Clinical Infectious Diseases

MAJOR ARTICLE

Potential Influence of Menstrual Status and Sex Hormones on Female Severe Acute Respiratory Syndrome Coronavirus 2 Infection: A Cross-sectional Multicenter Study in Wuhan, China

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Background. Recent studies have indicated that females with coronavirus disease 2019 (COVID-19) have a lower morbidity, severe case rate, and mortality and better outcome than those of male individuals. However, the reasons remained to be addressed.

Methods. To find the factors that potentially protect females from COVID-19, we recruited all confirmed patients hospitalized at 3 branches of Tongji Hospital (N = 1902), and analyzed the correlation between menstrual status (n = 509, including 68 from Mobile Cabin Hospital), female hormones (n = 78), and cytokines related to immunity and inflammation (n = 263), and the severity/clinical outcomes in female patients <60 years of age.

Results. Nonmenopausal female patients had milder severity and better outcome compared with age-matched men ($P < .01$ for both). Menopausal patients had longer hospitalization times than nonmenopausal patients (hazard ratio [HR], 1.91 [95% confidence interval {CI}, 1.06–3.46]; $P = .033$). Both anti-Müllerian hormone (AMH) and estradiol (E2) showed a negative correlation with severity of infection (adjusted HR, 0.146 [95% CI, .026–.824], $P = .029$ and 0.304 [95% CI, .092–1.001], $P = .05$, respectively). E2 levels were negatively correlated with interleukin (IL) 2R, IL-6, IL-8, and tumor necrosis factor alpha in the luteal phase ($P = .033$, $P = .048$, $P = .054$, and $P = .023$) and C3 in the follicular phase ($P = .030$).

Conclusions. Menopause is an independent risk factor for female COVID-19 patients. AMH and E2 are potential protective factors, negatively correlated with COVID-19 severity, among which E2 is attributed to its regulation of cytokines related to immunity and inflammation.

Keywords. menstrual status; female hormones; SARS-CoV-2; cross-sectional study; E2.

Beginning in December 2019, coronavirus disease 2019 (COVID-19), caused by the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), rapidly spread across the world, causing widespread concern. It was observed that there was a definite sex difference associated with COVID-19 morbidity and mortality. According to recent data from the Chinese Center for Disease Control and Prevention (CCDC), the ratio of male to female infection reached 2.7:1 [1]. Among 72 314 patients in China, the morbidity and case fatality rates (48.6% and 1.7%, respectively) of women were lower than those of men (51.4% and 2.8%, respectively). More deaths were found in males than females (63.8% vs 36.2%) [2]. Recent studies considered that biological sex differences contribute to male-biased death [3, 4]. These results indicate that females are less susceptible to COVID-19 than males, and have better outcomes.

Severe acute respiratory syndrome coronavirus (SARS-CoV) and Middle East respiratory syndrome coronavirus (MERS-CoV), "sisters" of SARS-CoV-2, also showed sex bias in their infection of humans, with males experiencing higher case fatality rates than females. Angiotensin-converting enzyme 2 (ACE2) has been shown to be the receptor of both SARS-CoV and SARS-CoV-2. Interestingly, its level of expression is higher in the lungs of males compared with females [5]. Consistently, the expression of ACE2 is downregulated by estradiol (E2) [6]. This was confirmed in animal experiments, showing that estrogen mitigates the susceptibility and severity of the phenotype for SARS-CoV, while ovariectomy or intervention with estrogen receptor inhibitors increased the mortality of female mice [7]. An

Received 6 June 2020; editorial decision 14 July 2020; accepted 20 July 2020; published online July 22, 2020.

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Clinical Infectious Diseases® 2020;XX(XX):1–9

DOI: 10.1093/cid/ciaa1022

antiviral drug study showed that estrogen receptor inhibitors are highly effective in antiviral activity screening for MERS-CoV, SARS-CoV, and other RNA viruses, and also have an anti-Ebola effect in mice [8]. These studies suggest that E2 might play a protective role in SARS-CoV or MERS-CoV infection, with lower susceptibility and severity as well as preferable outcomes in the respective diseases. The female sex hormones estrogen and progesterone, periodically secreted by the ovary, stimulate the uterus so as to cause regular menstruation. However, the effect of sex hormones and related menstrual status on the severity and outcomes of COVID-19 has remained unknown.

Our objective was to address how menstrual status and sex hormones could affect the progress and outcomes of COVID-19, with the aim of learning the possible mechanism in females that protects against COVID-19.

METHODS

Study Design and Participants

This collaborative clinical study evaluated aspects of menstrual status and sex hormones on severity and outcome of patients in China with COVID-19 in 3 cohorts. In cohort 1, sex differences in severity and clinical outcome were evaluated in all 1902 patients from 3 branches of Tongji Hospital (Sino-French New City Branch, Optical Valley Branch, and Hankou main campus) between 28 January and 8 March 2020. Women in this cohort who reported menstrual history were divided into 2 groups according to their menopausal status: nonmenopausal (still have menstruation, regular or irregular) or menopausal (amenorrhea >1 year), and compared with age-matched men. In cohort 2, differences in menstruation in relationship to severity and a composite clinical outcome (intensive care unit admission, ventilation, or death) were evaluated in 509 female patients who were <60 years of age (441 from Tongji Hospital, 68 from Mobile Cabin Hospital). They were queried by telephone follow-up regarding menstrual status and gynecologic history, and 435 patients were included in the study. Women in pregnancy were ruled out. None of them took hormone therapy within the past 3 months such as contraceptives or menopausal treatments. In cohort 3, which included the 435 patients in cohort 2, the relationship of illness severity and sex hormones (78 women aged <60 years) or serum cytokine levels were studied (263 women), excluding those who denied our request. Patients who still have menses were divided into 2 menstrual cycle groups according to the days after initiation of menses: day 5–12 was regarded as the follicular phase whereas day 15–28 was considered the luteal phase if the menstrual cycle was regular. If irregular, progesterone <1.5 ng/mL was regarded as the follicular phase and progesterone ≥1.5 ng/mL the luteal phase.

