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Original Article

Mechanism of new coronavirus pneumonia agreement prescription on 2019 novel coronavirus-infected pneumonia based on network pharmacology analysis and the validation

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Abstract: Purpose: To investigate the mechanism of action underlying the effective treatment of New Coronavirus Pneumonia Agreement Prescription (NCPAP) on 2019 Novel Coronavirus-Infected Pneumonia (2019-NCIP) using network pharmacology. Methods: In this retrospective study, 50 patients with 2019-NCIP were recruited, including 16 who received symptomatic treatment and 34 that received NCPAP formula treatment on the basis of symptomatic treatment. Hospitalization and lymphocyte percentages were served as efficacy evaluation indicators. Moreover, pharmacological analysis was performed to identify the target disease of NCPAP. Active ingredients in herbs were screened using the Traditional Chinese Medications Systems Pharmacology (TCMSP) database, and related target genes were identified. We then queried therapeutic target data for coronavirus-associated genes. The protein-protein interaction network was constructed to examine the relationships between these targets. Gene Ontology (GO) and the Kyoto Encyclopedia of Genes and Genomes (KEGG) network enrichment analyses were conducted using the Database for Annotation, Visualization and Integrated Discovery (DAVID) database. Results: NCPAP significantly reduced hospitalization time and increased both the absolute value and percentage of lymphocytes. Bioinformatics and cytokine analysis suggested that preventing cytokine storm syndrome and regulating immune response are the key mechanisms of NCPAP in treating 2019-NCIP. Conclusions: The possible mechanisms of NCPAP in the treatment of 2019-NCIP are reduction of cytokine storms and regulation of the immune response.

Keywords: Network pharmacology, 2019 novel coronavirus-infected pneumonia, cytokine storm, regulating immune response

Introduction

Since December 2019, the novel coronavirus (SARS-CoV-2, 2019-nCoV) has been spreading rapidly worldwide [1]. The virus particles are spherical or ellipsoidal in shape with widths ranging from 60 to 140 mm and a protective envelope, exhibiting pleomorphism [1, 2]. Epidemiological studies indicate that the most common symptoms of 2019 Novel Coronavirus-Infected Pneumonia (2019-NCIP) include fever, dry cough, and fatigue. Patients exhibit symptoms like nasal congestion, tongue pain, muscle aches, and constipation [3-6]. Currently, most patients have a favourable prognosis, with a few in critical condition. Elderly patients and those with chronic diseases tend to have poorer outcomes. Like most viral pneumonia, a key feature of 2019-NCIP is the presence of normal or decreased white blood cell count and lymphopenia (a decrease in the number of lymphocytes in the peripheral blood) during the early stages [5, 7].

Initially, there is no specific drug, and early antiviral and symptomatic treatments were the

foundation of care [5]. The current strategy involves controlling infection sources, employing personal protection measures to reduce transmission risk, and promptly diagnosing, isolating, and providing supportive care for affected patients. Antibacterial agents are ineffective [4]. Recently, Paxlovid has been recommended for coronavirus infection; however, its high-cost limits accessibility for all patients. The composition of NCPAP includes Citrus reticulata Blanco, Pinellia ternata (Thunb.) Breit, Poria cocos (Schw.) Wolf, Atractylodes macrocephala Koidz, Amomum villosum Lour, Agastacherugosus (Fisch. et Mey) O. Ktze, Schisandra chinensis (Turcz.) Baill, Adenophora tetraphylla (Thunb.) Fisch, and Glehnia littoralis F. Schmidtex Miq. During the practice in Leishenshan Hospital, it was found that the NCPAP effectively improved clinical symptoms in patients with 2019-NCIP, offering an affordable treatment option. Based on the affirmation of clinical efficacy, this study investigated the pharmacological mechanism of the NCPAP in treating 2019-NCIP by employing network pharmacology.

Methods

2019-NCIP diagnostic criteria

The diagnostic criteria for 2019-NCIP are based on the Diagnosis and Treatment Protocol for 2019-NCIP (Trial Version 7) combined with epidemiological history and clinical manifestations. 2019-NCIP can be confirmed either by etiological or antibody evidence, including a positive nucleic acid test for the novel coronavirus from throat, nose, or lower respiratory tract samples; virus genome sequencing showing significant similarity to other new coronaviruses; serum novel coronavirus-specific IgG antibody turning from negative to positive or recovering slowly during the acute phase; positive serum novel coronavirus-specific IgM and IgG antibodies; or a 4-fold or greater increase in serum novel coronavirus-specific IgG antibody levels [7-10].

2019-NCIP clinical classification

2019-NCIP clinical classification has been determined as follows (Trial Version 7) [10]: (1) Mild: No signs of pneumonia as indicated by imaging and mild clinical symptoms. (2) Normal: Pneumonia indicated by imaging and accompanied by symptoms such as fever and respiratory symptoms. (3) Severe: Individuals who fit the following criteria: a) Breathlessness with a respiratory rate of ≥30 beats per minute; b) Oxygen saturation level of ≤93% at rest; and c) PaO_2/FiO_2<300 mmHg (40.00 KPa). At high altitudes (>1000 meters), the value is adjusted according to the formula: PaO_2/FiO_2 × [Atmospheric pressure (mmHg)/760] [10].

Clinical data

In this retrospective study, we examined the clinical data of 50 2019-NCIP patients (24 males, 26 females) diagnosed between February 1 and March 31, 2020, at Shanghai's Seventh People's Hospital and Leishenshan Hospital. The diagnostic and therapeutic approach was based on above mentioned criteria. The patients were categorized into 39 normal cases (18 males, 21 females) and 11 severe cases (6 males, 5 females) according to disease severity. Based on the admission time and voluntary participation principle, 34 patients were assigned to the NCPAP group and the rest 16 to the control group. Participants or their legal guardians provided informed consent, and this study was approved by hospital ethics committee (Ethics approval number: 2021-7th-HIRB-049).

Inclusion criteria

Patients aged >18 years; patients diagnosed with 2019-NCIP (normal or severe type) and treated according to therapy protocol (Trial Version 7); and those voluntarily provided necessary information.

