Association of the traditional Chinese medicine treatment intensity and negative conversion time of SARS-CoV-2 nuclear acid in mild COVID-19 patients

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Abstract

The association between traditional Chinese medicine (TCM) treatment intensity (TI) and the clinical therapeutic outcomes of patients with mild to moderate COVID-19 symptoms has not been fully understood. A retrospective cohort study was undertaken to assess the therapeutic effect of traditional Chinese medicine (TCM) on patients with mild COVID-19. Between April and May 2022, a total of 6,120 confirmed COVID-19 cases were enrolled from temporary hospitals in Shanghai City. Patients receiving TCM treatment were identified through their daily prescriptions. The nonlinearity and cutoff point of this relationship were determined using a restricted cubic spline model. The study included 6,120 mild COVID-19 cases with a median (range) age of 43.0 (2.0, 75.0) years, a median (range) hospitalization duration of 9.7 (4.1, 22.5) days, a median (range) of taking TCM of 2.0 (0.0, 15.0) days, and the median (range) negative conversion time of SARS-CoV-2 nucleic acid was 6.67 (95% confidence interval [CI]: 4.1, 22.5) days, with 1854 women (30.3%) among the participants. The restricted cubic spline model revealed significant nonlinear association between TCMTI and the negative nucleic acid conversion time, and the statistical test results of all three parameters were statistically significant. The cutoff value of TCMTI is 0.65. The high TCMTI group showed a significant reduction in the median negative conversion time of SARS-CoV-2 nucleic acid (hazard ratio [HR]=1.769 [95% CI: 1.575, 1.986]) and a decrease in hospitalization duration (coefficient=-0.146, \(P<0.001\)) compared with the low TCMTI group after adjustment for confounding variables. The restricted mean negative conversion time of SARS-CoV-2 nucleic acid of high TCMTI group was 1.909 (1.675, 2.144) days shorter than that of the low TCMTI group \(P<0.001\). These findings could be utilized to recommend the use of TCM to treat mild COVID-19 patients and prevent overburdening of medical facilities.

Trial Registration: The trial was prospectively registered on Chinese Clinical Trial Registry (ChiCTR, ChiCTR2200063151, [http://www.chictr.org.cn/](http://www.chictr.org.cn/))

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Keywords: traditional Chinese Medicine, Coronavirus disease 2019, SARS-CoV-2, negative conversion time, hospitalization days

1 | INTRODUCTION

The continuous mutation of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) has caused the coronavirus disease 2019 (COVID-19) pandemic to persistently affect the entire world[1, 2], posing ongoing threats to global health and resulting in significant morbidity and mortality worldwide[3, 4]. The SARS-CoV-2 genome mutations led to the emergence of concerning variants, involving Alpha, Beta, Gamma, Delta, and Omicron[2, 5, 6]. Current evidences indicate that Omicron variant exhibits elevated infectivity, higher viral load, and a shorter incubation period, as well as enhanced transmission and immune evasion capabilities relative to earlier variants[4, 5]. The asymptomatic nature of most Omicron variant infection presents a formidable challenge to the identification and control the outbreaks, ultimately posing a significant threat to public health.

Over the past three years, novel chemical synthesis drugs have been developed, or some drugs have been expanded the scope of pharmaceutical practice in emergency COVID-19 treatment[7, 8], for instance, modern synthetic drugs such as Nirmatrelvir/Ritonavir, Remdesivir, and Molnupiravir have been urgently authorized for treating mild or moderate patients with underlying conditions[9-11]. The primary objective of these drugs is to prevent moderate cases from progressing to severe illness, thereby reducing the burden on healthcare systems through decreased hospitalization and mortality rates[12]. Additionally, the introduction of COVID-19 vaccines in 2021 has been recognized as a critical tool in controlling the pandemic and reducing mortality[11, 13-15]. However, when the highly transmissible SARS-CoV-2 Omicron variant became the dominant in 2022 year, immunological studies indicated evidence of immune evasion by Omicron variant, with decreased neutralization from both vaccine sera and monoclonal antibodies compared to previous
variants[13, 14]. Moreover, protection against Omicron decreases over time following the booster dose, and COVID-19 treatments are still unavailable in resource-limited countries and emergency situations[13, 14]. Therefore, healthcare providers are exploring various therapeutic regimens and continuously refining treatment processes to identify effective drugs that can shorten the duration of COVID-19 and hasten recovery.

The clinical application of traditional Chinese medicine (TCM) in fighting infectious diseases has a rich history, dating back to the first recorded in 243 BC in Shi Ji (Historical Records) in China[16, 17]. TCM theories related to infectious diseases were developed during the Tang, Song, Yuan, and Qing dynasties in China[17]. Comprehensive descriptions and systematic summaries of etiology, pathogenesis, syndrome, and treatment for acute epidemic diseases can be found in ancient Chinese medical books, such as the Inner Canon of the Yellow Emperor, Central Treasury Canon, Exogenous Febrile Disease, Golden Chamber, Pestilential Theory, and Compendium of Materia Medica[18, 19]. In recent years, Chinese herbs have been widely used to treat SARS in 2003 and H1N1 in 2009. Currently, Chinese herbs are frequently combined with modern medicine for COVID-19 treatment, demonstrating multiple therapeutic effects in China, and it was particularly used as an intervention drugs in clusters of infectious cases with acute respiratory diseases. The World Health Organization (WHO) Expert Meeting on the Evaluation of Traditional Chinese Medicine in the Treatment of COVID-19 suggests that TCM is beneficial in treating COVID-19[20, 21]. TCM can reduce the risk of progression from mild-to-moderate cases to severe COVID-19, and when administered as an adjunct to conventional treatment, may shorten the time for viral clearance and hospitalization, resolution of clinical symptoms[22, 23]. Consequently, the ninth version of the national guideline “Diagnosis and Treatment Protocol for Novel Coronavirus Pneumonia” recommends several TCM therapeutic regimens as general formulas for treating mild-to-moderate COVID-19 patients[16, 20].

