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Effects of acute severe acute respiratory syndrome coronavirus 2 infection on male hormone profile, *ACE2* and *TMPRSS2* expression, and potential for transmission of severe acute respiratory syndrome coronavirus 2 in semen of Asian men

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Objective: To confirm if severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) can be detected in semen of men with acute coronavirus disease 2019 and if their male hormone profile (testosterone, follicle-stimulating hormone, luteinizing hormone, sex hormone binding globulin, and free androgen index) is adversely affected during the acute phase of infection and any relation to the *ACE2* and/or *TMPRSS2* expression in human semen.

Design: Clinical study.

Setting: National University Hospital, Singapore.

Patient(s): Asian men aged 21–55 years who were admitted to National University Hospital, Singapore, with a laboratory-confirmed diagnosis of SARS-CoV-2 infection via nasopharyngeal swab in the acute phase of the infection, within 2–14 days of the development of symptoms or contact history, were recruited for the study.

Intervention(s): Blood was collected in the morning to assess the male hormone profile. Human semen were obtained by masturbation and sent to the molecular diagnostic laboratories to detect the presence of SARS-CoV-2 RNA and assess the *ACE2* and *TMPRSS2* expression.

Main Outcome Measure(s): Male hormone profile level and expression of SARS-CoV-2 RNA, *ACE2*, and *TMPRSS2* in human semen.

Result(s): A total of 63 men of Asian ethnicities agreed to participate in the study. Subsequently, 65% of recruited men had completely normal levels of male hormone profile. Moreover, 27% were noted to have higher luteinizing hormone levels between 6.6 and 16.1 IU/L (normal range, 0.8–6.1 IU/L), and 10% had higher follicle-stimulating hormone levels between 13.6 and 41.6 IU/L (normal range, 1.5–12.4 IU/L); all had normal testosterone levels. No SARS-CoV-2 RNAs were detected in all human semen. The *ACE2* and *TMPRSS2*

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expression was undetectable in 26 samples, whereas 23 samples only had a detectable *TMPRSS2* expression and 4 only had an *ACE2* expression. The remaining 3 expressed both *ACE2* and *TMPRSS2*.

Conclusion(s): Severe acute respiratory syndrome coronavirus 2 could not be found in the semen of a cohort of young to middle-aged Asian men with mild acute SARS-CoV-2 infection. However, there was a detectable expression of *ACE2* and *TMPRSS2* in semen, although not causal, and it may be correlated to changes in male hormone profiles and male age. (Fertil Steril Sci® 2022;3:29–34. ©2021 by American Society for Reproductive Medicine.)

Key Words: Acute COVID-19 infection, semen, male hormone profile, *ACE2* and *TMPRSS2* expression, SARS-CoV-2 expression

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Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), which is the novel coronavirus that has had a global impact through the coronavirus disease 2019 (COVID-19) pandemic, enters cells via binding to the *ACE2* receptor, followed by its priming by *TMPRSS2* (1). These receptors are widely expressed in bronchial cells, hence the predilection for this virus to cause respiratory disease. Interestingly, the *TMPRSS2* and *ACE2* receptors are also found in the prostate (2) and human testes, respectively, suggesting that the semen (produced from the prostate/seminal vesicles) and the spermatogonia, Leydig cells, and Sertoli cells (in human testes) (3) are also the targets of SARS-CoV-2 infection. The blood–testis barrier does not protect against SARS-CoV-2 (4), and impaired gonadal function may lead to an abnormal expression of male sex hormones (5). Several epidemiological studies have shown a male predominance of SARS-CoV-2 infection and delayed viral clearance of SARS-CoV-2 in men compared with that in women (6, 7), possibly because of the higher expression of *ACE2* in the testes (8). The delayed clearance can have implications for transmission of the virus. On the basis of limited published data, the possibility of SARS-CoV-2 harboring in the human testes and prostate is highly plausible. This has been noted with other viral infections such as Zika infection, whereby the Zika virus RNA detected in the semen of men who are infected and virus shedding has persisted for up to 144 days (9). In addition to transmission, implications to male fertility are possible as there are reports suggesting that the male hormonal profile can be adversely affected (3), and normal functions of male hormones are necessary for normal spermatogenesis. In assisted reproductive technologies, semen analyses and semen processing for intrauterine insemination and in vitro fertilization work require exposure to human semen. If SARS-CoV-2 is transmitted in human semen, then there is a potential risk of transmission to assisted reproductive technology laboratory personnel especially if there are breaches in laboratory protocols. This study aimed to confirm if SARS-CoV-2 (using established SARS-CoV-2 testing polymerase chain reaction [PCR] assays) can be detected in semen of men who are acutely infected with SARS-CoV-2 and if their male hormone profile (testosterone, follicle-stimulating hormone [FSH], luteinizing hormone [LH], and sex hormone binding globulin [SHBG]) is adversely affected during the acute phase of infection and related to the *ACE2* and/or *TMPRSS2* expression in human semen.