Exclusion criteria

Patients with severe bacterial infections; pregnant or lactating women; those with severe ventricular arrhythmias, acute cerebral infarction/hemorrhage, severe heart failure/shock, coagulopathy and bleeding tendency, or severe primary diseases (e.g., kidney, endocrine, liver, respiratory, hematopoietic); individuals with advanced malignant tumors or mental disorders; and patients unwilling to receive NCPAP or exhibiting poor compliance.

Discharge standards

(1) With stabilized and normal temperature for over 3 days. (2) Significant improvement in

Table 1. Composition of NCPAP

Formula	Herbal Components Latin name	Herbal Components Chinese name	Amount (g)	Function
NCPAP	Pinellia ternata (Thunb.) Breit	BanXia	9	Drying dampness and resolving phlegm
	Amomum villosum Lour	ShaRen	6	Promoting the circulation of qi
	Poria cocos (Schw.) Wolf	FuLing	15	Diuresis and dampness
	Atractylodes macrocephala Koidz	BaiZhu	15	Invigorating Spleen to remove dampness
	Citrus reticulata Blanco	ChenPi	10	Drying dampness and resolving phlegm
	Agastacherugosus (Fisch. et Mey) O. Ktze	HuoXiang	10	Resolving dampness
	Schisandra chinensis (Turcz.) Baill	WuWeiZi	6	Tonifying qi
	Adenophora tetraphylla (Thunb.) Fisch	NanShaShen	12	Nourishing yin to clear away the lung-heat
	Glehnia littoralis F. Schmidtex Miq	BeiShaShen	12	Dispelling phlegm and relieving cough

Note: NCPAP, New Coronavirus Pneumonia Agreement Prescription.

respiratory symptoms. (3) Substantially improved acute exudative lung lesions on MRI. (4) Negative nucleic acid results of respiratory tract samples for two consecutive days (≥24 hours apart).

Data collection

The clinical data of patients were collected, including nucleic acid results, coagulation function, routine blood tests, and liver and kidney function. Main indicators included: white blood cell count (WBC), platelet count (PLT), red blood cell count (RBC), hemoglobin (HGB), aspartate aminotransferase (AST), alanine aminotransferase (ALT), creatinine (Cr), blood urea nitrogen (BUN), prothrombin time (PT), activated partial thromboplastin time (APTT), etc. 1.5-2 mL of fasting elbow venous blood was collected from the 50 patients and analyzed using an automatic blood cell counter (Hitachi Limited, Japan). The data collection and entry were completed by 2 physicians who participated in the first-line rescue mission throughout the process.

Treatment method

The control group was given symptomatic treatment, such as antiviral and antipyretic treatments. The "NCPAP group" was administered with NCPAP prescription: Breit, Citrus reticulata Blanco, Pinellia ternata (Thunb.) Poria cocos (Schw.) Wolf, Atractylodes macrocephala Koidz, Amomum villosum Lour, Agastacherugosus (Fisch. et Mey) O. Ktze, Schisandra chinensis (Turcz.) Baill, Adenophora tetraphylla (Thunb.) Fisch and Glehnia littoralis F. Schmidtex Miq. The prescription was administered twice daily for a seven-day course. The ingredient's concentration and quantity details are listed in **Table 1**.

NCPAP ingredients identification and HPLC/ESI-MS

The NCPAP formula was purchased from Leishenshan Hospital (Wuhan, China) and Seventh People's Hospital (Shanghai, China). The extraction process was as follows: 1000 g of the ingredient drugs were boiled in 90% ethanol for 1.5 hours before being sifted through a 120-mesh sieve. After filtering the ethanol, the residue was condensed to obtain a viscous extract. The leftovers were then sifted through a 120-mesh screen after being decocted with water for an hour. The obtained extracts from previous two cycles was condensed under a vacuum at 50°C and desiccated using lyophilization (120 g). Based on the Pharmacopoeia of People's Republic of China (2015), Dr. Cheng Lu isolated the extract of the prescription. The composition of NCPAP ingredients is shown in **Table 1**.

The compound separation was performed using a quaternary pump, degasser, autosampler, automated thermostatic column chamber, digital analyzer detector (DAD), and LC/MSD Trap XCT ESI mass spectrometer (all from Agilent Technologies, MA, USA) built into an Agilent 1100 HPLC system (Agilent Technologies MA, USA). The separation was performed at 35°C on a GS-120-5-C18-BIO chromatography column (5 μm, 250 4.6 mm i.d.). A linear gradient elution of A (0.1% formic acid water) and B (acetonitrile) was used with the gradient procedure as follows: 0 min, B 5%, to 60 min, B 40% (v/v). We injected 10 μL at a 1 mL/min flow rate and set the DAD wavelength to 210 nm.

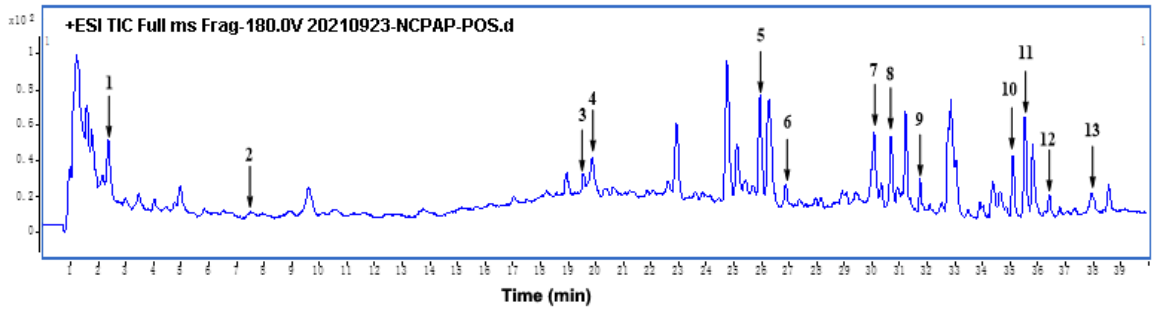

Figure 1. Positive mode HPLC/ESI-MS chromatogram of the water extract in HPLC/ESI-MS chromatogram which is in line with the molecular weights of thirteen compounds. Comparing the retention periods and the MS data with the reference standards allowed for the clear identification of 13 different compounds. HPLC/ESI-MS, High Performance Liquid Chromatography coupled with electrospray MassSpectrometry.