This study was reviewed and approved by the Medical Ethical Committee of Tongji Hospital of Huazhong University of Science and Technology (TJ-IRB20200214). Oral informed consent was obtained from each enrolled patient. The flowchart of the study is shown in Figure 1.

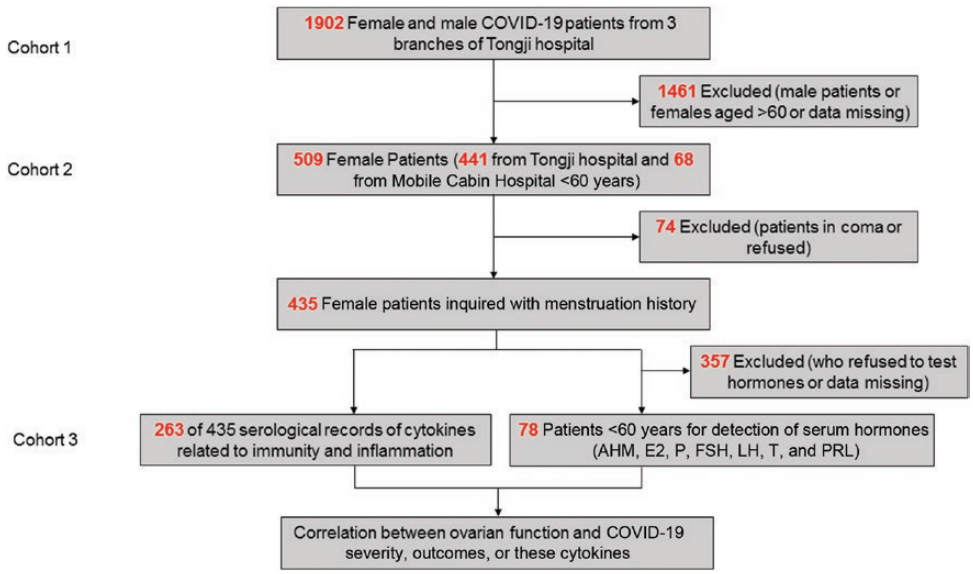

Figure 1. Flowchart of patient recruitment procedure. Patients (N = 1902) from the 3 branches of Tongji Hospital were included to explore the sex differences in coronavirus disease 2019 (COVID-19) prognosis. Female patients (n = 509 [441 from Tongji Hospital and 68 from Mobile Cabin Hospital]) were surveyed by telephone follow-up regarding menstrual status and gynecologic history. A total of 435 patients with complete medical history were included. We then tested serum cytokines related to immunity and inflammation for 263 patients and sex hormone levels for 78 patients except those who denied our request. Finally, we determined the correlation between menstruation status/sex hormones and severity, and outcomes of COVID-19. Abbreviations: AMH, anti-Müllerian hormone; COVID-19, coronavirus disease 2019; E2, estradiol; FSH, follicle-stimulating hormone; LH, luteinizing hormone; P, progesterone; PRL, prolactin; T, testosterone.

Data Collection

A confirmed case of COVID-19 was defined as a positive result on real-time reverse-transcription polymerase chain reaction assay of throat swab specimens [9]. We grouped patients from all 3 cohorts into severe and nonsevere SARS-CoV-2 infection on admission according to the American Thoracic Society/Infectious Diseases Society of America guideline for community-acquired pneumonia [10] and the published article of COVID-19 by Guan et al [11]. Recorded information included exposure history, clinical symptoms, comorbidities, laboratory findings, chest computed tomographic scans, and clinical outcome data, which were monitored up to 8 March 2020. Comorbidities refer to coexisting chronic diseases. Main antiviral treatments included arbidol and oseltamivir.

The blood samples using in this study were collected once in hospital before any treatment to know the patients' baseline conditions. Laboratory tests included a complete blood count, C-reactive protein, and cytokines related to immunity and inflammation. All laboratory testing was performed according to the patient's clinical care needs.

Hormone Detection

Serum follicle-stimulating hormone, luteinizing hormone, E2, testosterone, prolactin, and progesterone levels were measured using a chemoluminescence-based immunometric assay on an UniCelDxI 800 immunoassay system (Beckman Coulter). Serum concentrations of anti-Müllerian hormone (AMH) were measured using the Elecsys AMH kit (Roche). All of these samples were measured in the same laboratory immediately after collection. The intra- and interassay coefficients of variation were all <15%. The lowest amount of AMH that could be detected with a 95% probability in a sample was 0.01 ng/mL.

