Efficacy and Safety of Chinese Medicine in the Treatment of Chikungunya Fever: A Real-World Observational Study

Study Protocol and Statistical Analysis Plan

Official Title:	Efficacy and Safety of Chinese Medicine in the Treatment of Chikungunya Fever: A Real-World Observational Study
Sponsor:	The Second Affiliated Hospital of Guangzhou University of Chinese Medicine (Guangdong Provincial Hospital of Chinese Medicine)
Principal Investigator:	Zhang Zhongde
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Program Summary

Study Name	Efficacy and Safety of Chinese Medicine in the Treatment of Chikungunya Fever: A Real-World Observational Study
Purpose	1. To explore the epidemiological characteristics and Chinese medicine syndrome distribution of Chikungunya fever; 2. To evaluate the effectiveness and safety of Chinese medicine in the treatment of Chikungunya fever.
Overall Design	A prospective observational study design based on real-world data
Sample size	Routine observation: all patients that can be collected during the observation period.
Diagnostic criteria	Suspected or confirmed cases of Chikungunya fever were included based on the diagnosis criteria of Chinese Guidance of the Diagnosis and Treatment for Chikungunya Fever (2017 Edition) published by the Chinese Center for Disease Control and Prevention.
Inclusion and exclusion criteria	**Inclusion Criteria** (1) Meets the suspected or confirmed diagnostic criteria for chikungunya fever; (2) Symptom onset ≤3 days before enrollment; (3) Actual prescribed treatment aligns with the study's group assignment (Chinese medicine, Western medicine, or combined therapy); (4) Sign the informed consent form. **Exclusion criteria** (1) Severe chikungunya fever; (2) Participation in another drug clinical trial within the past 3 months; (3) Any other condition deemed by the investigator as unsuitable for enrollment.
Observation Grouping	**Chinese medicine group:** Patients receive only Chinese medicine treatments, which may include herbal formulas or acupuncture, tailored to their syndrome differentiation (e.g., heat-clearing, detoxifying, or dampness-resolving therapies); **Western medicine group:** Patients receive only standard Western medical care, which may include antipyretics (e.g., acetaminophen), NSAIDs for pain, and supportive treatments (e.g., hydration); **Integrated Therapy Group:** Patients receive both Chinese and Western treatments.
Observation period **Visit**	Maximum observation period: 28 days Visit time: Screening period/baseline: First visit D0, with lab assessments: complete blood count, CRP, and chikungunya nucleic acid tests; Treatment observation period: D1-D5, daily follow-up with clinical and lab assessments,

	D5 or before discharge: complete blood count, CRP, and chikungunya nucleic acid tests; Follow-up after treatment; telephone follow-up on D7, D14, and D28.
General information and medical history	1. Demographic information: gender, age, height, weight, ethnicity, etc.; 2. Medical history: past medical history, current medical history, epidemiological history and allergy history.
Effectiveness evaluation	**1. Joint Pain and Swelling Relief Rate (DAS44 Score)** **2. Change in Rash Severity (Modified EASI Score)** **3. Time to Complete Fever Resolution** 4. Time to Viral RNA Clearance (RT-PCR Negative) 5. Recovery of Laboratory Markers 6. Time to Symptom Recovery 7. Incidence of Complications
Safety evaluation	Incidence of adverse events/adverse reactions.

Flow chart

Stage	**Screening Period / Baseline**	**Treatment observation period**						**Follow-up after treatment (telephone follow-up)**		
Visit point (D, day)	Visit 1 D0	Visit 2 D1	Visit 3 D2	Visit 4 D3	Visit 5 D4	Visit 6 D5	Visit X Before discharge	Visit 7 D7	Visit 8 D14	Visit 9 D28
Informed consent form	×									
Chikungunya RNA test	×					×	×			
Epidemiological data	×									
General information and medical history	×									
Physical examination, vital signs	×			×		×	×			
Body temperature	×	Patient diary card for body temperature record								
Chikungunya Symptom Scale	×	×	×	×	×	×	×	×	×	×
Complication		×	×	×	×	×	×	×	×	×
Severe / critical illness		×	×	×	×	×	×	×	×	×
Laboratory tests	×					×	×			
Patient diary card collection	×					×	×			
Medication records	×	×	×	×	×	×	×			
Adverse Events		×	×	×	×	×	×	×	×	×

Program text

1 Background

Chikungunya fever is an acute infectious disease caused by chikungunya virus (CHIKV), transmitted by Aedes mosquitoes, and characterized by fever, rash, and joint pain. The first outbreak of chikungunya fever was confirmed in Tanzania in 1952, and the virus was isolated in 1956. The disease is mainly prevalent in Africa and Southeast Asia. Since 2004, outbreaks of chikungunya virus have become more frequent and widespread, partly because the virus has adapted to make it easier to spread through Aedes albopictus, and people of all ages are susceptible. In areas where Aedes mosquitoes exist, when Aedes mosquitoes reach a certain density and natural conditions are suitable, if CHIKV is introduced, it may cause an epidemic or outbreak.

On July 8, 2025, an imported case of Chikungunya fever occurred in Foshan, Guangdong. Till July 19th, there have been a total of 1,873 confirmed cases had been reported in Foshan. The rapid spread of the epidemic has brought certain treatment pressures to medical institutions. At present, there are no available vaccines or specific drugs in China, and the clinical treatment plan is mainly symptomatic treatment. In order to effectively respond to the recent surge in Chikungunya fever patients in medical institutions, the Chinese medicine expert team of Guangdong has discussed and formed a Chinese medicine agreement formula plan and applied it in clinical practice. In order to evaluate the effectiveness of this plan, this observational study is planned to be carried out.

2 Purpose

(1) To explore the epidemiological characteristics and Chinese medicine syndrome distribution of Chikungunya fever;

(2) To evaluate the effectiveness and safety of Chinese medicine in the treatment of chikungunya fever.

3 Overall study design

Prospective observational study design based on real-world data.

3.1 Sample size

Routine observation: all patients that can be collected during the observation period.