The mass spectrometer operated with a 1:3 split ratio. In the negative ion mode, ultrahigh-purity helium was used as the collision gas, while nitrogen was used as both nebulizer (35 psi) and drying gas (10 L/min). The drying temperature was 350°C, HV was 3500 V, mass scan range was 100-2200 m/z, target mass was 500 m/z, and compound stability and trap drive level were 100%. Statistical analyses were conducted using Chemstation.

UHPLC-Q-Orbitrap HRMS analysis

Chromatography-quadrupole/electrostatic field orbitrap high-resolution mass spectrometry (UHPLC-Q/Exactive) was used to evaluate the main bioactive components subjectively and numerically, allowing for better quality control over the NCPAP extraction process. The extract's chromatography signature is depicted in **Figure 1**. A. The ultra high-performance liquid chromatography system (Thermo Fisher, San Jose, CA, USA) with an autosampler, quaternary gradient pump, and quadrupole/electrostatic field orbitrap high-resolution mass spectrometry detector was employed for the studies. Components were extracted using a mixture of acetonitrile (I) and 0.1% formic acid in water (II) (0-2 minutes, 10% II; 2-9 minutes, 90% II) [12-14].

Pharmacological network analysis

Screening the active ingredients of the prescription: The prescription's active components were vetted and compiled from two sources, including the Traditional Chinese Medications Systems Pharmacology database (TCMSP, https://www.ncbi.nlm.nih.gov) and the Bioinformatics Analysis Tool for Molecular Mechanism of Traditional Chinese Medicine (BATMAN-TCM, http://bionet.ncpsb.org/batman-tcm) [11, 15, 16]. Moreover, physiological measures of potent drugs, such as drug similarity (DL), oral bioavailability (OB), and Caco-2 permeability (Caco-2) were predicted using an ADME algorithm, with thresholds of ≥30%, ≥0.18, and ≥-0.4, respectively.

Predicting the ingredient-related target genes: The target genes of active components were obtained from TCMSP [11, 15, 16]. The official gene identities were acquired by converting the various ID forms of the target genes into UniProt (https://www.uniprot.org/) IDs with the restriction of "Homo sapiens" as the species.

Retrieving the 2019-NCIP-related target genes: Two datasets were searched to identify genes associated with 2019-NCIP: the DisGeNET (http://www.disgenet.org) and the therapeutic target database (TTD, https://db.idrblab.org/ttd) [17-19]. Target IDs were converted to UniProt IDs with "Homo sapiens" as the species restriction.

Protein-protein interaction and network construction: To further elucidate the possible mechanism of the prescription in treating 2019-NCIP, we constructed a protein-protein interaction (PPI) network to study interactions between the overlapping targets. In this investigation, we used STRING to examine the interaction between the screened targets [20]. Furthermore, the networks of these targets were mapped using Cytoscape (3.7.0).

Gene function analysis and Kyoto Encyclopedia of Genes and Genomes (KEGG) signaling pathway enrichment analysis: Enrichment analysis

Table 2. The retention times and pseudomolecular ion peaks for the 13 identified compounds, as observed in the HPLC/ESI-MS chromatogram

Compound Number	Compound Name	Retention Time (mins)	Pseudomolecular Ion Peak (m/z)
1	Threonin	2.3	120.0776 [M + H]+
2	Isoborneol	7.5	177.0508 [M + H]+
3	Naringin	19.6	581.1838 [M + H]+
4	Cerevisterol	19.9	453.3317 [M + H]+
5	Hesperridin	26.4	611.1880 [M + H]+
6	5-O-methylvisammioside	26.9	453.3364 [M + H]+
7	Schisandrin	30.0	887.2656 [2 M + Na]+
8	Lupenone	30.7	447.1297 [M + Na]+
9	Secoisolariciresinol	31.2	725.2282 [2 M + H]+
10	Schisanhenol	35.1	403.1218 [M + H]+
11	β-sitosterol	35.6	415.2062 [M + H]+
12	Tangeretin	36.4	373.1201 [M + H]+
13	Schisandrin B	37.9	401.1908 [M + H]+

of gene ontology (GO) terms and KEGG (Kyoto Encyclopedia of Genes and Genomes) signaling pathways was carried out using the David 6.8 database [21] (https://david.ncifcrf.gov/). We utilized the OmicShare service (https://www.omicshare.com/) and chose the top 20 in the bubble graphic based on their *P* values.

Construction of a "drug-component target-disease" network: We built a database including active components, targets, diseases, and networks using Cytoscape 3.7.0 to gain a comprehensive knowledge of the interconnections of the herbs in the prescription [20].

Topology analysis: The topology study was performed with Cytoscape 3.7.0's "network analyst" tool, which provides a centrality and a degree. We color-coded and sized each component in descending order of degree [21].

Statistical analysis

The statistical program SPSS 25.0 was used to conduct all analyses on the experimental data, and the findings were presented as the mean ± standard error of the mean (SEM) (SPSS Inc., Chicago, IL, USA). The Levene test was utilized to examine the level of uniformity in the variance. The data underwent a one-way analysis of variance, and a t-test for the least significant difference was used to determine the level of difference between the two groups. A *P*-value less than 0.05 was considered statistically significant.

Results

Identification of 13 compounds in NCPAP

High-performance liquid chromatography combined with electrospray mass spectrometry (HPLC/ESI-MS) in a positive-ion mode was utilized to evaluate the water preparation of the standardized Chinese pharmaceutical materials (**Figure 1**). The findings identified the 13 different compounds based on the retention time. **Table 2** presents the retention times, compounds, and pseudomolecular ion peaks used to determine the 13 compounds. These compounds were positively identified by cross-referencing retention times and MS data with standards (**Table 2**).

Moreover, we utilized ADME parameters to screen and identify active ingredients from various plant species for their potential therapeutic effects on 2019-NCIP. A total of 78 active ingredients were obtained after eliminating repetitive and non-targeted components. Subsequent target prediction analysis revealed 53 potential therapeutic targets for 2019-NCIP distributed among the plant species (**Table 3**). The technology roadmap detailing the screening and identification process is presented in **Figure 2**.