MATERIALS AND METHODS

Subjects

Asian men aged 21–55 years who were admitted to National University Hospital, Singapore, with a laboratory-confirmed diagnosis of SARS-CoV-2 infection via nasopharyngeal swab were recruited for the study. These men were diagnosed with SARS-CoV-2 in the acute phase of the infection, within 2–14 days of the development of symptoms or contact history. Only men who were clinically stable with mild respiratory symptoms were recruited for the study. Men who were treated with any medications known to affect male hormone profile or treated for SARS-CoV-2 infection with specific therapies such as remdesivir were excluded from the study. The study was approved by the Institutional Ethics Review Board (2020/00526), and informed consent was obtained from the men before participating in the study.

Measurement of Male Hormone Profile

Five milliliters of blood was collected in the morning to assess the male hormone profile—the LH, FSH, total testosterone (T), SHBG, and free androgen index (FAI), calculated as per formula: $\text{total testosterone (nmol/L)} \times 100/\text{SHBG (nmol/L)}$. The FAI is superior to T measurements when T is borderline (7.5–12 nmol/L) to the estimate of free testosterone (10). The morning blood draw was to ensure that the total testosterone was determined accurately (11). The male hormone profile assays were performed in National University Hospital using the laboratory automation system according to the manufacturer's instructions. The laboratory automation system includes centrifugation set at 3,000 rpm for 8 minutes and measurement of the aforementioned hormones in the serum (LH, FSH, SHBG [Sandwich IA; Beckman Coulter, Inc., Brea, California] and T [Competitive IA; Beckman Coulter, Inc.]).

Collection and Measurement of SARS-CoV-2 RNA in Human Semen

Men who consented to participate in the study were instructed to collect their semen after they washed their hands and penis with clean water to avoid contaminating the semen sample during collection. The semen was collected via masturbation into a sterile container within 24–48 hours on admission to hospital after a confirmed laboratory diagnosis of SARS-CoV-2 infection via nasopharyngeal swab. The semen samples

were then bagged as per hospital protocol and transported to the hospital molecular laboratory for further analyses. Total nucleic acid was extracted from 140 μ L of semen sample using the QIAamp Viral RNA Mini Kit (QIAGEN, Hilden, Germany) and eluted in 60 μ L of elution buffer, according to the manufacturer's recommendations. Real-time reverse transcription PCR for SARS-CoV-2 detection was performed using the MiRSES Fortitude Kit 2.1 (Fortitude; MiRSES Pte. Ltd., Singapore) on LightCycler 480 Instrument II (Roche Molecular Systems, Branchburg, New Jersey). Of note, the settings for the Fortitude assay on LightCycler 480 Instrument II were adapted from the settings provided for the CFX96 Real-Time PCR Detection System (Bio-Rad Laboratories, Inc., Hercules, California). The Fortitude amplifies and detects 2 different regions within the ORF1ab gene of the SARS-CoV-2 for specific SARS-CoV-2 detection. In addition, the assay detects a synthetic nucleic acid sequence that serves an internal control to rule out failure of amplification because of the presence of inhibitory substances.