Maximum observation period: 28 days.

3.2 Visit

Screening period/baseline: First visit D0, complete blood count, CRP, chikungunya nucleic acid and liver and kidney function tests

Treatment observation period: D1-D5, daily follow-up, filling in the CRF, collecting the complete blood count, CRP, and Chikungunya fever nucleic acid results on D5/Discharge day.

Follow-up after treatment; telephone follow-up was conducted on D7, D14, and D28.

4 Case selection

4.1 Chikungunya fever diagnostic criteria

Suspected or confirmed cases of Chikungunya fever were included based on the diagnosis criteria of Chinese Guidance of the Diagnosis and Treatment for Chikungunya Fever (2017 Edition) published by the Chinese Center for Disease Control and Prevention.

4.1.1 Basis of diagnosis

1) Epidemiological data: living in Chikungunya fever endemic areas or having a travel history to epidemic areas within 12 days, and having a history of mosquito bites within 12 days before onset of the disease.

2) Clinical manifestations: Acute onset, with fever as the first symptom, rash appears 2 to 5 days after the disease, and severe pain in multiple joints.

3) Lab assessment: Chikungunya RNA detection from patient blood samples.

4.1.2 Diagnostic criteria

1) Suspected diagnosis: patients with the above-mentioned epidemiological history and clinical manifestations; or patients without epidemiological history but with the above-mentioned typical clinical manifestations.

2) Confirm the diagnosis: Patients with positive result of Chikungunya virus nucleic acid.

4.2 Inclusion Criteria

(1) Meets the suspected or confirmed diagnostic criteria for chikungunya fever;

(2) Symptom onset ≤3 days before enrollment;

(3) Actual prescribed treatment aligns with the study's group assignment (Chinese medicine, Western medicine, or combined therapy);

(4) Sign the informed consent form.

4.3 Exclusion criteria

(1) Severe chikungunya fever;

(2) Participation in another drug clinical trial within the past 3 months;

(3) Other conditions that the researchers consider unsuitable for inclusion .

4.4 Source of Cases

At each research center, all eligible patients will serve as observation subjects for this cohort study. Patients will be assigned to respective observation cohorts based on their actual prescription status, with enrollment for each group continuing until the target number is reached.

4.5 Withdrawal Criteria

Eligible patients will undergo a 28-day follow-up, or until the occurrence of any of the following events:

(1) Allergic reactions or serious adverse events (SAEs) that, in the investigator's judgment, warrant discontinuation of observation;

(2) Severe comorbidities or special pathological changes during the study that, in the investigator's opinion, make continuation inadvisable;

(3) Post-enrollment discovery of significant protocol violations by the patient;;

(4) Patient death, loss to follow-up, or voluntary withdrawal.

4.6 Handling of dropout cases

If a patient drops out, the investigator must document the reason in the eCRF, attempt to contact the patient, complete all feasible assessments, and fill out the end-of-treatment follow-up record. The last medication time should be recorded whenever possible, and follow-up should continue until the study endpoint. If the dropout is due to an adverse event (AE) later judged to be related to the study treatment, this must be recorded in the eCRF.

4.7 Criteria for case exclusion:

(1) Violation of inclusion criteria by the patient;
(2) Absence of any post-enrollment data;
(3) Joint determination by statisticians and investigators prior to database locking;
(4) Other criteria to be determined during data review based on actual circumstances.

4.8 Termination criteria

(1) Patient requests to withdraw from the study.

5 Observation Cohorts and Treatments

5.1 Treatment Allocation by Cohort

- Chinese Medicine Group: Chinese treatments, which may include herbal formulas (e.g., decoctions, granules) or acupuncture, tailored to their syndrome differentiation (e.g., heat-clearing, detoxifying, or dampness-resolving therapies).;

- Western medicine group: Patients receive only standard Western medical care, which may include antipyretics (e.g., acetaminophen), NSAIDs for pain, and supportive treatments (e.g., hydration);
- Integrated therapy group : receive both Chinese and Western treatments.

5.2 Observation period and follow-up

Maximum observation period: 28 days

Visit time:

Screening period/baseline: First visit D0, with lab assessments: complete blood count, CRP, and chikungunya nucleic acid tests;

Treatment observation period: D1-D5, daily follow-up with clinical and lab assessments, D5 or before discharge: complete blood count, CRP, and chikungunya nucleic acid tests;

Follow-up after treatment; telephone follow-up on D7, D14, and D28.

On D0, D5 or before discharge, collect 8 ml of whole blood (anticoagulant tube) for storage.

6 Observation items and evaluation during the study period

6.1 Clinical characteristics

(1) Patient demographic characteristics: gender, age, height, weight, ethnicity, etc.;

(2) Epidemiological data: living in chikungunya fever endemic areas or having a travel history to epidemic areas within 12 days, and having a history of mosquito bites 12 days before onset of illness;

(3) Past medical history and treatment history: past medical history, past medication history, surgical history, allergy history, history of adverse drug reactions, etc.;

(4) Concomitant diseases and medications: disease name, treatment drugs, purpose of medication, method of medication, time of medication, etc.

(5) The current illness situation: onset time, highest axillary temperature within 72 hours before consultation, and diagnosis and treatment.

6.2 Effectiveness Observation Items

(1) Joint swelling and pain relief rate: After the first dose, DAS 44 score was < 1.6, and decreased by more than 0.6 before and after treatment.

Disease Activity Score of 44 Joints Based on CRP (DAS 44 -CRP): It assesses the degree of joint swelling and pain. The calculation formula is as follows:

$$\text{DAS44 - CRP} = 0.54 \times \sqrt{RAI} + 0.065 \times \sqrt{SJC44} + 0.17 \times \ln(CRP + 1) + 0.072 \times GH + 0.45$$

44 assessed joints: including both hand metacarpophalangeal joints (MCP1-5), proximal interphalangeal joints (PIP2-5), thumb interphalangeal joints, both wrists, both elbows, both shoulders and both knees, as shown in the figure below:

Swollen joint count (SJC): The number of swollen joints among 44 joints was recorded .