Prediction of shared target genes between the NCPAP formula and 2019-NCIP

In total, 229 targets of NCPAP were acquired from the TCMSP databases. TTD searches

Table 3. Active ingredients and potential therapeutic targets for each plant species related to 2019-NCIP

Plant Species	No. of Active Ingredients Screened	No. of Active Ingredients Related to 2019-NCIP	No. of Potential Therapeutic Targets
Citrus reticulata Blanco	5	5	94
Pinellia ternata (Thunb.) Breit	11	5	167
Poria cocos (Schw. Wolf)	15	6	30
Atractylodes macrocephala Koidz	7	4	23
Amomum villosum Lour	10	9	84
Agastache rugosus (Fisch. et Mey) O. Ktze	9	6	243
Schisandra chinensis (Turcz.) Baill	8	7	30
Glehnia littoralis F. Schmidtex Miq	8	8	250
Adenophora tetraphylla (Thunb.) Fisch	5	3	44

Note: 2019-NCIP, 2019 Novel Coronavirus-Infected Pneumonia.

yielded 103 targets associated with 2019-NCIP, while DisGeNET searches yielded 1832 targets. After removing 48 duplicates, 1887 unique targets remained. The Venn diagram of NCPAP compound targets and 2019-NCIP disease targets was drawn, and 105 common disease targets were obtained for the treatment of the 2019-NCIP (**Figure 3**).

Protein-protein interaction (PPI) network diagram

Cytoscape 3.7.0 was used to integrate the results of a scan of the STRING database, and the findings revealed 1557 associations between 105 disease targets for the therapy of the 2019-NCIP. According to the graph, a node's significance, size, and visibility increased with its volume and degree. In the PPI network diagram, IL-6, TNF, AKT1, etc., were highly related to other proteins and can be used as the key targets of the party (**Figure 4**).

The GO and KEGG enrichment analysis

The GO enrichment function analysis involving BP (biological process), CC (cell structure), and MF (analysis function) was conducted using DAVID database. The results showed that 410 biological processes, 55 cell structures, and 50 molecular functions were involved. The biological processes include the response to the drug, positive regulation of transcription from RNA polymerase II promoter, response to hypoxia, and positive regulation of nitric oxide biosynthetic process. The cell structure mainly focuses on the extracellular space, extracellular region, plasma membrane, extracellular exosome and so on. The molecular functions mainly involve identical protein binding, enzyme binding, protein binding, transcription factor binding, etc. The KEGG enrichment analysis identified 110 signaling pathways and revealed that the TNF signaling pathway, cancer pathways, HIF-1 signaling pathway, and T cell receptor signaling pathway were the most likely routes for NCPAP to exert pharmacological effects in treating 2019-NCIP (**Figure 5**).

Network construction and analysis

According to the screened compounds, predicted targets, and GO/KEGG analysis, the "*drug-component target-disease*" network was constructed (**Figure 6**).

The NCPAP formula reduced hospital stays and boosted lymphocyte counts

Patients with the 2019-NCIP diagnosed in the isolation ward of Shanghai Seventh People's Hospital and Leishenshan Hospital from February to March 2020 were selected. No significant differences were observed between the two groups regarding gender, comorbidities, clinical classification, or age (all $P>0.05$). The number of hospitalization days was reduced ($P<0.05$), and there was a decline tendency in disease progression in the NCPAP group compared to the control group. The blood routine showed that lymphocyte percentage was significantly increased in the NCPAP group as compared to the control group ($P<0.05$) (**Table 4** and **Figure 7**).

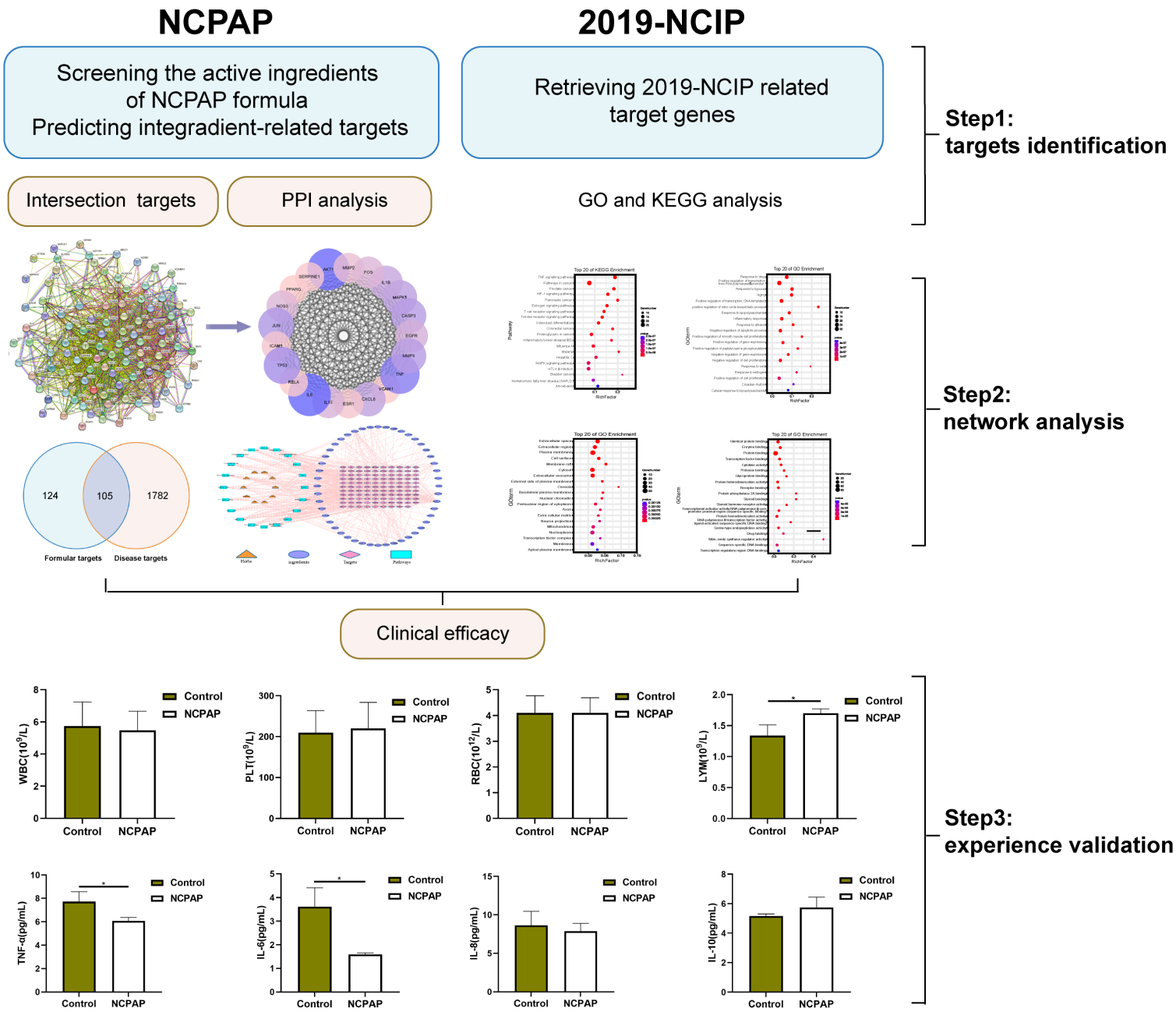
NCPAP
2019-NCIP
Screening the active ingredients of NCPAP formula Predicting integradient-related targets
Retrieving 2019-NCIP related target genes
Step1: targets identification
Intersection targets
PPI analysis
GO and KEGG analysis
124
105
1782
Formular targets
Disease targets
Step2: network analysis
Clinical efficacy
Control
NCPAP
WBC(10^9/L)
PLT(10^9/L)
RBC(10^12/L)
LYM(10^9/L)
TNF-α(pg/mL)
IL-6(pg/mL)
IL-8(pg/mL)
IL-10(pg/mL)
Step3: experience validation