Although TCM has been extensively utilized for treating COVID-19 patients in China, no studies have investigated the association between TCMTI and therapeutic efficacy. In other words, no research has examined the response of TCMTI to the negative conversion time of SARS-CoV-2 ribonucleic acid (RNA) and hospitalization duration (days). Therefore, the primary objective of the present study was to evaluate the clinical efficacy of TCMTI on COVID-19 patients presenting mild or moderate symptoms. The results will offer evidence-based recommendations for using TCM, further assisting in the provision of personalized and precise treatment, saving lives, and promoting the health of millions.

2 | METHODS

2.1 | Study design and setting

A retrospective cohort study was conducted using administrative data from four wards (A, B, C, and D) in a temporary hospital that was transformed from Shanghai Jinshan Fangcang Hospital between April 15th and May 31st, 2022. The temporary hospital, designated to treat mild COVID-19 patients, had an indoor space of 50 000 m² and 5030 hospital beds. The study aimed to explore the potential association between TCMTI and the clinical outcomes of individuals with mild COVID-19. The data pertaining to SARS-CoV-2 infected patients were integrated into the electronic medical record system of the temporary hospital and the vital statistics database, using a unique anonymous identifier. The medical records system contained comprehensive inpatient data for all COVID-19 patients, and their identities were encoded using non-traceable codes to guarantee utmost confidentiality.

The protocol was approved by the Ethics Committee of Longhua Hospital affiliated to Shanghai University of Traditional Chinese Medicine (No.2022LCSY065), and it was registered at the Chinese Clinical Trial Registry (ChiCTR2200063151). For each research participant, oral informed consent was obtained either through face-to-face interviews with the individual or via telephone communication with their legal guardian. An independent data monitoring committee oversaw the monitoring of participant safety, efficacy, and trial conduct. Additionally, the study followed the Strengthening the Reporting of Observational Studies in Epidemiology reporting guideline for cohort studies (STROBE, the supplementary material 1).

2.2 | Participants
COVID-19 severity is categorized into mild, moderate, severe, and critical illness based on laboratory findings, clinical characteristics, and chest imaging (chest X-ray and/or computed tomography scan, CT) examination results[24]. Mild COVID-19 is defined as a case with positive results from SARS-CoV-2 infection and mild clinical symptoms (fever, dry cough, fatigue, vomiting, body aches, loss of appetite, etc) without evidence of pneumonia on imaging, according to the 'Guidelines on Diagnosis and Treatment of Novel Coronavirus Pneumonia (Trial 9th edition) proposed by the National Health Commission of the People’s Republic of China[24]. Confirmed SARS-CoV-2 infection is determined by meeting one of the following pathogenic or serological criteria: 1) positive SARS-CoV-2 nucleic acid detection by real-time reverse transcriptase-polymerase chain reaction (RT-PCR) from an oropharyngeal and/or nasal swab; 2) simultaneous positive IgG and IgM antibody tests for SARS-CoV-2 antigen using enzyme-linked immunosorbent assay (ELISA)[24]. Throat swab or nasopharyngeal aspirate specimen was collected daily from each recruited patient upon admission and during hospitalization.

Patients who fulfilled all the following criteria were eligible: 1) individuals with confirmed SARS-CoV-2 infection who were admitted to the temporary hospital; 2) COVID-19 patients who met the diagnostic criteria for mild or moderate COVID-19 as outlined above; and 3) patients who provided informed consent. Patients were excluded from the study if they met any of the following criteria: 1) inability to care for themselves or cooperate in completing self-rating and professional evaluation, 2) disease progression worsening or death occurring within 48 hours after admission to the temporary hospital, 3) recent severe psychological trauma, 4) severe psychiatric disorder, 5) severe heart, liver, kidney, nervous system diseases or other serious medical conditions, or 6) participation in other clinical trials for treating COVID-19 or drug withdrawal during treatment in other clinical trials.

2.3 | Exposure variables and assessment

Patients receiving TCM treatment were identified through their daily prescriptions. Our primary analysis modeled TCM treatment as a continuous exposure. To assess duration, TCM treatment intensity (TCMTI) was defined as the percentage of total prescribed TCM therapy relative to the length of hospitalization (days) for each patient, as shown in formula (1).

\[
TCMTI = \frac{\text{Total days of TCM treatment during in temporary hospital}}{\text{Total length of hospitalization (days) in temporary hospital}}
\]

In subsequent analyses, exposure was discretized into a categorical variable using an optimal threshold value determined by a plain grid search method[25]. Consequently, the all patients was divided into high TCMTI group and low TCMTI group.