Measurement of ACE2 and TMPRSS2 RNA Transcripts in Human Semen

All RNA extracts from human semen underwent heat inactivation in a ThermoMixer at 60°C for at least 30 minutes before being transferred to the research laboratory. During heat inactivation, a thermometer was placed in a control tube with water to ensure that the temperature reached 60°C before starting the 30-minute countdown. The RNA was then treated with DNase I (NEB) and converted into complementary DNA (cDNA) using qScript XLT cDNA SuperMix according to the manufacturer's instructions. Complementary DNA was diluted 10 times before PCR with SYBR Green with primers for *ACE2* and *TMPRSS2* transcripts. For negative control (NC), water was used for cDNA conversion and quantitative PCR. The RNA of human embryonic stem cells and human embryonic stem cell-derived cardiomyocytes were used for positive controls. Positive *ACE2* and *TPMRS22* expressions in the human semen samples were confirmed if the cycle threshold (Ct) value of these genes in the sample was statistically significant ($P < .05$), less than the Ct value in NC, which meant that the delta Ct (NC sample) was more than 0; otherwise, we considered this as no expression. The Student *t*-test, correlation efficient analysis, and graph synthesis were performed using GraphPad Prism 7 and R, respectively. *P* values of $< .05$ were considered statistically significant.

RESULTS

Demographics of Subjects

Sixty-three of the 100 men approached agreed to participate in the study. They were admitted to National University Hospital, Singapore, from June 21, 2020, to September 16, 2020, and were all of Asian ethnicities (Chinese, Indian, Bangladeshi, Thai, Filipino, and Burmese); their mean age was 46.17 ± 6.84 years (range, 23.25–53.08 years), and their diagnosis of SARS-CoV-2 infection was made between 2 and 14 days (mean, 3–4 days) of enrolment in this study. These men were generally well, had minimal to mild symptoms, and

were only on medications to treat their symptoms of cough and sore throat, which included antihistamines, antitussives, and antipyretics. None were on antivirals or immunomodulatory agents.

Male Hormone Profile in Men with Acute SARS-CoV-2 Infection

Forty-one (65%) of 63 recruited men had completely normal levels of male hormones. More than a quarter (17/63, 27%) were noted to have higher LH levels between 6.6 and 16.1 IU/L (normal range, 0.8–6.1 IU/L). Almost 10% (6/63) of men had higher FSH levels between 13.6 and 41.6 IU/L (normal range, 1.5–12.4 IU/L), and all had normal testosterone levels. Approximately 8% (5/63) had low testosterone levels of 3–7.48 nmol/L (normal range, 9.90–27.80 nmol/L). Incidentally, it was noted that all men were aged ≥ 45 years although their FAI was normal. Four of 5 men with low testosterone levels had normal FSH and LH levels (Table 1).

No SARS-CoV-2 RNAs were Detected in Human Semen

Among the 63 men who participated in this study, 1 was unable to produce his semen sample before discharge from the hospital. Another 6 semen samples spilled as the caps of the containers were not secured, resulting in too low a volume remaining for RNA extraction. A total of 56 semen samples were processed, and no SARS-CoV-2 RNAs were detected in any of the semen samples. It was ensured that the PCR reactions were validated with internal controls to rule out any possibility of failure of amplification because of the presence of inhibitory substances.

Expression Levels of ACE2 and TMPRSS2 in Human Semen

The 56 available semen samples were processed to assess the expression abundance of *ACE2* and *TMPRSS2* transcripts. The *ACE2* and *TMPRSS2* expression was undetectable in 26 samples, whereas 23 samples only had a detectable *TMPRSS2* expression and 4 only had an *ACE2* expression. The remaining 3 expressed both *ACE2* and *TMPRSS2*.

We attempted to determine if any deviation of the male hormone profile was associated with the expression abundance of *ACE2* and/or *TMPRSS2* in human semen (Table 2). Men with normal FSH/LH/T/FAI levels were classified under the normal male hormone profile group, whereas men with derangements in any of the indices were grouped under abnormal male hormone profile. A higher proportion of men with abnormal male hormone profile had a *TMPRSS2* expression ($5\% + 50\% = 55\%$) compared with men with no *TMPRSS2* expression ($5\% + 40\% = 45\%$). On the other hand, most men with normal male hormone profile ($50\% + 40\% = 90\%$) had no *ACE2* expression. Although this finding did not reach statistical significance because of the small number of men in the study (Fig. 1, correlation coefficient [*ACE2* vs. LH] = 0.049, correlation coefficient [*ACE2* vs. FSH] = 0.232, correlation coefficient [*ACE2* vs. T] = -0.04, correlation coefficient [*TMPRSS2* vs. LH] = 0.188, correlation

TABLE 1

Male hormone profile in Asian men with acute severe acute respiratory syndrome coronavirus 2 infection.