Figure 2. The technology roadmap. The network pharmacology method diagram for displaying the processes involved in the NCPAP formula's action against 2019-NCIP. NCPAP, New Coronavirus Pneumonia Agreement Prescription; 2019-NCIP, 2019 Novel Coronavirus-Infected Pneumonia.

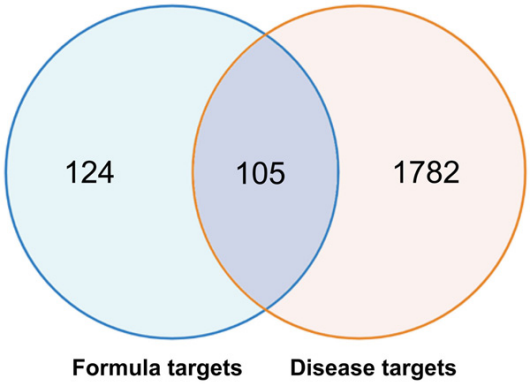

Figure 3. Prediction of common target genes shared by NCPAP formula and 2019-NCIP. 105 disease targets of NCPAP were obtained for the treatment of the 2019-NCIP. NCPAP, New Coronavirus Pneumonia Agreement Prescription; 2019-NCIP, 2019 Novel Coronavirus-Infected Pneumonia.

Verification of the mechanism obtained by the network pharmacology analysis and safety evaluation

In the treatment of 2019-NCIP patients, TNF, HIF-1, and all the main signaling pathways associated with NCPAP were evaluated. The NCPAP intervention significantly reduced the levels of TNF-α and IL-6 ($P<0.05$) without causing significant adverse effects on the liver, kidney or blood coagulation functions of the patients (**Figure 8**).

Discussion

The 2019-NCIP has become a global health concern [18]. Mortality risks differ significantly between severe and mild cases. If the disease is not treated promptly and reasonably, the mortality rate of Severe patients may rise to approximately 15% [1]. Accurate assessment of a patient's condition is crucial for determining treatment timing and plans, ultimately reducing the incidence of critical illness and mortality [3, 22]. No effective treatment was available for 2019-NCIP management at the beginning [23, 24]. Recently, Paxlovid has been recommended for coronavirus infection; however, its high-cost limits accessibility for all patients in China. The NCPAP, comprising Chinese medicinal plants such as Pinellia ternata, Citrus reticulata Blanco, Poria cocos, Atractylodes macrocephala, Amomum villosum, Agastache rugosus, Schisandra chinensis, Adenophora tetraphylla, and Glehnia littoralis, has demonstrated significant clinical efficacy. NCPAP comprised of numerous active ingredients, produces synergistic effects on various levels and targets. The complex mechanisms underlying these pharmacological actions remain elusive. Recently, network pharmacology has been employed to explore the complex chemical interactions in Chinese remedies for treating severe diseases. The information about drug, gene, protein, and disease database was analyzed to construct a "*drug-component target-disease*", revealing how drugs interact with the diseases at the network level. We employed a network pharmacology approach to describe in detail the intricate biochemical process underlying the NCPAP for the management of 2019-NCIP.

Our study revealed that NCPAP significantly reduced hospitalization days, suggesting its effectiveness in treating 2019-NCIP patients. The term "cytokine storm" describes an inflammatory reaction that begins locally but rapidly spreads through the bloodstream, which is developed due to an immune system dysregulation. In the absence of an effective adaptive immune response, the persistence of the virus and the subsequent enhanced innate immune response lead to immune failure and an elevated inflammatory state caused by a cytokine storm [25, 26]. Patients with severe 2019-NCIP have severe lung inflammation and cytokine storms [26, 27]. Therefore, the treatment of cytokine storms is important for the treatment of 2019-NCIP. Excessive and abnormal host cytokine storms are the key factors leading to strong immune pathology and fatal outcomes. A retrospective study published in Lancet found that the levels of IL-6 and TNF-α in the plasma of critically ill patients were significantly increased, suggesting the presence of inflammation-related cytokines in the plasma of 2019-NCIP patients [16]. As inflammatory cytokines, IL-6 and TNF participate in the inflammatory response through autocrine, paracrine, and systemic actions to play a role