CRP level: Record laboratory test results in mg/dL (CRP).

VAS score: Patients mark the disease activity on a 0-100 mm visual analog scale based on their own feelings, with 0 representing no activity and 100 representing extreme activity.

2) Disease activity grading of joint swelling and pain (based on DAS 44 score):

Low activity (DAS 44 < 1.6)

Moderate activity (1.6≤DAS44 < 2.4)

High activity (2.4 ≤DAS 44 ≤ 3.7)

Severe activity (DAS 44 > 3.7)

3) Evaluation of treatment response at the time of disease remission (based on reduction in DAS 4 4 score):

Good response (DAS 44 >1.2)

General response (0.6<DAS 44 ≤1.2)

No response (DAS 44 ≤ 0.6)

(2) Rash scores compared with baseline: Changes in rash scores compared with baseline on days 3 and 5 after medication ;

Affected area : The maximum affected area of each body part is 100% , and the affected area is locally scored according to the following table, 0-6.

Affected area	0	1-9%	10 - 29%	30 - 49%	50 - 69%	70 - 89%	90 - 100%
cal scoring	0	1	2	3	4	5	6

Clinical symptom score: The severity of each symptom is rated from 0 to 3:

Severity of illness	none	light	middle	Heavy
score	0	1	2	3
✓ The average severity of the affected area. Half-grade points can be recorded between each symptom grade, e.g. 2.5.				

Erythema: 0 = none; 1 = mild (barely visible, pink); 2 = moderate (clearly visible, dark red); 3 = severe (deep red or fiery red)

Papules: 0 = none; 1 = mild (barely noticeable raised areas); 2 = moderate (clearly visible raised areas but not prominent); 3 = severe (prominent raised areas)

Rash Scoring Scale:

Body Parts	erythema (0-3)	Edema / pimples (0-3)	Local scoring (0-6)	Body Parts Score

neck	(+	+	)	
trunk	(+	+	)	
Upper limbs	(+	+	)	
Lower limbs	(+	+	)	

VAS (Visual Analog Scale) pruritus score

No itching at all 0--1--2--3--4--5--6--7--8--9 — 10 Worst itching point

(3) Complete fever reduction time: Complete fever reduction is defined as the body temperature dropping to 37.2℃ or below after the first dose and maintained for 24 hours or more;

(4) Nucleic acid negative conversion time;

(5) Time for single symptom to disappear;

(6) Overall symptom disappearance time;

(7) Time for white blood cell count to return to normal: the time from the first dose to the first observation of white blood cell count returning to normal;

(8) Time for platelet count to return to normal: the time from the first dose to the first observation of platelet count returning to normal;

(9) Incidence of Chikungunya-related complications: Proportion of patients with recorded AEs for Chikungunya-related complications (shock, DIC, encephalitis, acute liver injury, acute kidney injury, myocarditis, pericarditis, pneumonia, respiratory failure, rhabdomyolysis, acute pancreatitis) after the start of treatment.

6.3 Safety Information

(1) Laboratory tests such as complete blood count/liver and kidney function , chest CT/X-ray, electrocardiogram: collected based on actual clinical situations ;

(2) Incidence of adverse events/adverse reactions.

7 Safety observation

7.1 definition

(1) Adverse Event, AE)

In drug clinical trials, any adverse medical event that occurs after a subject receives a test drug is called an adverse event. Adverse events can manifest as diseases , symptoms, signs or laboratory abnormalities, and adverse events do not necessarily have a causal relationship with the investigational drug.

Events that meet the definition of adverse events include:

Exacerbation of a pre-existing chronic or intermittent illness, including an increase in frequency and/or severity of illness;

New diseases detected or diagnosed after administration of the investigational drug, even though they may have existed before the start of the study;

signs, symptoms, or clinical sequelae of suspected drug interactions;

Signs, symptoms, or clinical sequelae of suspected overdose of the investigational drug or co-administered drug (overdose itself is not reported as an adverse event/serious adverse event).

(2) Serious adverse events Adverse Event, SAE)

It refers to adverse medical events such as death, life-threatening, permanent or severe disability or loss of function, the need for hospitalization or prolonged hospitalization, congenital abnormalities or birth defects, etc. that occur after the subject receives the investigational drug.

(3) Important adverse events

Refers to any clinical adverse event or significant abnormality in laboratory tests other than SAE that leads to targeted medical measures (such as discontinuation of drug, dose reduction and/or symptomatic treatment).

(4) Adverse drug reactions drug reaction, ADR ）

In drug clinical trials, harmful or unexpected reactions to the human body caused by the test drugs are called adverse reactions.

(5) Suspected and unexpected serious adverse reactions

Refers to suspected and unexpected serious adverse reactions whose nature and severity of clinical manifestations are beyond the existing information in the investigator's brochure of the investigational drug, the instructions for use of the marketed drug, or the summary of product characteristics.

7.2 Recording of adverse events

The name, severity, time of occurrence, duration, treatment measures, and process of treatment of adverse events, important adverse events, and adverse reactions that occur from the beginning of the treatment period to the last visit should be recorded in the research medical record, and their relevance to the trial drug should be evaluated based on comprehensive consideration of comorbidities and concomitant medications, and recorded in detail by the researcher.

When adverse events or adverse reactions are found, researchers can determine whether to discontinue observation based on the patient's condition. Cases in which medication is discontinued due to adverse events or adverse reactions should be followed up and the results recorded in detail.

If any abnormality is found in the safety test indicators such as vital signs, physical examination, complete blood count, urine routine, liver function, kidney function, electrolytes, etc. during or after treatment, the test must be repeated at an appropriate time and comprehensively analyzed with the subjects' onset and treatment to determine whether it is related to the test drug.