Male hormone profile	Normal (%)	High (%)	Low (%)	Remarks
Luteinizing hormone (LH) N = 63	41 (65)	17 (27)		5 men have both FSH and LH levels elevated, but their T level and FAI are normal
Follicle-stimulating hormone (FSH) N = 63	41 (65)	6 (10)		
Testosterone (T) N = 63	41 (65)		5 (8)	1 man has low T level with only elevated LH level and normal FAI

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coefficient [*TMPRSS2* vs. FSH] = 0.212, correlation coefficient [*TMPRSS2* vs. T] = 0.048, it suggested that there were a potential correlation between no *ACE2* expression and normal male hormone profile and a potential correlation between *TMPRSS2* expression and abnormal male hormone profile. We also found that there was a significantly negative correlation between age and T and FAI levels (Fig. 1, correlation coefficient [age vs. T] = -0.422 [$P < .01$], correlation coefficient [age vs. FAI] = -0.478 [$P < .001$]), suggesting that abnormal male hormone levels were attributable to increasing age.

DISCUSSION

We have attempted to answer the question as to whether acute SARS-CoV-2 infection affected male reproductive function or posed a transmission risk. Similar to the smaller study of young Chinese men performed by Song et al. (12), we did not find evidence of SARS-CoV-2 in semen. In our study, we had a larger cohort of Asian men ($n = 56$) of different ethnicities, as well as older ages, ranging from 23–53 years, and we have confirmed the absence of SARS-CoV-2 in semen in the acute phase of infection. We also noted that in a study by Pan et al. (13), men showed no evidence of SARS-CoV-2 in the semen 1 month after their diagnosis of the infection, which suggested that despite the high *ACE2* expression in the testes (14) and prostate, it did not result directly in the transmission of the virus into prostatic fluids or testicular reservoir. This is reassuring in terms of making late seminal transmission of SARS-CoV-2 less likely.

On the other hand, there were direct male hormonal changes noted in men with acute SARS-CoV-2 infection.

This could be linked to the predilection of the virus to the *ACE2* and *TMPRSS2* receptors, which are both detected in Leydig cells in the testes (14). Leydig cells regulate testosterone synthesis in the testis, which is vital for spermatogenesis. We noted that more than a quarter of the men in the acute phase of the infection had elevated LH levels, which could suggest the possibility of the virus binding to the *ACE2* receptors and competing with binding to the LH receptors on the Leydig cells whereby both the *ACE2* and LH receptors were expressed, potentially resulting in the higher levels of LH observed. In the acute phase of infection, despite changes of LH from Leydig cells, the production of testosterone did not appear to be affected. This may not be the case for individuals with more severe infections or with higher viral loads as our cohort all had mild illness. We did notice a small number of men with high FSH and LH levels. However, this may be attributable to the men's age (15) as the mean age of our men were much older (approximately 46 years) when compared with men from earlier studies (12, 13). However, it is also possible that hormonal changes are not directly related to the *ACE2* and *TMPRSS2* expression but rather a consequence of other systemic factors, such as severity of illness, although most of our patients had mild disease. The FSH receptors are found on Sertoli cells to initiate spermatogenesis, but the current study was unable to perform semen analyses for any of the men because of biosafety restrictions in the laboratory.