Statistical Analysis

Continuous variables were expressed as mean and standard deviation or median and interquartile range (IQR) as appropriate. Categorical variables were summarized as the counts and percentages in each category. Mann-Whitney *U* tests were applied to continuous variables, and χ^2 tests and Fisher exact tests were used for categorical variables as appropriate. Multivariate Cox proportional hazards models were used to evaluate the independent effect of menstrual status on prolonging hospitalization time, controlling for age, severity, and comorbidity. A multiple logistic regression model was used to assess the independent adjusted relationship between menstrual status/sex and the severity of infection, controlling for age. Pearson correlation analysis was used to explore the relationship between cytokines related to immunity and inflammation and severity, and composite endpoint of infection or E2 level. All analyses were conducted with SPSS version 19.0 and R version 3.5 software. $P < .05$ was considered significant. Data were analyzed by qualified statisticians.

RESULTS

Sex Difference in Severity and Clinical Outcome of Patients With COVID-19

To explore the sex difference in severity and outcomes, a cohort of patients (n = 1902) infected with SARS-CoV-2 was recruited from the 3 branches of Tongji Hospital (Supplementary Table 1). We then divided the female patients into the nonmenopausal and menopausal groups depending on their menstrual status, and compared them with age-matched males. No significant differences of age or comorbidities existed in nonmenopausal females and age-matched males ($P = .98$ and $P = .24$, respectively), or in menopausal women and age-matched men ($P = .96$ and $P = .15$, respectively). Comparing the disease severity and clinical outcomes in menopausal women and age-matched men, no apparent differences were observed ($P = .83$ and $P = .49$, respectively). However, obvious differences existed in nonmenopausal females and age-matched males in both disease severity and clinical outcomes. Compared with age-matched males, fewer nonmenopausal females suffered severe COVID-19 disease (76/124; $P < .01$) and died eventually (0/16; $P < .01$) (Table 1). Collectively, these results suggested that nonmenopausal women have potential protective factors.

Relationship Between Menstrual Status and COVID-19 Severity or Outcomes in Females

To reduce the extreme value from older menopausal women, 509 patients <60 years of age from Tongji and Mobile Cabin Hospitals were compared and classified into nonsevere (386 [75.8%]) and severe (123 [24.2%]) groups. The median age was 49 years (IQR, 38–56 years); for patients >55 years of age, the proportion was significantly increased in the severe group ($P < .0012$). Fifty-two patients had a body mass index of >24 kg/m^2, 138 patients described a recent mental disorder, and 443 of 509 lived in Wuhan; there were significant differences in severity ($P = .0046$) and composite endpoint ($P = .031$) between nonmenopausal and menopausal COVID-19 patients, even those aged <60 years (Table 2). But if adjusted for age variations, the significances were reduced ($P = .26$ vs $P = .544$, respectively; Supplementary Table 2). At admission, the most common symptoms were fever (393 [77.2%]), cough (156 [30.6%]), and dyspnea (54 [10.6%]). About one-quarter (119/506 [23.5%]) of patients had 1 or more comorbidities, and 90 of 250 (24.4%) had 1 or more gynecological diseases. Nearly half (206/433 [47.6%]) of patients received antiviral treatment, and 198 of 433 (45.7%) received antibiotic treatment (Table 2).

In univariate Cox regression analysis, age and disease severity showed significant correlation with probability of hospitalization (hazard ratio [HR], 0.36 [95% confidence interval {CI}, .23–.57], $P < .0001$; HR, 0.38 [95% CI, .2–.72], $P = .0020$, respectively), and menstruation showed no significance with probability of hospitalization (HR, 0.67 [95% CI, .41–1.1]; $P = .11$) (Figure 2, Supplementary Table 2). However, when discharge was used as an endpoint and variables including

Table 1. Disease Severity and Clinical Outcomes Between Males and Females in 1902 Patients With Coronavirus Disease 2019

Characteristic	Total	Male (Age-matched)	Female (Nonmenopausal)	*P* Value	Total	Male (Age-matched)	Female (Menopausal)	*P* Value
Age, y, mean ± SD	328	42.49 ± 12.98	42.45 ± 13	.98	1402	64.49 ± 11.23	64.52 ± 10.93	.96
Disease severity								
Nonsevere	128	40 (24.39%)	88 (53.66%)	<.01	579	275 (40.86%)	304 (41.7%)	.83
Severe	200	124 (75.61%)	76 (46.34%)		823	398 (59.14%)	425 (58.3%)	
Clinical outcomes								
Discharged	52	14 (8.54%)	38 (23.17%)	<.01	87	44 (6.54%)	43 (5.9%)	.49
Remained in hospital	260	134 (81.71%)	126 (76.83%)		1280	609 (90.49%)	671 (92.04%)	
Death	16	16 (9.76%)	0 (%)		35	20 (2.97%)	15 (2.06%)	
Comorbidities[a]				.24				.15
Yes	105	58 (35.37%)	47 (28.66%)		813	404 (60.03%)	409 (56.1%)	
No	223	106 (64.63%)	117 (71.34%)		589	269 (39.97%)	320 (43.9%)	

Data are presented as no. (%) unless otherwise indicated. Mann-Whitney *U* test was applied to continuous variables; χ^2 test and Fisher exact test were used for categorical variables as appropriate.

Abbreviation: SD, standard deviation.