2.4 | Covariates

The epidemiological characteristics, clinical manifestations, and the sequence of RT-PCR test results were collected, and the data were independently extracted and cleaned by two researchers. Information for each participant was retrieved from the electronic medical record systems of the temporary hospital. The key baseline covariates that were identified as potential predictors included: 1) demographic characteristics, such as age, gender and marital status; 2) risk factors, such as patient recruitment time month, food allergy, COVID-19 vaccine inoculation, volunteer status, and underlying diseases and comorbidities (tumor, hypertension, diabetes, cardiovascular and cerebrovascular diseases, chronic obstructive pulmonary disease, pulmonary infection, etc.).

Additionally, the post-baseline covariates comprised the administration of drugs for respiratory, circulatory, and digestive system diseases, prescription drug information of antibiotics, non-steroidal anti-inflammatory drugs (NSAIDs), and other medications during hospitalization. For COVID-19 cases with the aforementioned underlying diseases and comorbidities, medications were prescribed by doctors and provided to patients daily in the temporary hospital. Treatment intensity (TI) for each type of drug was calculated as the ratio of the days of administration of each drug to the total hospitalization days, respectively.

2.5 | Intervention
The study aimed to investigate the therapeutic impact of TCMTI on mild COVID-19. The key independent variable was TCMTI, which was examined both as a continuous variable and as a dichotomous variable (high TCMTI group vs. low TCMTI group).

The TCM treatment protocol for each mild COVID-19 case adhered to China’s "Guidelines on Diagnosis and Treatment of Novel Coronavirus Pneumonia (Trial 9th Edition)"[16, 24]. Basic symptomatic treatment and general healthcare (pyretolysis, cough alleviation, sputum dissolution, pain relief, antiasthma, etc.) were provided to patients based on their clinical manifestations. Additionally, treatment drugs for underlying diseases were administered to COVID-19 patients if necessary.

2.6 | Definition and therapeutic efficacy evaluation

Participants underwent an oropharyngeal swab for SARS-CoV-2 RT-PCR testing daily during hospitalization. The primary outcome was the negative conversion time of SARS-CoV-2 nucleic acid, defined as the time interval (days) from the date of entering the temporary hospital to the date of the first negative test with two consecutive negative results. The negative conversion was confirmed when COVID-19 case had at least two consecutive negative results of real-time RT-PCR testing taken at least 24 hours apart. SARS-CoV-2 nucleic acid was considered negative if the cycle threshold (Ct) values of the ORF1ab gene and the N gene were both above 35[24].

Secondary efficacy endpoint was the hospitalization days for COVID-19 cases, which were defined as the time interval (measured in days) between the date of hospital admission and the date of hospital discharge. The discharge criteria required laboratory evidence of SARS-CoV-2 clearance, which was confirmed by negative RT-PCR tests for nasopharyngeal swabs taken at an interval of over 24 hours[24].

2.7 | Statistical Analysis

For quantitative data, the normality assumption diagnosis was conducted using the Kolmogorov-Smirnov test with Lilliefors correction significance. The Kolmogorov-Smirnov test with a P-value < 0.05 was used to determine non-normally distributed variables, which included, age, hospitalization days, negative conversion time of SARS-CoV-2 nucleic acid, respiratory drug TI, circulatory drug treatment intensity, antibiotic TI, digestive drug TI, NSAIDs TI, and other drug TI. Alternatively, some variables (hospitalization days, etc.) underwent log-transformation and were compared using the Mann-Whitney U test. Non-normally distributed variables were described with median and interquartile ranges (IQRs) and compared using the Mann-Whitney U test between low TCMTI and high TCMTI group. For categorical variables (gender, marital status, patient recruitment time, food allergy, COVID-19 vaccine inoculation, volunteer status, comorbidities, each type of drug for each disease mentioned above, etc.), frequency and percentage were used to describe these values. The univariate analysis for the differences between the low TCMTI and high TCMTI groups was performed using either the chi-square test or Fisher’s exact test. There were no missing values in continuous or categorical variables in the study.

The probability of SARS-CoV-2 nucleic acid turning negative at 7 day and TCMTI was evaluated visually, and a mathematical analysis was conducted to identify a cutoff point using threshold regressions with the R package chngpt (v.2021.5-12)[26]. In addition, the continuous TCMTI data were employed in restricted cubic spline (RCS) regression models. The potential cutoffs for TCMTI were explored with the RCS model with four knots(5th, 35th, 65th, and 95th percentiles)[27, 28]. Finally, the cutoff value was determined to 0.65(Supplementary material 2).

To assess the association between the negative conversion time of SARS-CoV-2 nucleic acid and TCMTI, survival curves for high and low TCMTI groups were plotted using the Kaplan-Meier method and analyzed with the log-rank test. The proportional hazards assumption was verified by plotting log(-log(survival)) against log (survival time), and was tested using the Schoenfeld residuals method. When proportionality assumptions were unmet, the time splitting-based approach was considered for Cox proportional hazard multivariate analysis. Multiple models were employed to explore the association, including a multivariate analysis using a lasso cox regression model, restricted mean survival time model (RMST), and cox regression
model of restricted cubic spline (RCS) with four knots (at the 5th, 35th, 65th, and 95th percentiles of TCMTI) following Harrell’s recommendations[29-31]. The statistical significance of the overall association and the non-linearity of the risk curves were evaluated at a significance level of 0.05 using the Wald test. Different sets of predictors (baseline covariates, post-baseline covariates) were employed in multiple models to evaluate the stability of the results in the aforementioned statistical models. Base Model (Model 0) only included TCMTI. Model 1 was adjusted for gender and age, and Model 2 additionally adjusted for marital status, patient recruitment time, volunteer status, and ward type. Model 3 further adjusted for various comorbidities and food allergies. Finally, the comprehensive Model 4 adjusted for all baseline covariates and post-baseline covariates, the latter including respiratory drug TI, circulatory drug TI, antibiotic TI, digestive drug TI, NSAIDs drug TI, and other drug TI.