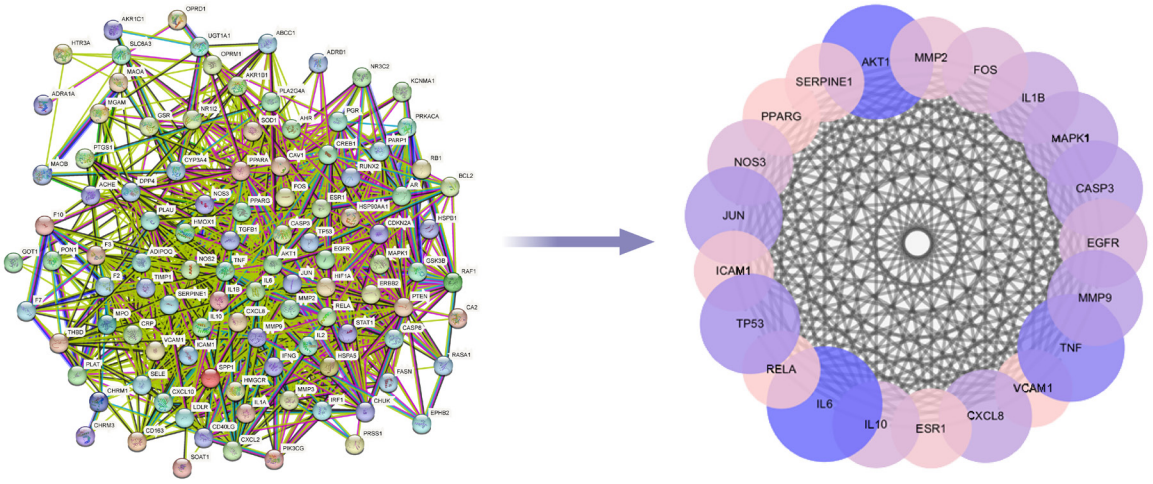

Figure 4. PPI network diagram. A protein-protein interaction (PPI) network was constructed to illuminate the relationships among the shared target genes.

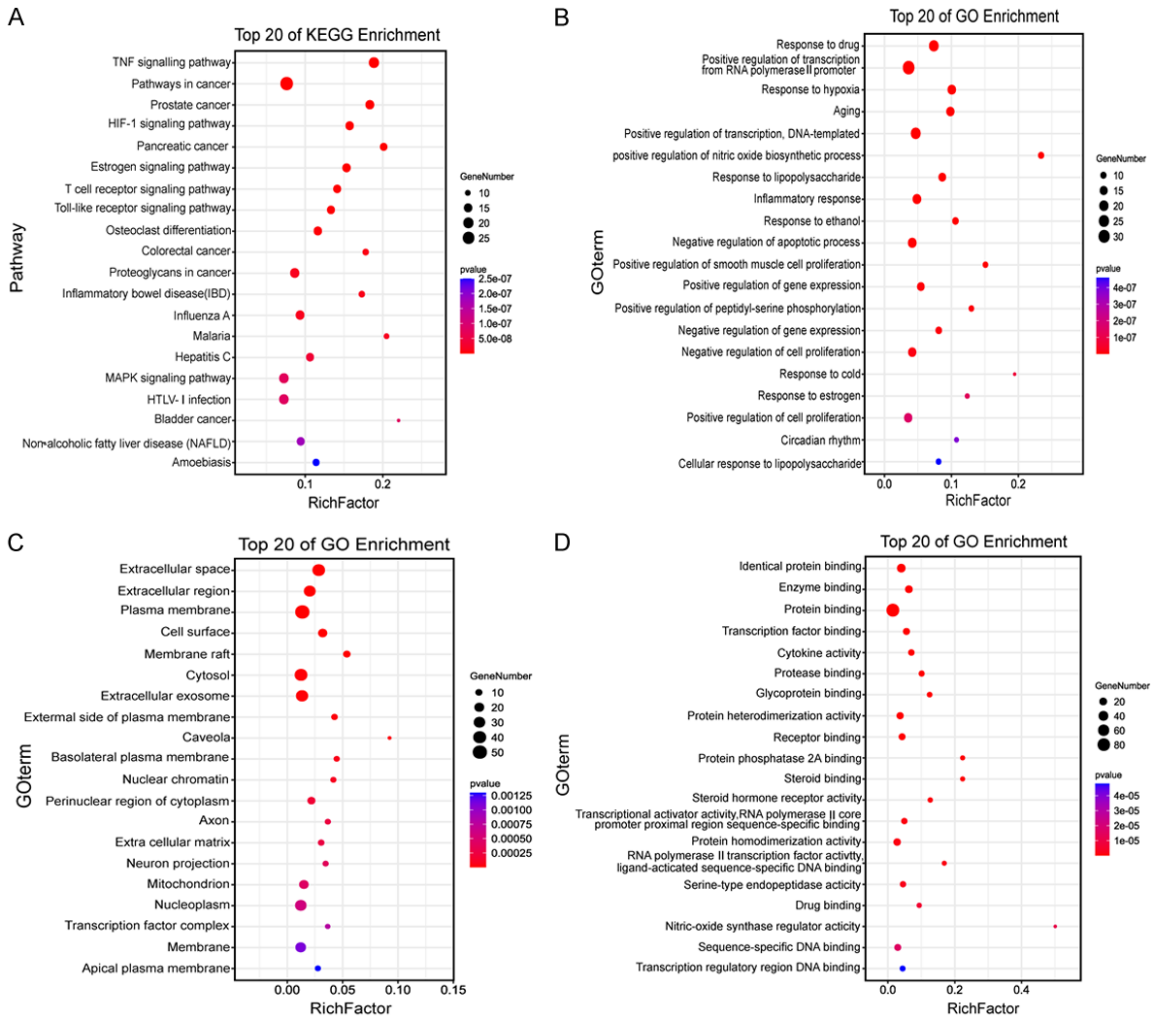

Figure 5. GO and KEGG study of the NCPAP formula's targets against 2019-NCIP. A. Related biological processes; B. Cell structures; C. Part of molecular functions; D. KEGG analysis of the NCPAP targets implicated in the 2019-NCIP activity. NCPAP, New Coronavirus Pneumonia Agreement Prescription; 2019-NCIP, 2019 Novel Coronavirus-Infected Pneumonia; GO, gene ontology; KEGG, Kyoto Encyclopedia of Genes and Genomes.

