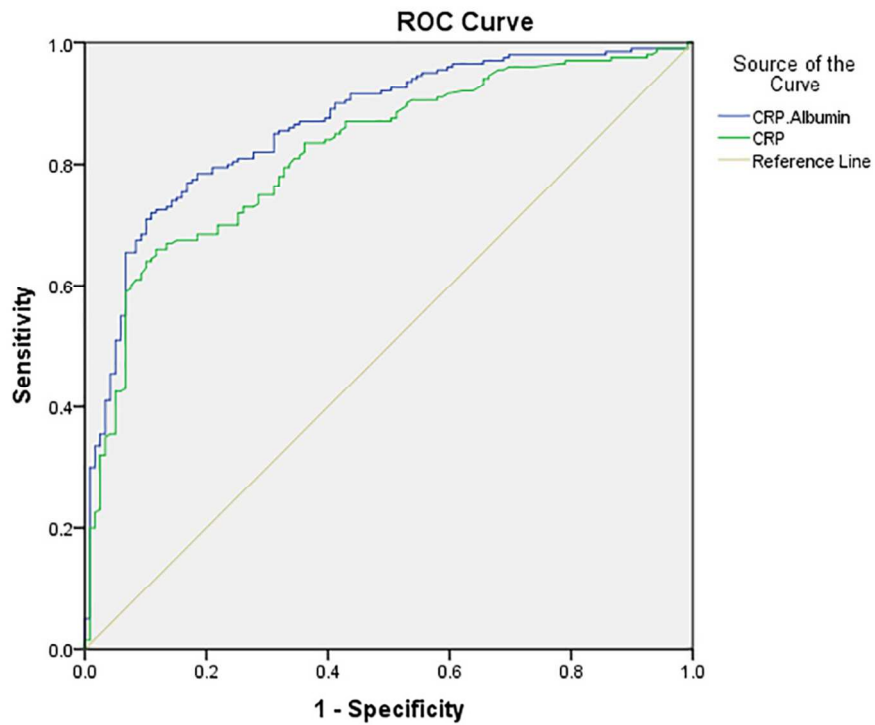
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19 464 computed investigating the relationship between CRP/albumin and BMI, adjusting for the

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21 465 variables found to associate with CRP/albumin (insulin, free testosterone, progesterone and

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23 466 adiponectin) plus age stratified by PCOS diagnosis.

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Diagonal segments are produced by ties.

33 Figure 1. Receiver Operating Characteristic (ROC) curve plotting the true positive rate against the false
34 positive rate for CRP/Albumin (green line) and CRP (blue line) in differentiating women with and without
35 PCOS. The area under the curve (AUC) for CRP/Albumin: 0.865, 95% CI: 0.824-0.905; for CRP: 0.820, 95%
36 CI: 0.773-0.867.

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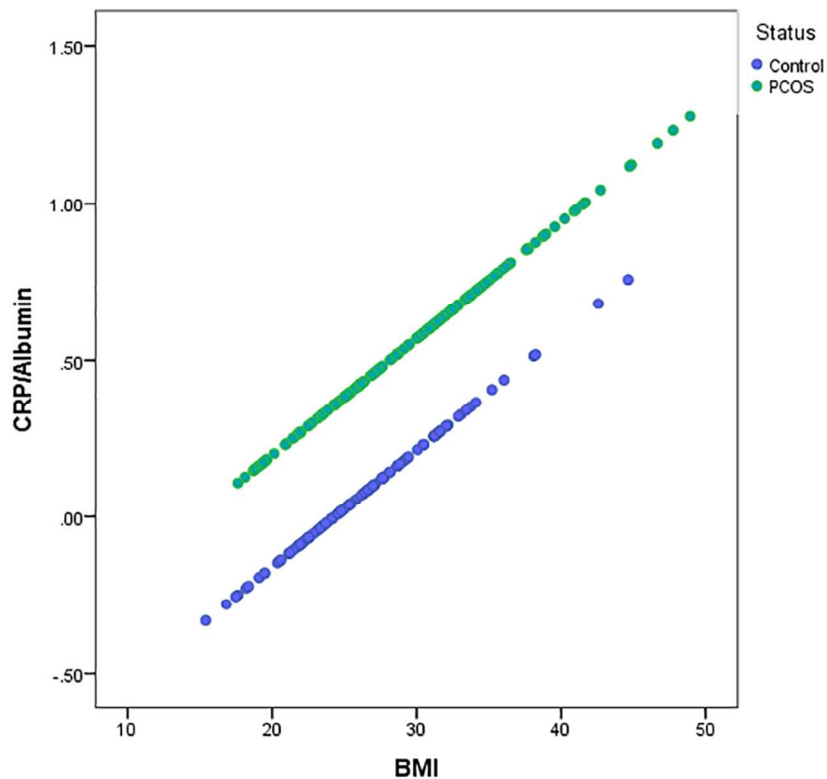