To evaluate the association between TCMTI and the log-transformation of hospitalization days, a linear regression model (LRM) was used, and a LRM of RCS was also performed with four knots (at the 5th, 35th, 65th, and 95th percentiles of the TCMTI distribution). The likelihood ratio test was conducted to examine the significance of nonlinear terms based on the statistics derived from these models mentioned above. To control confounding, covariates related to both TCMTI and the log-transformation of hospitalization days were adjusted, and four models were built with increasing numbers of covariates. Model 0 only included TCMTI. Model 1 adjusted for gender and age, Model 2 further adjusted for marital status, patient recruitment time, volunteer status, and ward type. Model 3 additionally adjusted for various comorbidities and food allergies. The comprehensive Model 4 further adjusted for all post-baseline covariates, including respiratory drug TI, circulatory drug TI, antibiotic TI, digestive drug TI, NSAIDs drug TI, and other drug TI to assess potential confounding.

To validate the robustness of the main findings, a sensitivity analysis was conducted to address the following question: to what extent is TCMTI associated with the outcome after controlling for all confounding factors? In other words, how strong would any uncontrolled confounding factors have to be to overturn our statistically significant results? Sensitivity analyses for measured and unmeasured confounding were performed using the full model (model 4). Measured confounding was assessed using an inverse probability of treatment weighting (IPTW) Cox regression model with propensity score. Standardized mean differences (SMD) were employed to assess covariate balance, with SMD values less than or equal to 0.10 indicating acceptable balance. The sensitivity analysis method for unmeasured confounding was in accord with the reference[32]. Two synthetic variables were created and assigned at various levels, including gamma, which under certain assumptions can be interpreted as the direct effect of the unmeasured confounder on the negative conversion time of SARS-CoV-2 nucleic acid and TCMTI, and delta, which can be interpreted as the the association between the unmeasured confounder and the exposure indicator. The values of gamma and delta ranged from 0.00 to 1.00 and the step was set to 0.25. By introducing hypothetical unmeasured confounders, the re-fitted results were confounded by these additional factors with different correlation coefficients that can be observed.

All statistical analyses were performed using R software version 4.2.3 (The R Foundation for Statistical Computing, https://cran.r-project.org/). All statistical tests were two-tailed, and the significance level was set at $P < 0.05$.

3 | RESULTS

3.1 | Characteristics of participants

A total of 6,120 COVID-19 patients were included in the study, all of whom were recruited from a temporary hospital during the period of April to May in 2022. No instances of loss to follow-up or mortality were found. The median age of all mild COVID-19 patients was 43.0 years (range: 20.0-75.0), with a median duration of TCM usage of 2.0 days (range: 0.0-15.0) and a median TCMTI of 0.23 (range: 0.00-1.00). The median negative conversion time of SARS-CoV-2 nucleic acid was 6.67 days (range: 6.64-6.69), while the median hospitalization duration was 9.70 days (range: 4.10-22.50). The median log-transformation of hospitalization days was 2.28 (range: 1.40-3.24). In addition, all mild COVID-19 cases included 1,854 women (30.3%), 3,928 married individuals (64.2%), 1,188 unvaccinated individuals (19.4%), 206 cases with one vaccine dose
(3.4%), 1,714 individuals with two vaccine doses (28.0%), and 3,012 individuals who completed intensive immunization with three doses (49.2%). Additionally, 64 patients (1.0%) had a history of food allergy, and 648 participants (11.6%) had underlying diseases, including 444 with circulatory system diseases (7.3%), 79 with endocrine system diseases (1.3%), 33 with digestive system diseases (0.5%), and 92 with diseases of other systems (1.5%). 569 (9.3%) received respiratory drugs, 205 (3.3%) received circulatory system drugs, 260 (4.3%) received antibiotics, 250 (4.1%) received digestive system drugs, and 404 (6.6%) received NSAIDs (Table S1 in supplementary material 2).

Furthermore, a significant non-linear positive correlation was observed between log-transformation of hospitalization days and the negative conversion time of SARS-CoV-2 nuclear acid ($P < 0.001$, Figure 1).

### 3.2 | Association between TCMTI and negative conversion time of SARS-CoV-2 nucleic acid

#### 3.2.1 | The association between TCMTI and negative conversion time of SARS-CoV-2 nucleic acid was analysed by cox proportional risk model

The results of the univariate cox proportional hazards regression analysis were presented in Table 1. Several factors were found to be significantly associated with the negative conversion time of SARS-CoV-2 nucleic acid, including age, gender, patient recruitment time, hospital ward in the temporary hospital, vaccine inoculation, marital status, respiratory drug TI, circulatory system drug TI, antibiotics TI, gastrointestinal drug TI, NSAIDs TI, and other drugs TI (all $P < 0.001$, Table 1). However, no statistically significant linear correlation was observed between TCMTI and the negative conversion time of SARS-CoV-2 nucleic acid ($HR = 0.873$, 95% CI : 0.756, 1.007, $P = 0.062$). A nonlinear relationship was observed between TCMTI and the negative conversion time of SARS-CoV-2 nucleic acid by modeling a RCS cox regressions with four knots. The likelihood ratio test indicated that the nonlinear model was considerably better compared to the linear model ($\chi^2 = 701.223$, $P < 0.001$). In addition, smoothed plots revealed that there was significant nonlinear association between TCMTI and negative conversion time of SARS-CoV-2 nucleic acid (Figure S1, table S2).