[a]Comorbidities refer to coexisting chronic diseases including hypertension, diabetes, cardiovascular disease, chronic obstructive pulmonary disease, cerebrovascular disease, hyperthyroidism, and chronic nephrosis; excludes any gynecological diseases.

comorbidities, disease severity, age, and menstrual status (nonmenopausal vs menopausal) were brought into a multivariate Cox regression analysis, the nonmenopausal group showed a definite protective effect. Nonmenopausal patients tended to have a lower hospitalization proportion and to be discharged earlier than patients in menopause, controlling for age and severity (HR, 1.91 [95% CI, 1.06–3.46]; *P* = .033) (Figure 2, Supplementary Table 2).

Relationship Between Serum Hormone Levels and COVID-19 Severity

The baseline clinical characteristics of the 78 patients who agreed to test for serum hormone are shown in Supplementary Table 3. Age, phase of menstrual cycle, menstrual regularity in the preceding 12 months, and menstrual volumes in the preceding 3 months were similar without differences in terms of COVID-19 severity and composite endpoints. Therefore, sex hormones were analyzed as a continuous or statistical cutoff value considering their current effects regardless of menstrual status or menstrual cycle phase. The correlation between sex hormones and disease severity is shown in Table 3. Levels of E2, AMH, and testosterone seemed to be most closely related to infection severity. Composite endpoints were not discussed for this group, as all patients were alive and had not reached any of the composite endpoints at the end of observation. Since age strongly affects sex hormone levels, it was used as an adjustable variable. In this context, AMH still showed a protective effect against severity (adjusted HR [aHR], 0.146; *P* = .029). E2 showed a critical value (aHR, 0.335; *P* = .065). Considering that patients' menstrual phase was greatly affected by E2 levels and comorbidities may influence the severity of illness, these were used as another adjustable variable except age. E2 >70 pg/mL showed a clear protective effect against the disease (aHR, 0.304; *P* = .05; Table 3).

Correlation Analysis Between Cytokines Related to Immunity and Inflammation and E2 or COVID-19 Severity/Outcomes

The correlation between cytokines related to immunity, inflammation, and disease severity or composite endpoints is displayed and analyzed in Supplementary Table 4. Higher levels of interleukin (IL) 6 and IL-8 were found in the severe group than in the nonsevere group (*P* = .040 and *P* = .033, respectively; Figure 3, Supplementary Table 4). Higher levels of IL-2R, IL-6, IL-8, and IL-10 were also observed in patients with composite endpoints compared with patients without composite endpoints (*P* = .0091, *P* < .0001, *P* = .00017, and *P* = .040, respectively; Supplementary Table 4).

No significant correlation was found between the AMH level and the cytokines cited above. The correlation between the E2 level (either in luteal or follicular phase) and the level of these cytokines is shown in Figure 3. E2 levels were negatively correlated with levels of IL-2R, IL-6, IL-8, and tumor necrosis factor alpha (TNF-α) in the luteal phase (Pearson correlation = −0.592, −0.558, −0.545, and −0.623; *P* = .033, *P* = .048, *P* = .054, and *P* = .023, respectively) and with C3 in the follicular phase (Pearson correlation = −0.651; *P* = .030; Figure 3). Collectively, the levels of IL-6 and IL-8 were positively correlated with COVID-19 severity and composite endpoints. The E2 level was negatively correlated to levels of IL-6, IL-8, IL-2R, and TNF-α in the luteal phase and C3 in the follicular phase.

DISCUSSION

Whether sex might impact the severity and outcomes of COVID-19 patients is not completely understood. Additionally, the clinical outcomes of female patients were better than those of male patients, which is in accordance with recent studies [1, 11, 12]. Menstrual status and sex hormone, the unique characteristics of women being quite different from men, were first taken into consideration to further explore this mechanism.

Table 2. Baseline Clinical Characteristics of 509 Female Patients With Coronavirus Disease 2019, According to Disease Severity and Composite Endpoint