Figure 2. Linear regression analysis of adjusted CRP/albumin values by body mass index (BMI) in women with PCOS and controls. A univariate generalized linear model was computed investigating the relationship between CRP/albumin and BMI, adjusting for the variables found to associate with CRP/albumin (insulin, free testosterone, progesterone and adiponectin) plus age stratified by PCOS diagnosis.

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STROBE Statement

Checklist of items that should be included in reports of observational studies

Section/Topic	Item No	Recommendation	Reported on Page No
Title and abstract	1	(a) Indicate the study's design with a commonly used term in the title or the abstract	1
		(b) Provide in the abstract an informative and balanced summary of what was done and what was found	2,3
Introduction			
Background/rationale	2	Explain the scientific background and rationale for the investigation being reported	4-5
Objectives	3	State specific objectives, including any prespecified hypotheses	5
Methods			
Study design	4	Present key elements of study design early in the paper	5
Setting	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection	5-6
Participants	6	(a) <i>Cohort study</i> —Give the eligibility criteria, and the sources and methods of selection of participants. Describe methods of follow-up	5-6
		<i>Case-control study</i> —Give the eligibility criteria, and the sources and methods of case ascertainment and control selection. Give the rationale for the choice of cases and controls	
		<i>Cross-sectional study</i> —Give the eligibility criteria, and the sources and methods of selection of participants	
Variables	7	(b) <i>Cohort study</i> —For matched studies, give matching criteria and number of exposed and unexposed	5-7
		<i>Case-control study</i> —For matched studies, give matching criteria and the number of controls per case	
Data sources/measurement	8*	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable	5-7
Bias	9	For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group	6,7
Bias	9	Describe any efforts to address potential sources of bias	5,6
Study size	10	Explain how the study size was arrived at	5
Quantitative variables	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why	7,8
Statistical methods	12	(a) Describe all statistical methods, including those used to control for confounding	7,8
		(b) Describe any methods used to examine subgroups and interactions	-
		(c) Explain how missing data were addressed	-
		(d) <i>Cohort study</i> —If applicable, explain how loss to follow-up was addressed	5
		<i>Case-control study</i> —If applicable, explain how matching of cases and controls was addressed	
(e) Describe any sensitivity analyses	-		
		<i>Cross-sectional study</i> —If applicable, describe analytical methods taking account of sampling strategy	

Section/Topic	Item No	Recommendation	Reported on Page No
Results			
Participants	13*	(a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed	9
		(b) Give reasons for non-participation at each stage	-
		(c) Consider use of a flow diagram	-
Descriptive data	14*	(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders	Table 1
		(b) Indicate number of participants with missing data for each variable of interest	Table 1
		(c) <i>Cohort study</i> —Summarise follow-up time (eg, average and total amount)	
Outcome data	15*	<i>Cohort study</i> —Report numbers of outcome events or summary measures over time	
		<i>Case-control study</i> —Report numbers in each exposure category, or summary measures of exposure	9, Table 1
		<i>Cross-sectional study</i> —Report numbers of outcome events or summary measures	
Main results	16	(a) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included	9-11
		(b) Report category boundaries when continuous variables were categorized	Table 2, 11
		(c) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period	-
Other analyses	17	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses	Figures 1-2, Table 2
Discussion			
Key results	18	Summarise key results with reference to study objectives	12-13
Limitations	19	Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias	13-14
Interpretation	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence	14
Generalisability	21	Discuss the generalisability (external validity) of the study results	13, 14
Other Information			
Funding	22	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based	14

*Give information separately for cases and controls in case-control studies and, if applicable, for exposed and unexposed groups in cohort and cross-sectional studies.

Note: An Explanation and Elaboration article discusses each checklist item and gives methodological background and published examples of transparent reporting. The STROBE checklist is best used in conjunction with this article (freely available on the Web sites of PLoS Medicine at <http://www.plosmedicine.org/>, Annals of Internal Medicine at <http://www.annals.org/>, and Epidemiology at <http://www.epidem.com/>). Information on the STROBE Initiative is available at www.strobe-statement.org.

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