The study employed various statistical methods to determine the cutoff point for the association between the probability of nucleic acid turning negative at 7$^{th}$ day of entering the shelter hospital and the TCMTI in COVID-19 patients. First, the threshold regressions with changepoint (chngpt) were utilized to identify the cutoff point, which was established at 0.65. Additionally, the relationship between the 7-day survival probability and TCMTI was explored using restricted cubic splines (RCS) with four knots (located at the 5$^{th}$, 35$^{th}$, 65$^{th}$, and 95$^{th}$ percentiles of the TCMTI distribution), the results demonstrated that the cutoff point for this relationship was also established at 0.65. Furthermore, it was seen in Figure S1 (supplementary material 2) that the linear relationship between the 7-day survival probability and TCMTI when the TCMTI was over 0.65. Consequently, all COVID-19 patients were classified into either a low TCMTI group ($< 0.65$, n=5,725) or a high TCMTI group ($> = 0.65$, n=395).

#### 3.2.2 | RCS cox regression to explore the association between TCMTI and negative conversion time of SARS-CoV-2 nucleic acid

After adjusting for potential covariates, RCS cox multivariate models to investigate the association between TCMTI and the negative conversion time of SARS-CoV-2 nucleic acid, as demonstrated by the differences among the multiple models(Table 2). The inverse relationship between TCMTI and the negative conversion time of SARS-CoV-2 nucleic acid appeared to be non-linear (Table 2, Figure 2). All adjusted models displayed a negative association between the increase of TCMTI (continuous variable) and the negative conversion time of SARS-CoV-2 nucleic acid when TCMTI exceeded a threshold (0.65. All $P < 0.001$. Figure 2).

#### 3.2.3 | Time-dependent cox model to explore the association between TCMTI (high TCMTI group vs low TCMTI) and the negative conversion time of SARS-CoV-2 nucleic acid

The association between TCMTI (high TCMTI group vs. low TCMTI. Table S3) and the negative conversion time of SARS-CoV-2 nucleic acid was analyzed using Kaplan-Meier (K-M) survival curves (Figure 3a). The median days for negative conversion of SARS-CoV-2 nucleic acid were 8.47 [Range: 1.90, 25.54] days in the
low TCMTI group and 5.24 [Range: 2.65, 21.50] days in the high TCMTI group. The median days for negative conversion of SARS-CoV-2 nucleic acid were shorter in the high TCMTI group compared to the low TCMTI group ($\chi^2 = 103.988, P < 0.0001$).

However, the Schoenfeld residuals proportional hazards test indicated a violation of the proportional hazards assumption ($\chi^2 = 21.643, P < 0.0001$), as illustrated by the crossing of the Kaplan-Meier survival curves (Figure 3a). Consequently, a time-dependent cox model with time-split functions was established using a splitting time of 11.5 days. The product of splitting time and TCMTI (high TCMTI group vs low TCMTI) was then considered a time-dependent covariates in proportional hazards Cox regression models.

The findings of the multivariable time-dependent cox model analysis are presented in Table 3. Low TCMTI was identified as a significant hazard factor for prolonging the time for SARS-CoV-2 to turn negative in all five models (Table 3, all $P < 0.001$). The hazard ratios (HRs) for the effect of TCMTI on the time of SARS-CoV-2 turning negative varied slightly between 1.578 and 1.857 across the five models (Table 3), and a notable positive association was observed between low TCMTI and the length of negative conversion time of SARS-CoV-2 nucleic acid (Figure 3b). In other words, high TCMTI can potentially reduce the negative conversion time of SARS-CoV-2 nucleic acid for COVID-19 patients.

Furthermore, the outcomes of the lasso cox regression model revealed that seven independent variables served as significant predictors of the negative conversion time of SARS-CoV-2 nucleic acid for mild COVID-19 patients who were admitted to the hospital within the first seven days. These variables included TCMTI, age, ward, patient recruitment time, respiratory system drugs TI, digestive system drugs TI, and NSAIDs TI (all $P < 0.01$, Table S4).

### 3.2.4 RMST model to explore the association between TCMTI (high TCMTI group vs low TCMTI) and the negative conversion time of SARS-CoV-2 nucleic acid

Non-proportional hazard analysis with restricted mean survival time (RMST) at the 21st day after COVID-19 patients entering the hospital was applied to evaluate the effect of TCMTI. Subsequently, an adjusted RMST analysis truncated at the 21st days indicated a significant benefit associated with the high TCMTI group. In comparison to the low TCMTI group, COVID-19 patients in the high TCMTI group had a shortened negative conversion time of SARS-CoV-2 nucleic acid by 2.081 days in univariate analysis (model 0, $P < 0.001$. Table 4). Moreover, similar results were observed in multiple models adjusted for various potential confounding factors (model 1 to model 4. Table 4). The estimated difference of the negative conversion time of SARS-CoV-2 nucleic acid between COVID-19 patients receiving the high TCMTI group and those receiving the low TCMTI group was nearly 1.9 days in multiple models (model 1 to model 4, all $P < 0.001$. Table 4).