7.3 Adverse event severity grading

According to the Common Adverse Events Evaluation Criteria (Common Terminology Criteria for Adverse Events 5.0, CTCAE) evaluates the severity of adverse events that occur during the trial, and the severity of adverse events is divided into 5 levels:

Grade 1: Mild; asymptomatic or mild; clinical or diagnostic observations only; no treatment required.

Grade 2: Moderate; minor, local or non-invasive treatment required; limitation of instrumental activities of daily living commensurate with age (instrumental activities of daily living include cooking, shopping for clothes, using the telephone, managing finances, etc.).

Grade 3: severe or medically significant but not immediately life-threatening; leading to hospitalization or prolonged hospitalization; causing disability; limiting self-care activities of daily living (self-care activities of daily living include bathing, dressing and undressing, eating, washing, taking medicine, etc., and not being bedridden).

Grade 4: Life-threatening, requiring urgent medical attention.

Grade 5: Death related to AE.

Records: Adverse events must be recorded in the original documents and the eCRF adverse event page, and the investigator shall determine the relevance of the adverse event to the trial drug and sign.

7.4 Determination of causal relationship between adverse events and drugs

Technical Guidelines for the Evaluation of the Correlation of Adverse Events in Clinical Trials of Drugs (Trial Implementation) [14] issued by the CDE , a comprehensive evaluation of the correlation between individual adverse events in clinical trials and the test drugs was conducted based on five evaluation points (whether there is a reasonable time relationship, whether it is consistent with the known drug mechanism, characteristics or known adverse reactions of the drug, destimulation results, restimulation results, and whether other reasonable reasons can be used to explain it). According to different situations, the judgment results are divided into five categories: related, very likely related, possibly related, possibly unrelated, and unrelated. Among them, "related, very likely related, and possibly related" are judged as adverse reactions and included in the calculation of adverse reaction incidence. See Table 1 and Table 2 for details.

Table 1 Classification and basis for the results of adverse event correlation determination in drug clinical trials

Five-point method	Judgment basis	dichotomy
related	• Have a reasonable time relationship • Comply with known drug mechanisms of action, properties, or known adverse reactions • To stimulate positive • Re-stimulation positive • No other reasonable explanation	Related
Probably related	• Have a reasonable time relationship • Comply with known drug mechanisms of action, properties, or known adverse reactions • To stimulate positive • Lack of positive evidence for rechallenge • No other reasonable explanation	
May be related	• There is a reasonable time relationship ; • lack of positive evidence of rechallenge ; • Symptoms include any of the following: ① It is consistent with the known drug mechanism, characteristics or known adverse reactions , and the positive result is not stimulated, but it can also be explained by other reasonable reasons; ② The condition complies with known drug mechanisms, characteristics or known adverse reactions, lacks positive evidence of de-stimulation, and has no other reasonable explanation ; ③ It does not conform to the known drug mechanism of action, characteristics or known adverse reactions , and there is no other reasonable reason to explain the positive result. ④ It does not conform to the known mechanism of action, characteristics or known adverse reactions, lacks positive evidence of de-stimulation, and has no other reasonable explanation;	

Probably not relevant	• Time relations cannot be ruled out • Lack of positive evidence for dechallenge • Lack of positive evidence for rechallenge • Symptoms include any of the following: ① Although it is consistent with the known mechanism of action, characteristics or known adverse reactions, it can be explained by other more reasonable reasons; ② It does not conform to the known mechanism of action, characteristics or known adverse reactions, and can be explained by other reasonable reasons;	Not relevant
Unrelated	• No reasonable time relationship • Does not conform to known drug mechanisms of action, properties, or known adverse reactions • Lack of positive evidence for dechallenge • Lack of positive evidence for rechallenge • Other reasonable reasons can be used to explain	

Special Notes:

1. The correlation between adverse events and drugs shall be evaluated by relevant personnel with medical expertise.
2. Table 5 may not cover all situations in actual work. If it cannot completely correspond to the judgment basis in Table 6, you can refer to the professional judgment logic of the correlation between adverse events and drugs in the table to make the most reasonable judgment result.
3. When more information and evidence on the correlation between adverse events and drugs is collected during the progress of clinical trials, the previous correlation judgment results can be modified as needed, but sufficient reasons should be provided.
4. Lack of positive evidence for de-challenge includes the following situations: the de-challenge result is negative; de-challenge has not yet been performed; de-challenge is not applicable; Lack of positive evidence for re-challenge includes the following situations: the re-challenge result is negative; re-challenge has not yet been performed; re-challenge is not applicable.
5. In order to facilitate the work, the main contents of Table 1 can be simplified to Table 2. The special instructions for using Table 2 are the same as those for Table 1.

Table 2 Adverse events and drug causal relationship judgment table

Judgment result Judgment basis	Definitely related	Probably related	May be related		Probably not relevant		Unrelated
Is there a reasonable time relationship?	+	+	+		±		-
Does it conform to known mechanisms of action, properties	+	+	+	-	+	-	-

or known adverse reactions?									
To stimulate the results	+	+	+	- /?	+	-/?	- /?		- /?
Re-stimulation results	+	- /?	—/?				- /?		- /?
Are there other reasonable reasons to explain this?	-	-	+	-	-	-	++	+	+

Note:

+ indicates affirmative, or positive result;

- Indicates a negative result, or a situation where no result has been obtained yet;

± indicates that a temporal relationship cannot be ruled out;

++ means that there are other "more" reasonable reasons to explain it;

-/? indicates that the dechallenge/rechallenge result is negative, or dechallenge/rechallenge has not been performed, or dechallenge/rechallenge is not applicable.

7.5 Management of adverse events

If adverse events occur to the subjects during the trial, they shall be handled according to the following procedures:

After discovering that a subject has an adverse event, the researcher needs to inquire in detail about the subject's symptoms and signs at the time. If necessary, symptomatic treatment can be given first. The research physician will preliminarily assess the severity of the adverse event and its relevance to the trial drug, and provide further treatment advice:

① General adverse events: The outcome of the event can be closely and continuously observed or corresponding symptomatic treatment can be performed;

② Important adverse events: The research physician should inform the principal investigator promptly and take measures such as suspending treatment, adjusting drug dosage and providing targeted treatment according to the subject's condition. If necessary, the principal investigator may decide whether to urgently unblind the subject (if applicable).