		no./No. (%)				no./No. (%)		
			Disease Severity			Composite Endpoint[a]		
		All Patients (N = 509)	Nonsevere (386/509 [75.8%])	Severe (123/509 [24.2%])	*P* Value	No (487/509 [95.7%])	Yes (22/509 [4.3%])	*P* Value
Characteristic								
	Age, y							
	Median (IQR)	49.0 (38.0–56.0)	47.0 (38.0–55.0)	52.0 (42.8–57.0)	<.0012	48.0 (38.0–56.0)	54.5 (43.8–57.0)	.15
	Distribution							
	0–40 y	145/506 (28.7)	119/384 (31.0)	26/122 (21.3)		142/484 (29.3)	3/22 (13.6)	
	41–55 y	230/506 (45.5)	176/384 (45.8)	54/122 (44.3)		220/484 (45.5)	10/22 (45.5)	
	>55 y	131/506 (25.9)	89/384 (23.2)	42/122 (34.4)	.022	122/484 (25.2)	9/22 (40.9)	.15
	BMI >24 kg/m^2	52/147 (35.4)	42/127 (33.1)	10/20 (50.0)	.14	51/145 (35.2)	1/2 (50.0)	1.0
	Recent mental disorder	138/245 (56.3)	117/207 (56.5)	21/38 (55.3)	.89	138/243 (56.8)	0/2 (0.0)	.11
	Resident of Wuhan	443/485 (91.3)	335/362 (92.5)	108/123 (87.8)	.11	423/463 (91.4)	20/22 (90.9)	1.0
Symptoms on admission								
	Fever	393/509 (77.2)	297/386 (76.9)	96/123 (78.0)	.80	381/487 (78.2)	12/22 (54.5)	.010
	Cough	156/509 (30.6)	123/386 (31.9)	33/123 (26.8)	.29	149/487 (30.6)	7/15 (31.8)	.90
	Sore throat	12/509 (2.4)	11/386 (2.8)	1/123 (0.8)	.31	11/487 (2.3)	1/22 (4.5)	.42
	Fatigue	41/509 (8.1)	39/386 (10.1)	2/123 (1.6)	.0026	40/487 (8.2)	1/22 (4.5)	1.0
	Myalgia	36/509 (7.1)	34/386 (8.8)	2/123 (1.6)	.0068	35/487 (7.2)	1/22 (4.5)	1.0
	Dyspnea	54/509 (10.6)	46/386 (11.9)	8/123 (6.5)	.090	51/487 (10.5)	3/22 (13.6)	.72
	Diarrhea	35/509 (6.9)	30/386 (7.8)	5/123 (4.1)	.16	34/487 (7.0)	1/22 (4.5)	1.0
	Other symptoms	23/509 (4.5)	22/386 (5.7)	1/123 (0.8)	.023	22/487 (4.5)	1/22 (4.5)	1.0
Medical history								
	Comorbidities[b]	119/506 (23.5)	91/383 (23.8)	28/123 (22.8)	.82	112/484 (23.1)	7/15 (31.8)	.38
	Benign gynecological disease	61/250 (24.4)	53/207 (25.6)	8/43 (18.6)	.33	61/245 (24.9)	0/5 (0.0)	.34
	Gynecological surgery history	90/249 (36.1)	79/208 (38.0)	11/41 (26.8)	.17	89/247 (36.0)	1/2 (0.0)	1.0
Menstruation								
	Menstrual status							
	Menopausal	251/435 (57.7)	181/335 (54.0)	70/100 (70.0)		239/421 (56.8)	12/14 (85.7)	
	Nonmenopausal	184/435 (42.3)	154/335 (46.0)	30/100 (30.0)	.0046	182/421 (43.2)	2/14 (14.3)	.031
	Menstrual volume in last 3 mo							
	Stable	138/186 (74.2)	114/156 (73.1)	24/30 (80.0)		137/184 (74.5)	1/2 (50.0)	
	Decreased	36/186 (19.4)	34/156 (21.8)	2/30 (6.7)		35/184 (19.0)	1/2 (50.0)	
	Increased	12/186 (6.5)	8/156 (5.1)	4/30 (13.3)	.047	12/184 (6.5)	0/2 (0.0)	.45
	Dysmenorrhea	80/210 (38.1)	64/178 (36.0)	16/32 (50.0)	.13	80/208 (38.5)	0/2 (0.0)	.53
Treatment and outcome								
	Antiviral treatment	206/433 (47.6)	152/313 (48.6)	54/120 (45.0)	.51	198/411 (48.2)	8/22 (36.4)	.28
	Antibiotic treatment	198/433 (45.7)	153/313 (48.9)	45/120 (37.5)	.033	186/411 (45.3)	12/22 (54.5)	.39
	Discharged	111/438 (25.3)	84/330 (25.5)	27/108 (25.0)	.93	108/421 (25.7)	3/17 (17.6)	.58

Mann-Whitney *U* test was applied to continuous variables; χ^2 test and Fisher exact test were used for categorical variables as appropriate.
Abbreviations: BMI, body mass index; IQR, interquartile range.
[a]Composite endpoint: admission to intensive care unit, mechanical ventilation, or death.
[b]Comorbidities refer to coexisting chronic diseases including hypertension, diabetes, cardiovascular disease, chronic obstructive pulmonary disease, cerebrovascular disease, hyperthyroidism, and chronic nephrosis; excludes any gynecological diseases.

Our study indicated that nonmenopausal females presented milder disease severity and better outcomes than age-matched males, whereas the differences disappeared between menopausal women and age-matched men, indicating that female hormones of premenopausal females may provide protection.

A previous study indicated the correlation between symptom severity and menstruation with hormonal fluctuation [13]. Our study showed that menstrual status is associated with severity and composite endpoint, while the significance was reduced when adjusted for age variations. Interestingly, multivariate Cox regression analysis shows that menstruation status still has a significant protective function, and that nonmenopausal patients tended to have shorter hospitalization days and be discharged earlier than menopausal patients, when taking age and severity into account. Accordingly, a prospective study, which evaluated sex differences in respiratory physiology and the development of chronic obstructive pulmonary disease (COPD), reported that early menopause was associated with greater risk