### 3.3 The association between TCMTI and log-transformation of hospitalization days

The LRM univariate analysis revealed that the log-transformed hospitalization days were associated with age, patient recruitment time, hospital ward in temporary hospital, marital status, respiratory drugs TI, circulatory system drugs TI, antibiotics TI, gastrointestinal TI intensity, NSAIDs TI, and other drugs TI (all $P < 0.001$, Table S5). Additionally, the log-transformation of hospitalization days was 2.007±0.02 in the high TCMTI group and 2.378±0.02 in the low TCMTI group. The log-transformation of hospitalization days in the high TCMTI group was less than that in the low TCMTI group (model 0, coefficient=-0.162, 95% CI : -0.160, 0.164, $P < 0.002$, Table S6). Furthermore, LRM models with increasing covariate dimensions (model 1 to model 4) demonstrated a consistent negative association between high TCMTI and log-transformed hospitalization days ($P < 0.001$, equally, Table S6). In other words, mild COVID-19 patients who received high TCMTI treatment had a shorter hospitalization days.

In addition, a significant non-linear association between TCMTI and log-transformation of hospitalization days was found using LRM with an RCS of four knots (Figure S2. Table S7). Furthermore, the association between TCMTI and log-transformed hospitalization days investigated using the RCS model fitted LRM with four knots was considerably better than that of the common LRM model (root sum squares value:
Additionally, nonlinear association was observed in multiple RCS models fitted for LRM. All models were adjusted for different confounding factors (Figure S3). High TCMTI demonstrated a negative correlation with log-transformation of hospitalization days in all adjusted models, ranging from model 1 to model 4 (Table S8). The findings also revealed that a higher TCMTI value (closer to 1.0) corresponded to a smaller log-transformation of hospitalization days (Figure S3).

3.4 | Sensitivity analyses

Sensitivity analyses were conducted using IPTW in the fully adjusted model (model 4) to evaluate the impact of known confounding variables. The results demonstrated that high TCMTI had a positive and statistically significant effect on the probability of negative conversion time of SARS-CoV-2 nucleic acid at 7 day (HR =1.481, 95% CI: 1.302, 1.683, \( P < 0.001 \)), it was accordance with initial result. In addition, it was possible that unmeasured confounding factors, represented by two hypothetical variables named gamma and delta, may have also influenced the association between TCMTI and time of SARS-CoV-2 RNA turning negative, the adjusted HRs in model 4 ranged from 0.651 (95% CI: 0.580, 0.938) to 1.769 (95% CI: 1.575, 1.986) (Table S9, Figure S4, Figure S5, Table S10). Among the 25 HRs and their corresponding 95% CIs, nineteen HRs (95% CI) obtained similar results to the study, four HRs (95% CI) showed no significant difference as their CIs included zero (Table S10), and two HRs (95% CI) presented conflicting conclusions as their HRs ranged from 0.0 to 1.0 (Table S10). Thus it can be seen that the observed association between TCMTI and the negative conversion time of SARS-CoV-2 nucleic acid may be not influenced by measured confounder and unmeasured confounding factors.

4 | DISCUSSION

The retrospective cohort study, which included a large sample of mild COVID-19 patients, revealed that high TCMTI can shorten the SARS-CoV-2 nucleic acid negative conversion time and hospitalization days. These findings provide compelling evidence for a non-linear association between TCMTI and the therapeutic effects in mild COVID-19 patients, rather than a simple linear correlation. Understanding the underlying mechanisms of the association is crucial for making a significant impact on COVID-19 treatment outcomes. Consequently, the study’s conclusions can aid healthcare professionals in selecting the optimal TCM drug dosage or prescription duration to expedite recovery. Although effective antiviral drugs (such as Remdesivir, Nirmatrelvir/Ritonavir, Molnupiravir) were not available for these COVID-19 patients. The combination of TCM with standard care has demonstrated efficacy in treating mild COVID-19, and the reassuring and encouraging findings may provide clinical evidence supporting guidelines that recommend TCM for treating mild COVID-19 patients.

Various association patterns between interventions and effects have been observed in nature, including positive linear, S-curve, U-curve, and reverse-L-curve (or reverse J-curve). Nonlinear correlations are common in various medical contexts, such as the U-curve relationships observed between total cholesterol and mortality [33] or between dietary copper intake and obesity risk [34], L-curve associations between adipose tissue linoleic acid and all-cause mortality [35], and variability of the association between treatment intensity and mortality by stage and cause-specific mortality [36]. The study also revealed a clear nonlinear relationship between treatment intensity and disease outcome. Accurately exploring the association between intervention measures and clinical outcomes can assist healthcare professionals in precisely selecting therapeutic schedules or clinical treatment intensities to accelerate diseases recovery.