③ Serious adverse events: Handle and report as the following “serious adverse events”.

④ The research physician will treat the subject according to the condition of the patient. If the subject's injury exceeds the treatment capabilities of the research department, the relevant departments will be consulted and assist in handling the situation.

7.6 Management and reporting of serious adverse events

7.6.1 Handling of serious adverse events

(1) When an SAE is considered, the attending physician shall notify the principal investigator or other responsible physician to be present. If the condition is serious, the project leader should be notified while emergency treatment is being given. If necessary, the trial drug should be discontinued immediately.

(2) If it is judged to be an SAE, appropriate treatment or rescue measures will be taken immediately according to the clinical manifestations and the standards of clinical rescue treatment of each specialty. If it is severe toxicity caused by drug overdose, the researchers will decide to give rescue measures such as accelerating drug excretion, maintain the stability of the patient's vital signs as much as possible, and perform ECG monitoring when necessary. If necessary, the relevant departments can be consulted and assist in handling the matter.

(3) When an out-of-hospital subject is diagnosed with an SAE and is unable to come for treatment, it is recommended that the subject return to the hospital or go to a local hospital for treatment in a timely manner, and immediately inform the project leader to obtain further treatment advice; if receiving treatment at a local hospital, contact the attending doctor to understand the specific situation and provide treatment advice. If necessary, bring the unblinding envelope and go to the local hospital for treatment or return to this hospital for treatment.

7.6.2 Reporting of Serious Adverse Events

The reporting of SAEs should follow the SAE reporting procedures of the Chinese regulatory authorities or independent ethics committees. After an SAE occurs, the researcher must immediately report all SAEs in writing to the sponsor via email, and then provide a detailed, written follow-up report in a timely manner. For reports involving death events, the researcher should provide the sponsor and the ethics committee with the information they need, such as autopsy reports and final medical reports.

Sponsors should report SAEs to the relevant regulatory authorities in accordance with the requirements of regulatory authorities and local regulations, and during the implementation of the study, they will strictly abide by the "Standards and Procedures for Rapid Reporting of Safety Data during Drug Clinical Trials" to rapidly report suspected unexpected serious adverse reactions (SUSAR) to regulatory authorities. After receiving safety-related information from any source, the sponsor should immediately analyze and evaluate it, including the severity, relevance to the trial drug, and whether it is an expected event. Sponsors should rapidly report SUSAR to all researchers, clinical trial institutions, and ethics committees participating in clinical trials; sponsors should report SUSAR to drug supervision and administration departments and health and health authorities. After receiving the relevant safety information of the clinical trial provided by the sponsor, the researcher should sign for and read it in a timely manner, consider the treatment of the subjects, whether to make corresponding adjustments, communicate with the subjects as soon as possible when necessary, and report the SUSAR provided by the sponsor to the ethics committee.

The sponsor determines whether to report the event to the regulatory agency on an expedited basis based on the expected nature of the SAE and the results of the assessment of the relevance of the SAE to the investigational drug. The sponsor unblinds the case before the expedited report. SAEs for which the investigator has not assessed the causal relationship will be considered to be related to the investigational product. Therefore, the investigator should always record the most likely cause of the SAE in his or her judgment to avoid unnecessary unblinding of the case. After a more precise cause is

determined, the causal relationship of the investigational drug in subsequent follow-up reports of the trial can be revised.

During the implementation of the study, the "Standards and Procedures for Rapid Reporting of Safety Data during Drug Clinical Trials" will be strictly followed and the requirements of China's GCP and relevant regulations will be obeyed.

7.7 Adverse events that were not relieved during follow-up

Adverse events that have not yet resolved at the study endpoint or have not been resolved must be followed up until one of the following events occurs:

(1) Incident mitigation;

(2) The event is stable;

(3) if the baseline value is acceptable, the event returns to the baseline level;

(4) When it is impossible to obtain additional information (the subject or the person who takes care of him/her refuses to provide additional information, and the subject is lost to follow-up despite follow-up efforts (at least 3 telephone contacts)).

Follow-up methods can be hospitalization, outpatient clinic, home visit, telephone, correspondence, etc.

8 Data Management

8.1 Data Management Plan

This study uses an electronic data collection system (EDC) for data management. The data management plan is written by the project data manager based on the clinical trial protocol and registration observation form, describing the data management process, personnel division of labor, timetable and file archiving, etc., to ensure the consistency, effectiveness and standardization of data management work, promote communication and exchanges between various clinical research departments, and establish a high-quality database for statistical analysis. During the clinical trial, if the user needs to modify the data management plan, the data manager should be notified to revise and update it in real time, publish it to all relevant responsible personnel, and sign and confirm it.

8.2 Database Management

The design and testing of the database shall be completed by the data management personnel based on the finalized clinical trial plan and the registration observation form. The data management personnel shall also mark the registration observation form and formulate variable descriptions. Logical verification settings shall be performed for fields with logical relationships according to the data verification plan.

After the database is established, it should be tested and verified, fully tested by relevant personnel, and entered after review and confirmation by the head of the data management department.

8.3 Data collection and recording

The registration observation form was designed according to the research protocol, and data collection was completed by clinicians.

The registration observation form is used as the original record and is imported into the EDC system, the database of this project.

8.4 Data Monitoring

The data verification plan is formulated by the data management department, medical technology department and project manager after fully understanding the design of the plan and discussing all the verification points of the plan's data management content. If modifications are required during the implementation process, the data manager will revise and update the version, and the above departments will sign and confirm again.

8.5 Data cleaning

Questions during the data verification process were generated in two forms: questions automatically generated by the online system and questions raised manually. The researchers or their authorized CRCs answered the questions online until the data were cleaned.