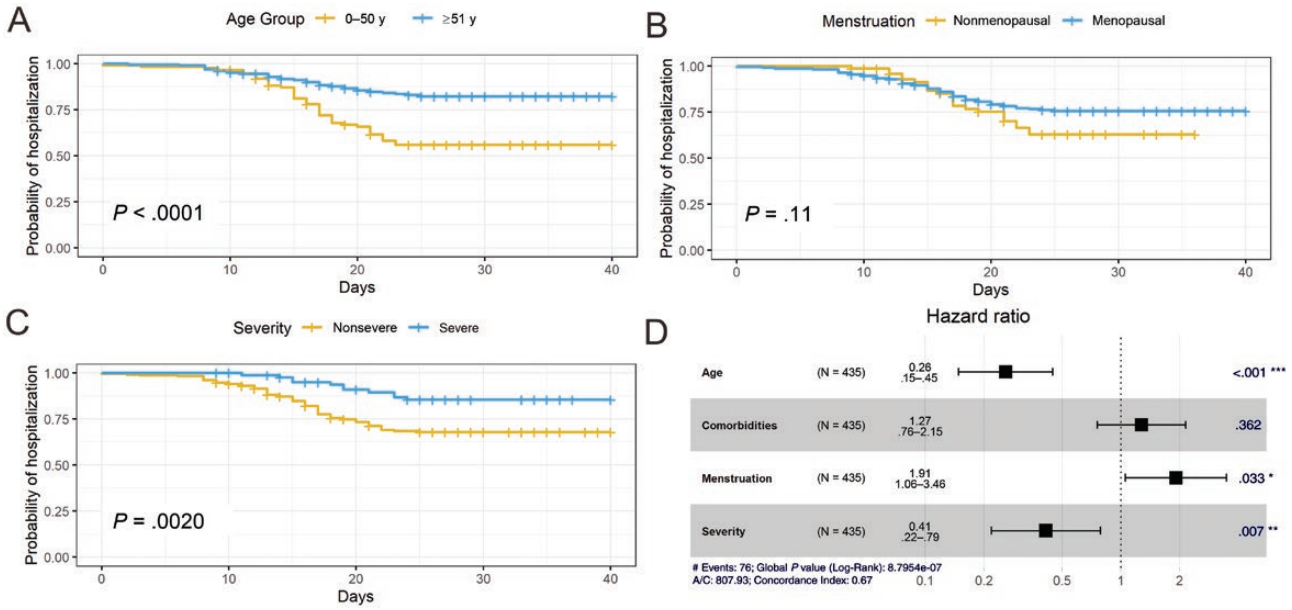

Figure 2. Cox analysis of age, menstrual status, and disease severity with probability of hospitalization. *A*, Univariate Cox regression of age with probability of hospitalization (hazard ratio [HR], 0.36 [95% confidence interval {CI}, .23–.57]; $P < .0001$). *B*, Univariate Cox regression of menstrual status with probability of hospitalization. Menstruation was divided into nonmenopause (regular or irregular menstruation) and menopause (HR, 0.67 [95% CI, .41–1.1]; $P = .11$). *C*, Univariate Cox regression of disease severity with probability of hospitalization (HR, 0.38 [95% CI, .2–.72]; $P = .0029$). *D*, In multivariate Cox analysis, the covariates age, menstruation, and severity were significant ($P < .001$, $P = .033$, and $P = .007$, respectively). However, the covariate comorbidities failed to be significant ($P = .362$, which is > .05). The HR for menstruation was 1.91, indicating a strong relationship between nonmenopause and reduced number of days in hospital. The HR for age and severity was 0.26 and 0.41, respectively, indicating that age and severity have a significant impact on risk of days in hospital. Abbreviation: AIC, Akaike information criterion

of COPD-related hospitalization or death [14]. Meanwhile, E2 in critically ill or injured adults was independently associated with mortality [15, 16], and low E2 was a diagnostic feature for prolonged hospital stay in critically ill surgical and trauma patients [17]. In addition, because menstruation could be affected by factors such as body mass index, drug intake, environment, and emotion [18], aside from the periodic fluctuation of sex hormones, further study of sex hormones was performed to more objectively evaluate the effect of sex difference.

Sex-based differences of disease severity and outcomes caused by viral infections are usually attributed to sex-dependent production of steroid hormones, different copy numbers of immune response X-linked genes, and the presence of disease susceptibility genes such as *TLR7*, *IFNβ*, and *IL6* in males and females [7]. Our data showed that E2 and AMH are positively correlated with infection severity. Estrogens are thought to protect nonmenopausal women from hepatitis C virus and other pathogens [19, 20]. Also, ovariectomized and estrogen receptor antagonist–treated female mice were more susceptible to SARS-CoV, which may be due to the protective effects of estrogen receptor signaling in SARS-CoV infection [7]. Here E2 might show the same protective pathway on SARS-CoV-2 infection, but further clinical studies are needed to verify the intrinsic mechanism. Suba [21] made the point that estrogen seems to be an ideal prevention and therapy against COVID-19 without any risk for adverse reactions. AMH changes slightly with menstrual cycle and drug intake and serves as the optimal marker for ovarian reserve and function. Logistic regression analyses showed that the levels of E2 and AMH in the nonsevere group were higher than those in the severe group, potentially playing vital roles in the progression of COVID-19.