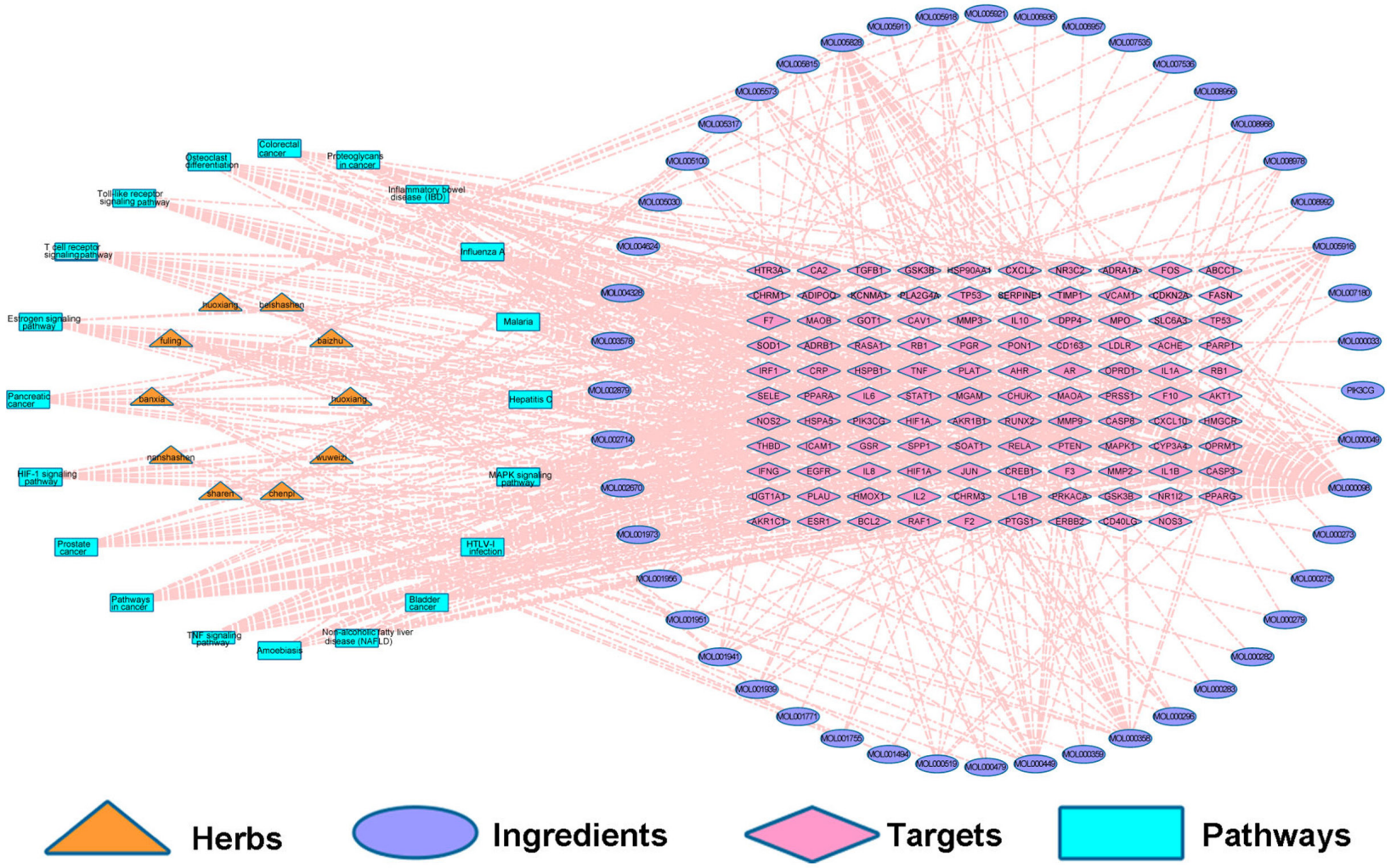

Figure 6. Network construction and analysis. The "drug-compound-target-disease" network was constructed according to the screened compounds, predicted targets, and GO/KEGG analysis. GO, gene ontology; KEGG, Kyoto Encyclopedia of Genes and Genomes.

Table 4. Basic information of patients

Group	Control group	Treatment group	t/X^2	P
N	16	34		
Male:Female (n)	9:7	15:19	X^2=0.642	0.423
Comorbidities (Yes/no)	11/5	20/14	X^2=0.500	0.455
Clinical classification (Severe/Normal)	5/11	6/28	X^2=1.173	0.279
Age (year)	54±18	52±14	t=-0.446	0.661
Disease course (day)	45.12±11.64	38.54±9.92	t=-2.127	0.054
Hospitalization (day)	33.31±6.22	16.61±5.04	t=-4.053	0.007