The mechanism behind the treatment of viral infections with Chinese herbs is complex. Viral respiratory infections, (e.g., SARS-CoV, SARS-CoV-2, H1N1) can trigger robust immune reactions and cytokine storms. Chinese herbs possess multi-component, multi-target, and multi-pathway characteristics [37], which play a crucial role in their broad-spectrum antiviral, anti-inflammatory, immune-regulatory, and organ-protective effects [37-39]. The therapeutic effect of TCM on COVID-19 is believed to be mainly based on the synergistic effect of various components from different herbs in one prescription, as suggested by previous studies [38-41]. Our study further supports this notion, indicating that when the TCMTI reaches a specific critical value,
numerous chemical components of herbs in the blood may achieve a particular threshold, enabling synergistic interactions that accelerate pathogen elimination through immune regulation and other mechanisms. The study revealed that TCMTI can shorten negative conversion time of SARS-CoV-2 nucleic acid and hospitalization duration even in the absence of effective antiviral drugs or prophylactic vaccines, other notable examples have also demonstrated potential in combating, albeit with varying levels of evidence[16, 42, 43]. Several studies have reported a significant reduction in the negative conversion time of SARS-CoV-2 nucleic acid and total hospitalization days in mild COVID-19 patients who received TCM treatment compared to those who did not receive such treatment[16, 43-45]. In contrast, a different study reported no statistically significant difference in the negative conversion time of SARS-CoV-2 RNA between mild COVID-19 cases with or without TCM treatment. Moreover, no clear benefit was observed in terms of viral clearance in the TCM treatment group[46]. This discrepancy in findings underscores the lack of consensus on whether TCM can enhance the therapeutic outcomes of COVID-19 patients, based on observational studies. Therefore, further randomized controlled trials (RCTs) are necessary to investigate the clinical efficacy of TCM in treating COVID-19 patients.

Infectious diseases have accompanied the development of human civilization and influenced the course of human history[47]. Despite three thousand years of medical experience in fighting against infectious diseases[38, 48], there are no specific drugs currently available for treating COVID-19. While modern medicine and treatment plans can relieve patient symptoms and prevent complications, the ultimate recovery of COVID-19 patients is entirely dependent on their own immunity to overcome the SARS-CoV-2. TCM adopts a holistic approach to examine symptoms and find their causes by combining corresponding manifestations and signs, and precise treatment is then applied to COVID-19 patients based on the perspective of syndrome differentiation[21, 49]. Further studies are needed to elucidate the mechanisms underlying the effectiveness of TCM in treating COVID-19.

Our study demonstrates that high TCMTI intervention could reduce hospitalization days by two days, with significant public health implications. It suggests that high TCMTI intervention should be administered to COVID-19 patients as early as possible to impede disease progression, and prevent severe illness or death in high-risk individuals. Additionally, high TCMTI can shorten the COVID-19 course during outbreaks and peak epidemic periods, accelerating patient recovery and discharge while preserving critical health resources such as beds, medicine personnel, drugs. This approach allows COVID-19 patients awaiting hospitalization to be admitted more expeditiously, thereby reducing family infections and community transmission. Therefore, there is an urgent need to establish a collaborative network beyond the boundaries of modern medical staff and traditional practitioners, scientists, and enterprises. Such a network could facilitate to bring adaptive, scientific, evidence-based and commercially viable medical products to more people during the epidemic[50, 51], it could potentially lead to a decrease in infections, severe cases, and deaths, especially in countries with large elderly populations and high-risk individuals with chronic illnesses.

Our study has found that the time to achieve negative RT-PCR results for SARS-CoV-2 and hospitalization days were significantly longer for elderly individuals, which is consistent with the conclusions of other studies[52, 53], these studies have shown that the severity rate and case fatality rate of COVID-19 are higher in elderly patients compared to younger ones. Furthermore, even in the absence of complications, elderly patients aged over 80 years take longer time to reach negative RT-PCR results for SARS-CoV-2 and have longer hospitalization periods than younger patients[52]. These differences may be due to the deterioration of various functions in the elderly, the relatively high viral load of SARS-CoV-2, and age-related reductions in T-cell numbers and functionality, which contribute to impaired control of viral replication[54]. Therefore, it is not surprising that these factors could also impact the time to achieve negative conversion of SARS-CoV-2 nucleic acid.

Based on our findings, viral RNA negative conversion was significantly slower in patients with respiratory or digestive diseases. This suggests that comorbidities may be associated with delayed negativization, indicating that COVID-19 patients with underlying conditions may experience slower recovery. Other studies have also reported longer viral clearance times in COVID-19 patients with conditions such as obesity, diabetes, or
hypoalbuminemia\cite{52, 55}, suggesting that these complications could be considered predisposing factors for delayed SARS-CoV-2 viral clearance\cite{52, 55}. One of the most important reasons for this may be that the immunity of individual with underlying conditions is compromised, leading to prolonged elimination time of SARS-CoV-2 nucleic acid. However, some studies have reported no increase in viral clearance time for COVID-19 patients with complicating diseases\cite{56, 57}.

Our investigation revealed that COVID-19 patients who were treated with NSAIDs experienced a prolonged period for viral clearance. This suggests that patients with COVID-19 who experience fever and other clinical symptoms may have a higher viral load than those who are asymptomatic. Our findings were consistent with previous studies that identified factors contributing to the duration of SARS-CoV-2 RNA negative conversion, where symptomatic patients exhibited significant delays in viral clearance\cite{52, 53, 58}. Moreover, the presence of symptoms was identified as a risk factor for early non-negativization in the COVID-19 patients under investigation\cite{52, 53}. This knowledge is crucial for the establishment of guidelines for the management of COVID-19.

Adverse events (AEs) and serious adverse events (SAEs) were recorded in several intervention studies. Our study showed that COVID-19 patients receiving either high or low TCMTI had relatively low incidences of AEs and SAEs, with no significant differences between the two groups. These findings suggest that high TCMTI does not increase the incidence of AEs or SAEs. This conclusion is supported by other studies reporting no statistical differences in AEs among COVID-19 patients treated with and without TCM\cite{59, 60}. Therefore, these findings provide evidence that TCM is generally safe for treating COVID-19.