8.6 Data review

Before the data review meeting, samples will be drawn for quality control according to the ratio specified in the relevant SOP of the Data Management Department for the queries manually closed by the data administrator. If the quality control error rate is within the specified range, then the quality control is passed; if the quality control error rate is not within the specified range, samples will be drawn again for quality control according to the specified ratio. If it still fails, 100% quality control will be performed on all manually closed queries.

After the data cleaning is completed, the sponsor will be notified to hold a data review meeting. The sponsor, principal investigator, data management personnel, and statistician will conduct a final review of unresolved data issues and discuss the data set division according to the data review report.

After all the data are confirmed, the principal researcher will electronically sign the electronic data.

8.7 Locking and unlocking the database

8.7.1 Database Lock

Data locking should meet the following prerequisites:

(1) All data have been collected and data processing such as data entry has been completed.

(2) All data queries have been answered and the database has been modified based on the data queries.

(3) If coding is required, ensure that the review of the medical data coding list has been completed to ensure the integrity and consistency of medical coding.

(4) The final data logic and consistency check has been completed.

(5) The data quality review has been completed and meets the requirements for statistical analysis.

(6) All trial related documentation was updated and saved.

After the data manager confirms that the trial meets the above conditions, he/she will submit a written lock application, which will be signed and dated by trial-related personnel (data manager, biostatistician, project manager, sponsor, principal investigator). Once the written approval document for database locking is obtained, the database will be locked.

8.7.2 Database Unlock

In principle, locked data will not be changed unless there is very clear evidence that there are errors in the data and that they significantly affect the analysis results. Unlocking should provide written modification instructions from researchers and sponsors, detailing the content and reasons for the modifications. The version before unlocking is retained. Written signatures of senior management (such as the head of clinical implementation, the head of biostatistics, and the head of data management) are required. All revised data have audit traces. The database is locked again following the same process as the first time the database was locked.

9 Quality Control of the Study

Clinical research quality control runs through the entire process from protocol design, project implementation, data management, statistical analysis to report writing. Possible quality problems in the research are controlled in advance. The possible quality problems in this study involve multiple links of the project. The main quality control plan is as follows:

9.1 Bias Control

9.1.1 Selectivity bias control

Mainly in the design and implementation stage. The target population of the program design is accurate, and the hospital and doctor selection ensure the representativeness of the sample cases; once the doctor starts to register, there is no selection of registered cases, etc.

9.1.2 Information Bias Control

Information bias refers to the systematic errors caused by human factors in the integrity, authenticity, logic and measurement of patient data when collecting patient data. This study mainly controlled the possible information bias through the following methods.

(1) Establish detailed data collection methods and strict quality control methods.

(2) Collect information in a timely manner and ensure its accuracy.

(3) Collect data strictly in accordance with the unified SOP to ensure consistency in data collection by all researchers.

(4) By verifying the original data, the authenticity and integrity of the data can be guaranteed.

(5) Regularly check missing values, abnormal values, logical relationships between indicators, etc. through electronic data logic verification and network verification, and send verification reports to the corresponding research centers for inspection, confirmation and modification.

9.1.3 Control of attrition bias

Loss-to-follow-up bias is inevitable in prospective studies. We should try to minimize the impact of patient loss-to-follow-up on the research results. We should strengthen physician education and increase physicians' attention to research. We should strengthen the responsibilities of research assistants and establish corresponding systems to minimize the number of lost-to-follow-up.

9.1.4 Control of confounding bias

The final data were controlled using statistical methods such as stratified analysis, standardization, multivariate analysis, and propensity score.

9.2 Program design and sample representativeness assurance

To ensure that the cases can represent the patients using Jingfang Heji, a multi-stage sampling method was used to select an estimated 10 hospitals from different regions and levels as case collection hospitals according to the regional distribution of clinical sales of Jingfang Heji and the hospital grade

distribution, as well as the sales share of hospitals of different grades. Based on the previous cases of using Jingfang Heji, a quota was used to allocate the sample size.

9.3 Monitoring and Auditing

9.3.1 Audit

At a ratio of 20%, we randomly select research centers to conduct audits to check problems encountered by researchers in data collection and by CRAs in the monitoring process. All centers are required to conduct self-inspections and make corrective actions based on the content and focus of the problems to ensure the quality of project research.

9.3.2 Monitoring

Formulate a strict monitoring plan, and CRA will regularly conduct routine monitoring of the research center to check the project progress and the implementation of the researcher's plan; the authenticity of the data and the traceability of the original data, etc. And promptly require the researcher to track and correct the problems in the monitoring report.

9.4 Project implementation SOP and training

(1) Formulate the SOP for this study and require that the project be implemented strictly in accordance with the corresponding SOP.

(2) Establish a strict training system and provide project training to all project participants (project managers, CRAs, researchers, and researcher assistants, etc.).

10 Statistical analysis

SAS version 9.4 or above was used for data statistical analysis.

All statistical tests were two-sided, and $P < 0.05$ was considered to be statistically significant.

The description of quantitative indicators will calculate the number of cases (N), mean (Mean), standard deviation (SD), median (Median), minimum (Min) and maximum (Max). When comparing between groups, t-test or analysis of variance was used. The description of categorical indicators uses the number of cases (N) and percentage (%) of each category. When comparing between groups, chi-square test or Fisher's exact probability method was used. The Kaplan-Meier (KM) test was used to estimate the survival function and calculate the median time for time-to-event variables. The survival curves between different groups were compared based on the Log-Rank test, and the corresponding P value was calculated.

11 Ethical requirements

Since this study is an observational study, it does not interfere with normal clinical medical services, nor does it interfere with patients' medical treatment and medication behavior. Therefore, in addition to passing the ethics review at the clinical lead unit, other research centers can conduct ethics filing as needed, or conduct ethics review of this project, but the focus of the review should be the feasibility of this project in the center. After the review and approval, if major modifications to the trial protocol are required, it should be submitted to the ethics committee of the clinical lead unit for review and approval again.