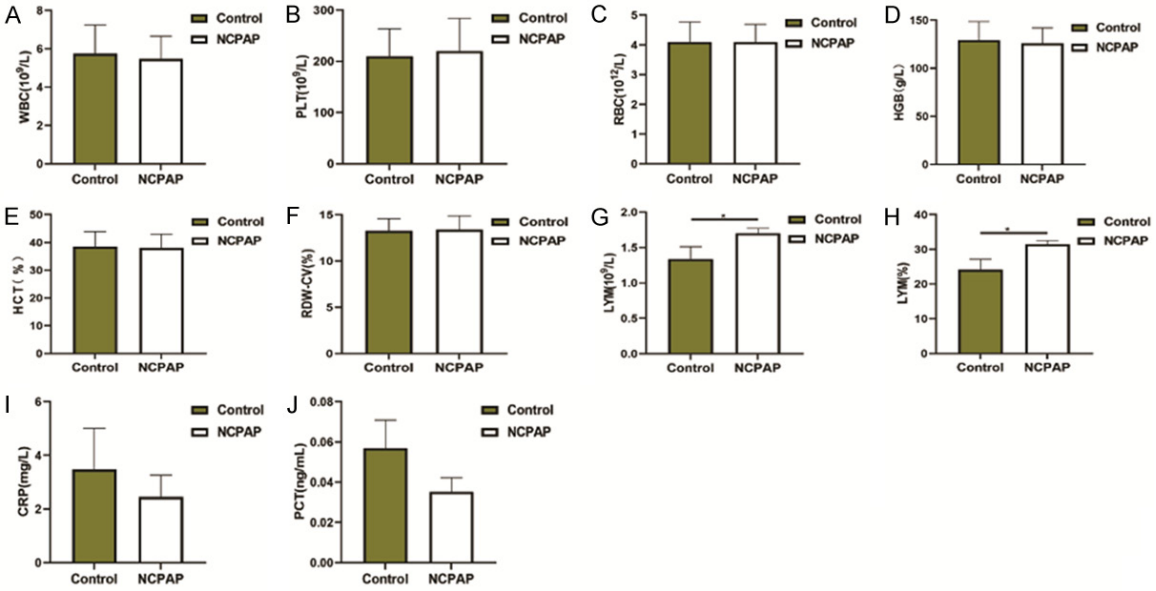

Figure 7. The routine blood tests results of the patients with the 2019-NCIP. A. The white blood cell (WBC) counts of the two groups (P>0.05); B. The platelet (PLT) counts of the two groups (P>0.05); C. The red blood cell (RBC) counts of the two groups (P>0.05); D. The hemoglobin (HGB) of the two groups (P>0.05); E. The hematocrit (HCT) of the two groups (P>0.05); F. The coefficient of variation of red blood cell distribution width (RDW-CV) of the two groups (P>0.05); G. The absolute value of lymphocytes (LYM) of the two groups (*P<0.05); H. The percentage of lymphocytes (LYM%) of the two groups (*P<0.05); I. The c-reactive protein (CRP) of the two groups (P>0.05); J. The procalcitonin (PCT) of the two groups (P>0.05). NCPAP, New Coronavirus Pneumonia Agreement Prescription; 2019-NCIP, 2019 Novel Coronavirus-Infected Pneumonia.

in disease progression. IL-6 is a prototypical proinflammatory factor that triggers cytokine cascades; it controls the early phase of the immune response and hematopoiesis and is crucial to the body's anti-infection immunological response [28]. TNF-α is mainly produced in monocytes, lymphocytes, macrophages, etc. It is one of the vasoactive factors, and it is a cytokine with a variety of biological functions. It mediates and promotes inflammation and the immune response. It constitutes the acute phase and is one of the reactive cytokines [28]. After viral infection, when the TNF-α expression is high and uncontrolled, inflammatory mediators are produced in large quantities, leading to the occurrence of a "cytokine storm". The significant increase in the IL-6 and TNF-α levels in patients after infection with 2019-NCIP indicates a strong inflammatory response in the patient's body. Intervention with NCPAP significantly reduced IL-6 and TNF-α levels, indicating that the inflammation and immune response has been decreased after the intervention.

This study still has a few limitations. First, we only used respiratory tract specimens for diagnosing 2019-NCIP through RT-PCR, and we did not include digestive tract specimens. Second, the 2019-NCIP database is incomplete for the

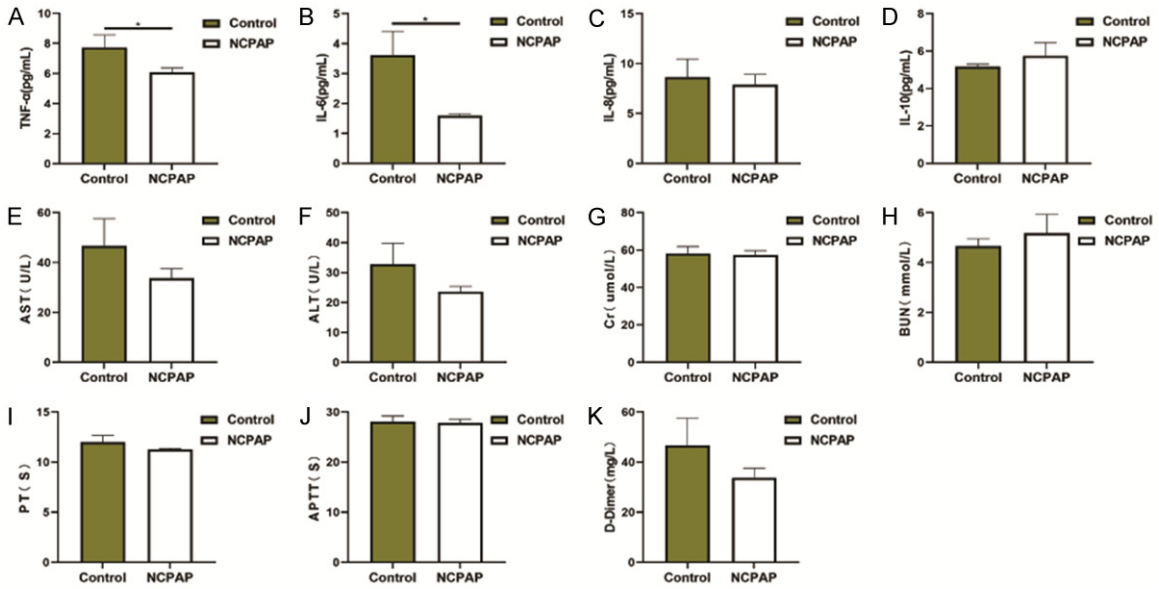

Figure 8. Verification of the mechanism obtained by the network pharmacology analysis and the safety evaluation of NCPAP. A. The comparison of the levels of TNF-α between the two groups (*P<0.05); B. The comparison of the levels of IL-6 between the two groups (*P<0.05); C. The comparison of the levels of IL-8 between the two groups (P>0.05); D. The comparison of the levels of IL-10 between the two groups (P>0.05); E. The comparison of aspartate aminotransferase (AST) between the two groups (P>0.05); F. The comparison of alanine aminotransferase (ALT) between the two groups (P>0.05); G. The comparison of creatinine (Cr) between the two groups (P>0.05); H. The comparison of blood urea nitrogen (BUN) between the two groups (P>0.05); I. The comparison of prothrombin time (PT) between the two groups (P>0.05); J. The comparison of activated partial thromboplastin time (APTT) between the two groups (P>0.05); K. The comparison of D-Dimer between the two groups (P>0.05). NCPAP, New Coronavirus Pneumonia Agreement Prescription; 2019-NCIP, 2019 Novel Coronavirus-Infected Pneumonia.

years 2020-2022. Lastly, we did not conduct follow-up on the patients involved in the study.

Conclusion

Our study shows that NCPAP treatment significantly reduces TNF-α and IL-6 levels, alleviates inflammation, and improves immunity in 2019-NCIP patients. The TNF signaling pathway may be crucial in the efficacy of NCPAP against 2019-NCIP. NCPAP also enhances lymphocyte count and regulates inflammation and immune function. This is consistent with network pharmacology predictions of the mechanism of NCPAP. Although our bioinformatics analysis is theoretical, further research and experimental verification are needed to strengthen the evidence for NCPAP in treating 2019-NCIP.

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Disclosure of conflict of interest

None.

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