Although this study provides valuable evidence and insights into TCM’s potential therapeutic role in treating COVID-19, there are several limitations that must be acknowledged. Firstly, this retrospective observational study cannot establish a causal relationship between TCM and clinical outcomes. Therefore, it is necessary to assess and verify these findings through large-scale, multi-center randomized controlled trials (RCTs). Secondly, the study was conducted solely on mild COVID-19 cases in Shanghai, China, which limits the generalizability of the findings and may result in a lower level of evidence. Thirdly, the study did not capture essential pre-admission information for COVID-19 patients, such as the exact time of virus infection and the time interval between SARS-CoV-2 infection onset and temporary hospital admission, which could have partially affected clinical treatment outcomes. Fourth, while regression models and sensitivity analyses were conducted, residual confounding effects from unmeasured or unknown factors cannot be entirely excluded. Fifthly, some electronic medical records primarily rely on the memories of COVID-19 patients, it may introduce memory bias. Finally, Chinese herbal medicine contains a large number of components, and it is necessary to identify these components and monomers before starting a clinical study. Additionally, the blood concentration and metabolism of these herbal components also need to be investigated before conducting a clinical trial. In the future, a large-scale multi-center RCT should be designed to objectively investigate the effect of TCM on treating COVID-19 patients. Such a study will provide objective knowledge regarding whether Chinese herbal medicine can improve the therapeutic effect for COVID-19 patients.

This cohort study provides evidence that early high TCMTI intervention is associated with a faster clearance of SARS-CoV-2 RNA and shorter hospitalization periods for mild COVID-19 patients. In light of TCM efficacy and safety profile, high TCMTI interventions may accelerate recovery during the early stages of the disease. Thus, in the face of a large number of COVID-19 patients and limited medical resources, TCM may be recommended for treating mild patients. In the future, large-scale multi-center trials will be necessary to confirm the therapeutic efficacy of TCM for COVID-19 patients.

AUTHOR CONTRIBUTIONS

LC and G-JM conceived and designed the research, WL, MW, X-XC, H-FW, XW, JL and DZ collected the data, MY and S-XZ analyzed the data, PY, YM, QF and X-XC contributed to the draft writing-reviewing-editing. S-XZ and YT contributed equally to this work, MY and Y-LC are common correspondent authors. All authors read and approved the final manuscript. ACKNOWLEDGMENTS The acknowledgment is forwarded for the study participants for their valuable information and data collectors, doctors, nurses and
supervisors for their commitment.

CONFLICT OF INTEREST STATEMENT

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

DATA AVAILABILITY STATEMENT

The data used to support the findings of this study are available from the author upon reasonable request (Ming Yang. Email: yangpluszhu@sina.com).

ETHICS STATEMENT

The protocol was approved by the Ethics Committee of Longhua Hospital affiliated to Shanghai University of Traditional Chinese Medicine (No.2022LCSY065), and it was registered at the Chinese Clinical Trial Registry (ChiCTR2200063151, http://www.chictr.org.cn/, date of Registration is Aug-31-2022). For each research participant, oral informed consent was obtained either through face-to-face interviews with the individual or via telephone communication with their legal guardian.

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FIGURE LEGENDS

**FIGURE 1** The correlation between log-transformation of hospitalization days and the negative conversion time of SARS-CoV-2 nuclear acid

**FIGURE 2** After adjustments in RSC cox multiple models, the sharp between log relative hazard of negative conversion time of SARS-CoV-2 nucleic acid less than seventh day and TCMTI. Model 0 only included TCMTI. Model 0 only included TCMTI. Model 1 was adjusted for gender and age. Model 2 was further adjusted for marital status, patient recruitment time, volunteer and wards. Model 3 was further adjusted for various comorbidities (hypertension, diabetes, cardiovascular and cerebrovascular diseases, chronic obstructive pulmonary disease(COPD), pulmonary infection, and so on) and food allergy. The fully model 4 further adjusted for all post-line covariants, it included respiratory drug TI, circulatory drug TI, antibiotic TI, digest drug TI, nsaids drug TI and others drug TI.

**FIGURE 3a** Kaplan-Meier survival analysis shown that median days of the negative conversion time of SARS-CoV-2 nucleic acid have significant differences between high TCMTI group and low TCMTI group.

**FIGURE 3b** The high TCMTI can reduce the negative conversion time of SARS-CoV-2 nucleic acid for mild COVID-19 patients. The calculation is based the model 4 (fully model), the fully model 4 were adjusted for all covaritants, it included gender, age, marital status, patient recruitment time, volunteer, wards, various comorbidities (hypertension, diabetes, cardiovascular and cerebrovascular diseases, chronic obstructive pulmonary disease, pulmonary infection, and so on), food allergy, respiratory drug TI, circulatory drug TI, antibiotic TI, digest drug TI, nsaids drug TI and others drug TI

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Number at risk

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<th>5</th>
<th>10</th>
<th>15</th>
<th>20</th>
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<td>4484</td>
<td>1812</td>
<td>439</td>
<td>45</td>
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<tr>
<td>High TCMT1</td>
<td>395</td>
<td>270</td>
<td>59</td>
<td>10</td>
<td>2</td>
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Adjusted Survival Probability

Survival function for treatment groups:

- Low TCMT1
- High TCMT1

Log Rank (Mantel-Cox) test: Chi-Square = 103.988, P = 0.0001

Hospitalization days (day)

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