Some reports have suggested that high doses of E2 may inhibit the production of inflammatory cytokines (eg, IL-1β, TNF-α), whereas stimulation with E2 at its physiological level enhances their production [22, 23]. After viral infection, proinflammatory cytokines/chemokines secreted by macrophages induced various antiviral mechanisms. Several clinical studies reported that levels of proinflammatory cytokines/chemokines, including IL-6, IL-8, interferon-γ, monocyte chemoattractant protein–1, and IL-10, are significantly elevated in patients with SARS-CoV, some of which were correlated with acute respiratory distress syndrome, respiratory syncytial virus infection, and human infection by avian influenza viruses [24, 25]. In our study, the levels of IL-6 and IL-8 were negatively related to both severity and composite endpoints, and IL-2R and IL-10 were related to composite endpoints, consistent with the findings of a recent study [26]. E2 has also been shown to regulate host immune response [27–30]. E2 fluctuates within menstrual cycles, and it was impossible for us to collect the blood samples on days 2–5 at a baseline level. Therefore, we divided the patients into 2 menstrual cycle phases (follicular and luteal) to make them comparable. The luteal phase has a relatively

Table 3. Sex Hormones and Disease Severity in 78 Female Patients With Coronavirus Disease 2019

		Disease Severity[a]		
	All Patients	Nonsevere	Severe	
Hormones	(N = 78)	(n = 61)	(n = 17)	*P* Value
E2				
Median (IQR), pg/mL	72.5 (42.5–165.0)	89.0 (49.5–172.0)	53.0 (20.0–93.5)	.0089
>70 pg/mL, no./No. (%)	39/78 (50.0)	34/61 (55.7)	5/17 (29.4)	.055
AMH				
Median (IQR), ng/mL	0.275 (0.030–1.715)	0.585 (0.085–1.815)	0.035 (0.010–0.265)	.023
>0.25 ng/mL, no./No. (%)	36/70 (51.4)	32/54 (59.3)	4/16 (25.0)	.016
LH				
Median (IQR), mIU/mL	5.34 (3.07–21.06)	4.89 (2.35–16.93)	9.83 (3.75–33.48)	.063
>5 mIU/mL, no./No. (%)	42/78 (53.8)	30/61 (49.2)	12/17 (70.6)	.12
Testosterone				
Median (IQR), ng/mL	0.390 (0.250–0.565)	0.410 (0.278–0.570)	0.320 (0.105–0.485)	.040
>0.4 ng/mL, no./No. (%)	36/77 (46.8)	30/60 (50.0)	6/17 (35.3)	.28
FSH				
Median (IQR), mIU/mL	6.345 (3.730–23.418)	6.060 (3.575–13.550)	19.900 (4.210–58.035)	.099
>6 mIU/mL, no./No. (%)	42/78 (53.8)	31/61 (50.8)	11/17 (64.7)	.31
FSH/LH ratio				
Median (IQR)	1.590 (0.891–2.168)	1.589 (0.851–2.098)	1.591 (0.959–2.752)	.87
>1.6, no./No. (%)	38/78 (48.7)	30/61 (49.2)	8/17 (47.1)	.88
Progesterone				
Median (IQR), ng/mL	0.77 (0.31–1.93)	0.88 (0.43–3.16)	0.36 (0.27–1.45)	.17
>0.8 ng/mL, no./No. (%)	38/78 (48.7)	32/61 (52.5)	6/17 (35.3)	.21
PRL				
Median (IQR), ng/mL	24.1 (18.6–32.3)	24.0 (18.2–32.9)	24.2 (19.7–28.9)	.84
>24.0 ng/mL, no./No. (%)	39/78 (50.0)	30/61 (49.2)	9/17 (52.9)	.78
	Logistic Regression[b]			
	aHR (95% CI)[c]	*P* Value	aHR (95% CI)[d]	*P* Value
E2 distribution				
>70 pg/mL	0.335 (.105–1.070)	.065	0.304(.092–1.001)	.05
AMH distribution				
>0.25 ng/mL	0.146 (.026–.824)	.029	…	
LH distribution				
>5 mIU/mL	2.388 (.733–7.783)	.15	…	
Testosterone distribution				
>0.4 ng/mL	0.556 (.181–1.702)	.30	…	

Abbreviations: aHR, adjusted hazard ratio; AMH, anti-Müllerian hormone; CI, confidence interval; E2, estradiol; FSH, follicle-stimulating hormone; IQR, interquartile range; LH, luteinizing hormone; PRL, prolactin.

[a]Mann-Whitney *U* test was applied to continuous variables; χ^2 test and Fisher exact test were used for categorical variables as appropriate.

[b]Multiple logistic regression model was used to assess the relationship between sex hormones and disease severity.

[c]Adjusted for age.

[d]Adjusted for age, menstrual phase (follicular, luteal, and menopause), and comorbidities.

fixed period of time (14 days), which has a relatively stable E2 level that reflects the real E2 level. In contrast, the follicular phase has a floating period—14 days or even longer, especially under infection with SARS-CoV-2—in which E2 may fluctuate largely and cover its correlation with cytokines. Our results indicated that E2 is negatively associated with C3 in the follicular phase and with IL-2R, IL-6, IL-8, or TNF-α in the luteal phase. Cytokines and chemokines play vital roles in immunity and immunopathology during a viral infection. The effects of E2 on the immune system are mainly due to its concentration, distribution, and different expression of 2 main estrogen receptors (ERα and ERβ) in various lymphoid cells. E2 was involved in both cell-mediated and humoral immune responses. E2 can also initiate the differentiation from monocytes into inflammatory dendritic cells by regulating the expression of cytokines and chemokines, and can promote greater internalization and antigen presentation to naive T cells [31]. Thus, E2 may be a protective factor for female COVID-19 patients by regulating cytokines or immunoproteins such as IL-2R, IL-6, IL-8, TNF-α, and C3. In females, severity of symptoms and outcomes may change during times of hormone fluctuation, which needs to be validated prospectively.