Only researchers and monitors involved in clinical research may have access to the personal medical records of the research subjects, and they will include confidentiality in the signed researcher statement or confidentiality commitment. The ethics committee and the drug supervision and administration department have the right to review the clinical research records. When processing data, the data will be anonymous, omitting information that can identify the individual identity of the research subjects. The medical records of the research subjects are kept in the data archives of the drug clinical research institution with strict security and confidentiality measures.

12 Data Retention

All paper and electronic materials (investigator's manual, research plan, case observation form, monitoring and audit record report, etc.) of this study will be kept by Guangdong Provincial Hospital of Chinese Medicine. All materials will be kept for at least 5 years after the termination of the study.

Efficacy and Safety of Chinese Medicine in the Treatment of Chikungunya Fever: A Real-World Observational Study

Informed consent form and informed disclosure page (research introduction)

Information Leaflet for Informed Consent

Dear Patient:

Your doctor has confirmed that you may have or have Chikungunya.

We will invite you to participate in an observational study on the treatment of Chikungunya fever with Chinese medicine . This study will observe the TCM syndrome manifestations and clinical characteristics of Chikungunya fever, and evaluate the effectiveness and safety of Chinese medicine in the treatment of Chikungunya fever.

Before you decide whether to participate in this study, please read the following content as carefully as possible. It can help you understand the study and why it is being conducted, the procedures and duration of the study, and the possible benefits, risks, and discomforts that may arise from participating in the study. If you wish, you can ask your doctor to explain it, or you can discuss it with your family members and friends to help you make a decision.

Research

1. Research background and purpose

Chikungunya fever is an acute infectious disease caused by chikungunya virus (CHIKV), transmitted by Aedes mosquitoes, and characterized by fever, rash, and joint pain. The first outbreak of chikungunya fever was confirmed in Tanzania in 1952, and the virus was isolated in 1956. The disease is mainly prevalent in Africa and Southeast Asia. Since 2004, outbreaks of chikungunya virus have become more frequent and widespread, partly because the virus has adapted to make it easier to spread through Aedes albopictus, and people of all ages are susceptible. In areas where Aedes mosquitoes exist, when Aedes mosquitoes reach a certain density and natural conditions are suitable, if CHIKV is introduced, it may cause an epidemic or outbreak.

On July 8, 2025, an imported case of Chikungunya fever occurred in Foshan, Guangdong . Since then, the epidemic has spread rapidly, with more than a thousand confirmed cases in a short period of time, which has brought certain treatment pressure to medical institutions. At present, there are no available vaccines or specific drugs in China, and the clinical treatment plan is mainly symptomatic treatment. In order to effectively respond to the recent surge in Chikungunya fever patients in medical institutions, a team of traditional Chinese medicine experts in Guangdong Province has formed a traditional Chinese medicine agreement formula plan after discussion and applied it in clinical practice. In order to evaluate the effectiveness of this plan, this observational study is planned to be carried out.

Under the guidance of the expert group of Guangdong Provincial Hospital of Traditional Chinese Medicine, this study will collect all patients that can be collected during the observation period in medical institutions in Foshan and other places where epidemics may occur in the future .

The principal investigator of this study is Professor Zhang Zhongde from Guangzhou University of Chinese Medicine and Guangdong Provincial Hospital of Chinese Medicine. As a member of the expert advisory group for the National Joint Prevention and Control Mechanism, Professor Zhang has participated in multiple TCM treatment guidance programs for emerging infectious diseases. Through these practical experiences, he has accumulated substantial expertise in applying traditional Chinese medicine to treat novel and sudden infectious disease outbreaks.

The Ethics Committee of Guangdong Provincial Hospital of Traditional Chinese Medicine has reviewed that this study complies with the principles of the Declaration of Helsinki and meets the requirements of medical ethics.

2. Who is suitable to participate in the study?

1. You can participate in this study if you meet all of the following conditions:

(1) Meets the suspected or confirmed diagnostic criteria for chikungunya fever;

(2) Symptom onset ≤3 days before enrollment；

(3) Actual prescribed treatment aligns with the study's group assignment (Chinese medicine, Western medicine, or combined therapy);

(4) Sign the informed consent form.

2. However, if you have any of the following conditions, you should not participate in this study:

(1) Severe Chikungunya fever;

(2) Participation in another drug clinical trial within the past 3 months;

(3) Any other condition deemed by the investigator as unsuitable for enrollment.

Your study doctor will evaluate you and tell you whether you are suitable for participating in this study. After obtaining your informed consent and signature, we will officially start the study.

3. What to do if you participate in the study

(1) Before you are selected for the study, the doctor will ask questions, record your medical history, and evaluate you to determine whether you can participate in the study.

(2) If you participate in the research, the research will be conducted as follows:

At the beginning of the study, the doctor will record your demographic information (gender, date of birth, etc.), medical history, symptoms and signs during the visit, test results, and treatment plan;

During your hospitalization, your treatment plan will be entirely determined by your attending physician and will not be affected by this study. You may receive Chinese medicine treatment, or only conventional symptomatic treatment of Western medicine, or both. At the same time, the doctor will follow up on you as follows:

On the 1st to 5th day after enrollment, the doctor will follow up with you every day to inquire about and record your medication status and symptoms and signs;

On the 7th day after enrollment, the doctor will make the first follow-up call to you to inquire about and record your medication status and symptoms and signs;

On the 14th day after enrollment, the doctor will make a second telephone follow-up call to inquire about and record your medication status and symptoms and signs;

On the 28th day after enrollment, the doctor will make a third follow-up call to you to inquire about and record your medication use and symptoms and signs.

Before you are discharged from the hospital, your doctor will follow up with you to ask and record your medication use and symptoms and signs.

If your actual hospitalization time does not meet the above time points, the doctor will conduct a final follow-up visit with you before you are discharged according to the actual time.

(3) In addition to routine diagnosis and treatment, we will collect additional blood samples from you for future medical and scientific research. The samples will be collected when you are enrolled, 5 days after enrollment, or before discharge. The number of samplings will be determined based on your actual hospitalization time, and 8 ml of venous blood will be collected each time.