The relationship between semen samples with the *ACE2* and/or *TMPRSS2* expression and abnormal fluctuations of male hormone profile is intriguing. In a review by Navarra et al. (16), the *ACE2* expression was noted to be present in the testis and highly expressed in the seminiferous ducts

TABLE 2

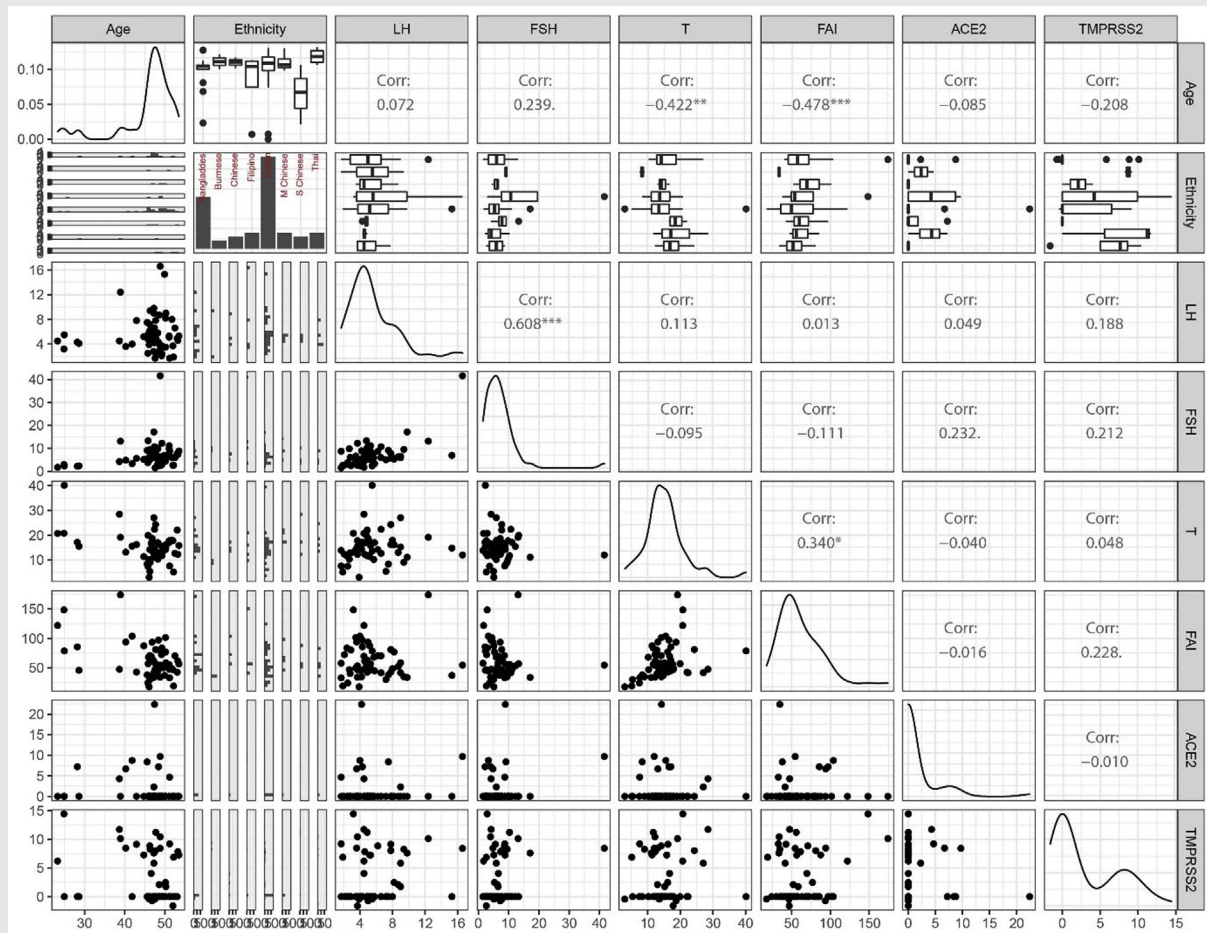
Expression of *ACE2* and *TMPRSS2* in human semen from men who were positive for coronavirus disease 2019.

	<i>ACE2</i> (+)/ <i>TMPRSS2</i> (+)	<i>ACE2</i> (+)/ <i>TMPRSS2</i> (-)	<i>ACE2</i> (-)/ <i>TMPRSS2</i> (+)	<i>ACE2</i> (-)/ <i>TMPRSS2</i> (-)	
Normal male hormone profile	2 (6%)	3 (8%)	13 (36%)	18 (50%)	36
Abnormal male hormone profile	1 (5%)	1 (5%)	10 (50%)	8 (40%)	20
	3	4	23	26	56

Note: % is calculated for different groups including *ACE2* (+)/*TMPRSS2* (+), *ACE2* (+)/*TMPRSS2* (-), *ACE2* (-)/*TMPRSS2* (+), and *ACE2* (-)/*TMPRSS2* (-) in the normal vs. abnormal hormone profile groups.