This study has several limitations. First, correlation analysis showed that E2 is negatively correlated with COVID-19

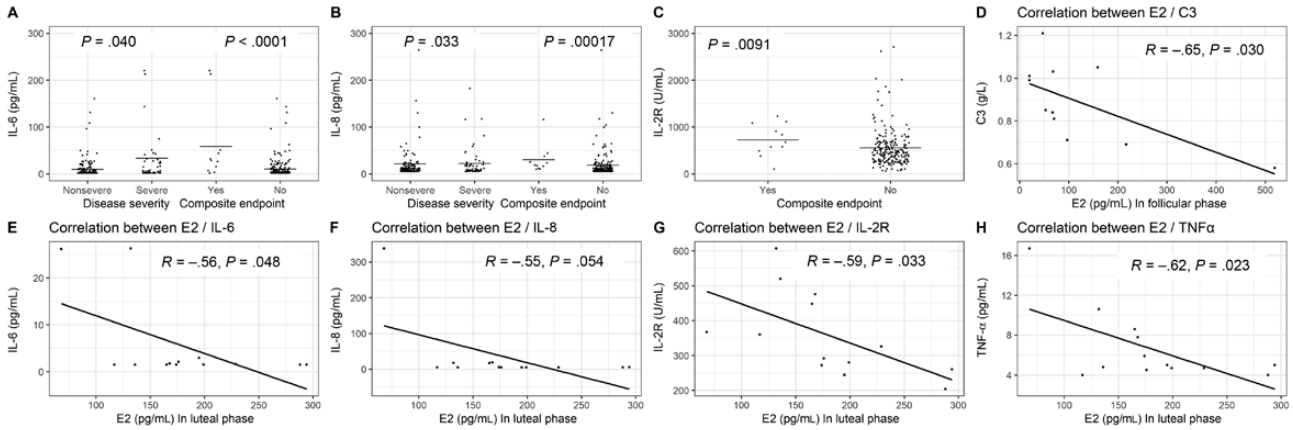

Figure 3. Correlation between immunity/inflammation-related cytokines and severity, composite endpoint, and estradiol (E2) in different phases. *A*, Correlation between interleukin (IL) 6 with disease severity and composite endpoint. *B*, Correlation between IL-8 with disease severity and composite endpoint. *C*, Correlation between IL-2R with composite endpoint. In *A–C*, the mean value is marked as a solid line. Results of Mann-Whitney *U* test indicate that patients in the severe group showed higher levels of IL-6 and IL-8 ($P = .040$ and $P = .033$, respectively) and that patients who reached the composite endpoint presented higher levels of IL-6, IL-8, and IL-2R ($P < .0001$, $P = .00017$, and $P = .0091$, respectively). *D*, Correlation between C3 and E2 of patients with coronavirus disease 2019 (COVID-19) in the follicular phase (n = 11). Result of Pearson correlation analysis indicates a significant inverse correlation between E2 and C3 ($R = –0.65$, $P = .030$). *E*, Correlation between IL-6 and E2 of COVID-19 patients in the luteal phase (n = 13). *F*, Correlation between IL-8 and E2 of COVID-19 patients in the luteal phase (n = 13). *G*, Correlation between IL-2R and E2 of COVID-19 patients in the luteal phase (n = 13). *H*, Correlation between tumor necrosis factor alpha (TNF-α) and E2 of COVID-19 patients in the luteal phase (n = 13). Results of Pearson correlation analysis indicate a significant inverse correlation between the E2 level and IL-6 ($R = –0.56$, $P = .048$), IL-8 ($R = –0.55$, $P = .054$), IL-2R ($R = –0.59$, $P = .033$), and TNF-α ($R = –0.62$, $P = .023$).

severity, but the causal relationship between them remains unclear. Second, the serum E2 level was determined from only 1 blood sample per patient regardless of the phase of menstrual cycle and was not collected before the SARS-CoV-2 infection, so the results may not completely reflect the average E2 level and its effects.

In summary, our data demonstrate that sex bias exists in COVID-19 patients, such that nonmenopausal females have milder severity and better outcomes than age-matched males. The difference disappeared when comparing postmenopausal women with age-matched men. To some extent, this is probably due to the protective role of female hormones, including AMH and E2, in nonmenopausal women. Among them, the most effective factor is believed to be E2, which may exert a protective effect by regulating cytokines related to immunity and inflammation. This is the first study to report on this, and the sample size was robust, which enabled us to control for potential confounders such as age and comorbidities. The levels of sex hormones and the status of menstruation may serve as potential markers for the clinical severity and outcomes of COVID-19. Clinical investigations regarding hormone supplements are urgently needed to further verify E2's protective and therapeutic effects on menopausal women, and even men, with COVID-19.

Supplementary Data

Supplementary materials are available at *Clinical Infectious Diseases* online. Consisting of data provided by the authors to benefit the reader, the posted materials are not copyedited and are the sole responsibility of the authors, so questions or comments should be addressed to the corresponding author.

Notes

Acknowledgments. The authors thank all of the study participants for giving their time despite the innate risks involved in sample collection and complex medical recording. They also thank the research staff, statistician, and administrators who contributed to the production of this article.

Financial support. This work was financially supported by the Clinical Research Pilot Project of Tongji Hospital, Huazhong University of Science and Technology (grant number 2019CR205).

Potential conflicts of interest. The authors: No reported conflicts of interest. All authors have submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest.

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