Your samples will be permanently stored in the Guangdong Provincial Hospital of Traditional Chinese Medicine's biological sample bank until they are used up. When samples are stored or sent for testing, they will be numbered instead of personal information to ensure that your privacy will not be leaked. Your samples will only be used by researchers at the Guangdong Provincial Hospital of Traditional Chinese Medicine for scientific research related to Chikungunya fever and will not be used for commercial purposes. Before researchers use your remaining samples for each study, they must obtain approval from the Ethics Committee.

(4) The study may be terminated if:

Patient withdrawal and termination of the study include the following situations: patients experience serious adverse events that are considered to be related to the study procedures.

The complete termination of research includes the following aspects: the researcher requests to terminate the research due to funding or management reasons and the government department requires to terminate the research .

4. Possible benefits of participating in the study

You will not benefit directly, but research using your identifiable information and identifiable biospecimens may help us understand health and disease, lead to improved medical care, make diagnoses and treatments safer or more effective, and advance new scientific knowledge.

5. Possible adverse reactions, risks, discomfort and inconvenience of participating in the study

This study is a non-interventional observational clinical study. In principle, it will not cause harm to your body. If you are harmed due to participating in the study, you will be given corresponding financial compensation in accordance with the law.

During the study, you will need to cooperate in completing some symptom information reporting. This information is collected during daily ward rounds and will help the doctor understand changes in your condition without causing you any additional trouble or inconvenience.

6. Relevant Fees

You do not need to pay any research-related expenses, such as blood collection fees and sample retention fees, to participate in the study. This study does not incur any additional costs for you. This study is only conducted during your hospital stay, and there will be no additional transportation costs.

This study is a non-interventional observational clinical study and will not cause any harm to your body in principle. If you are harmed as a result of participating in the study, you will be given appropriate financial compensation in accordance with legal provisions.

7. Is personal information kept confidential?

Your medical records (research medical records/CRFs, laboratory test reports, etc.) will be kept intact in the hospital, and the doctor will record the test results in your outpatient medical record. The researcher, the sponsor's representative (if applicable) and the ethics committee will be allowed to access your medical records. Any public report on the results of this study will not disclose your personal information. We will make every effort to protect the privacy of your personal medical information within the scope permitted by law.

8. How to get more information?

You may ask any questions about this study at any time. Your doctor or researcher will give you his or her phone number so he or she can answer your questions.

If you have any complaints about your participation in the study, please contact the Ethics Committee Office of Guangdong Provincial Hospital of Traditional Chinese Medicine (Tel: 020-81887233-35943).

Your doctor will promptly notify you if any important new information becomes available during the study that may affect your willingness to continue participating in the study.

9. Participants can voluntarily choose to participate in the study or withdraw from the study

Whether to participate in the study is entirely voluntary. You can refuse to participate in this study or withdraw from this study at any time during the study, which will not affect the relationship between you and the doctor, nor will it affect the loss of other interests in your medical treatment.

Your doctor or the investigator may terminate your participation in this study at any time if it is in your best interest.

If you do not take part in this study, or if you leave the study, it will not affect your treatment options. You do not have to take part in this study to get your disease treated.

If you withdraw from the study for any reason, you may be asked about your treatment. You may also be asked to undergo laboratory tests and a physical examination if your doctor thinks it is necessary. This is very important to protect your health.

10. What to do now?

Before you decide to take part in the study, please ask your doctor as many questions as possible until you fully understand the study.

It is up to you to decide whether to take part in this study. You can discuss this with your family or friends before making a decision.

Thank you for reading the above information. If you decide to participate in this study, please tell your doctor or research assistant, who will arrange all matters related to the study for you.

Please keep this information.

Informed consent form and consent signature page
Signature Leaflet for Informed Consent

Project Title: Efficacy and Safety of Chinese Medicine in the Treatment of Chikungunya Fever: A Real-World Observational Study
Sponsor/Project issuing unit: None
Ethics review approval committee: Ethics Committee of Guangdong Provincial Hospital of Traditional Chinese Medicine

Statement of consent

I have read the above description of this study and have had the opportunity to discuss and ask questions about this study with the physician. All of my questions have been answered to my satisfaction.

I understand the possible risks and benefits of participating in this study. I know that participating in the study is voluntary, I confirm that I have had enough time to think about it, and I understand that:

● I can always ask the doctor for more information.

● I can withdraw from this study at any time without being discriminated against or retaliated against, and my medical treatment and rights will not be affected.

I also understand that if I withdraw from this study midway, especially if I withdraw from the study due to medication reasons, it will be very beneficial to me and the entire study if I tell the doctor about the changes in my condition and complete the corresponding physical examination and physical and chemical examinations.

If I need to take any other medication due to my illness, I will seek the doctor's advice in advance or tell the doctor truthfully afterwards.

I agree that the drug regulatory authorities, ethics committee or sponsor's representative may review my research data.

I will be provided with a signed and dated copy of the informed consent form.

Finally, I decided to agree to participate in this study.

Subject's Signature:_____date:_____Daily contact number:_____

Guardian/Authorized Person Signature:_____ Relationship with the subject: _____
(Note: If the subject cannot sign the informed consent due to lack of/limited behavioral capacity, his/her guardian or authorized person shall sign it)

Contact Number:_____ date:_____

I consent□ or deny□ the use of my medical records and biological specimens for research other than this study.

Study Participant Signature:_____ date:_____

Guardian/Authorized Person Signature:____ Relationship with participants: _____ date:_____
(Note: If the participant is unable to sign the informed consent due to incapacity, etc., his/her guardian or authorized person shall sign it)

I confirm that the details of the trial, including their rights and possible benefits and risks, have been explained to the research participants and a copy of the signed informed consent form has been given to them.

Researcher’s Signature:_____ date:_____

Researcher's work phone number:_____ Phone number:_____

Contact number of the Ethics Committee Office of Guangdong Provincial Hospital of Traditional Chinese Medicine: 020-81887233-35943