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FIGURE 1



Scatter plots, box plots, and correlation coefficient analyses of different pairs among different groups, including age, ethnicity (Bangladesh, Burmese, Chinese, Filipino, Indian, M Chinese, S Chinese, and Thai), hormone (luteinizing hormone [LH], follicle-stimulating hormone [FSH], testosterone [T], and free androgen index [FAI]) level, and *ACE2* and *TMPRSS2* RNA expression. Corr = correlation coefficient. * $P < .05$, ** $P < .01$, *** $P < .001$ denotes statistical significance.

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and moderately expressed in the Leydig cells. *ACE2* and *TMPRSS2* were also expressed at low levels in the glandular cells of the seminal vesicles. However, *TMPRSS2* was not found to be expressed in the testis but expressed at moderate levels in the glandular cells of the epididymis and prostate. In our study, approximately 46.4% (26/56) of the semen samples had no detectable *ACE2* and *TMPRSS2* expression. Interestingly, men with no *ACE2* expression were likely to have normal male hormone profiles, and the *TMPRSS2* expression may be associated with abnormal male hormone profile in our study cohort with acute SARS-CoV-2 infection, although the hormone dependence of *ACE2* and *TMPRSS2* does not indicate the hormonal regulation of *ACE2* and *TMPRSS2* in the testis. This may not be due to SARS-CoV-2 infection per se as we did not have a control group of healthy men given that our study aimed to demonstrate the risk of potential sexual transmission of the virus. However, a recent study provides in vitro evidence that the expression of the

SARS-CoV-2 host cell receptor *ACE2* and coreceptor *TMPRSS2* is being regulated partly by androgens (17), which suggests the need for more detailed further studies to analyze the clinical implications of this.

Given our findings, despite demonstrating the *ACE2* and/or *TMPRSS2* expression in the semen of these Asian men with acute onset of SARS-CoV-2 infection, we were unable to detect the presence of SARS-CoV-2 in any of the semen samples. This could highlight the low risk of sexual transmission of SARS-CoV-2 or low risk to laboratory staff dealing with gametes.

The strengths of the study include a larger group of men ($n = 56$) (pan-Asian ethnicity) in the acute phase of SARS-CoV-2 infection. Male hormone profiles were obtained, and semen samples were sent to detect the presence of SARS-CoV-2. Other studies were smaller or did not investigate the acute phase of infection or correlation between the *ACE2* and *TMPRSS2* expression and male hormone profiles (18–23).

Unfortunately, we were not able to recruit a control group of men during the initial phase of COVID-19 pandemic in Singapore, and this is a limitation of the study. We managed to recruit 63 men who provided bloods for the assessment of male hormone profile, but only 56 of them had semen samples available for the assessment of SARS-CoV-2 and ACE2 and TMPRSS2 expression. These numbers remained relatively small. We also did not perform semen analyses on these men because the results obtained may not be accurate as it was dependent on the duration from the last ejaculate. Furthermore, as these men had mild illness and were only admitted for less than 2 days for isolation and workup during the peak of Singapore's COVID-19 pandemic before transfer to specific quarantine facilities, there was insufficient time to obtain semen in accordance to semen analysis protocol for the accurate assessment of semen parameters for workup.

In conclusion, SARS-CoV-2 could not be found in the semen of a cohort of young to middle-aged Asian men with mild acute SARS-CoV-2 infection. However, there was a detectable expression of ACE2 and TMPRSS2 in semen, which may be correlated with the changes in male hormone profiles. In the light of emerging data on potential nonrespiratory complications of SARS-CoV-2, more studies are needed on larger cohorts with longer follow-up, including potential vaccine breakthrough cases with novel SARS-CoV-2 viral variants, to determine the true impact on male reproductive health and overall well-being.

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