

STUDY PROTOCOL

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# Early identification and severity prediction of acute respiratory infection (ESAR): a study protocol for a randomized controlled trial

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## Abstract

**Background:** The outbreak of SARS-CoV-2 at the end of 2019 sounded the alarm for early inspection on acute respiratory infection (ARI). However, diagnosis pathway of ARI has still not reached a consensus and its impact on prognosis needs to be further explored.

**Methods:** ESAR is a multicenter, open-label, randomized controlled, non-inferiority clinical trial on evaluating the diagnosis performance and its impact on prognosis of ARI between mNGS and multiplex PCR. Enrolled patients will be divided into two groups with a ratio of 1:1. Group I will be directly tested by mNGS. Group II will firstly receive multiplex PCR, then mNGS in patients with severe infection if multiplex PCR is negative or inconsistent with clinical manifestations. All patients will be followed up every 7 days for 28 days. The primary endpoint is time to initiate targeted treatment. Secondary endpoints include incidence of significant events (oxygen inhalation, mechanical ventilation, etc.), clinical remission rate, and hospitalization length. A total of 440 participants will be enrolled in both groups.

**Discussion:** ESAR compares the efficacy of different diagnostic strategies and their impact on treatment outcomes in ARI, which is of great significance to make precise diagnosis, balance clinical resources and demands, and ultimately optimize clinical diagnosis pathways and treatment strategies.

*Trial registration* Clinicaltrial.gov, NCT04955756, Registered on July 9th 2021.

**Keywords:** Acute respiratory infection, mNGS, Multiplex PCR, Randomized controlled trials

## Background

Acute respiratory infection (ARI) is one of the major diseases that threatens human health worldwide, with high morbidity, high severity and high medical costs [1]. COVID-19 [2] in 2019 and the H1N1 influenza in 2009 [3] have imposed enormous healthcare burden and economic loss worldwide. According to World Health Organization (WHO), lower respiratory tract infections (LRTI) are the fourth leading cause of death globally in 2019 [4]. Respiratory tract infections can be caused by a wide spectrum of pathogens including

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bacteria, viruses, fungi, mycoplasma, chlamydia, etc., with similar clinical manifestations but completely different treatment. Besides, colonization and complex infection make it more difficult to make accurate diagnosis. Once diagnosed or treated improperly, severe pneumonia will develop, which is associated with high mortality and various complications [5].

Early and precise diagnosis for respiratory pathogens is of great significance for making appropriate treatment strategies, saving healthcare resources and reducing unnecessary use of antibiotics. The gold standard is culture or isolation from respiratory specimen [6], which is time-consuming along with low positivity. Nowadays, emerging technologies, such as multiplex polymerase chain reaction (PCR) and metagenomic next-generation sequencing (mNGS), have become more and more widely used in the rapid detection of respiratory tract infections. Multiplex PCR is relatively cost-saving and can report results within hours [7], which has become the most attractive test method and currently the most commonly used method in clinical laboratory [8]. Filmarray respiratory panel (FA-RP) is featured as automated sample preparation, nucleic acid extraction and nested multiplex PCR detection, which can detect more than 20 pathogenic microorganisms in one test, including 17 respiratory viruses and 3 atypical pathogens [7, 9]. QIAstat-Dx RP is another full-automated multiplex real-time PCR test for identifying common respiratory viral and bacterial pathogens within 1 h [10]. Although diagnostic performance of multiplex PCR has been proved to be satisfying [11], detection spectrum of microorganisms is still limited, especially in rare or emerging pathogens [12]. mNGS can identify pathogens without subjective bias within 48 h [13, 14], but is more time-consuming, more expensive and requires well-equipped laboratory to perform.

Although a variety of diagnosis tools have been applied clinically, there still lacks consensus on clinical diagnosis pathway for ARI. The impact of multiplex PCR and mNGS on prognosis of ARI is still unclear. Therefore, based on mNGS and multiplex PCR detection, the study proposes a multicenter, open, randomized controlled clinical trial to compare the efficacy of different diagnostic strategies and its impact on prognosis for ARI, which ultimately aims to optimize the diagnostic pathways and treatment strategies.

## Methods

### Study design

The study is a multicenter, open-label, randomized controlled, non-inferiority clinical trial recruiting patients with respiratory tract infections who meet inclusion and exclusion criteria (Table 1). Enrolled patients were divided into two groups in a 1:1 ratio [15]: Group I will be detected by mNGS for pathogens. Group II will first receive respiratory multiplex PCR, then mNGS in severe pneumonia (Table 2) if multiplex PCR results are negative or inconsistent with the clinical conditions. Disease condition and treatment status will be followed up every 7 days until reaching predefined outcomes within 28 days. If a participant with mild symptoms become severe during follow-up, respiratory samples should be collected again for mNGS detection. The detailed research process is shown in Fig. 1.

### Ethical and confidential considerations

The study protocol and informed consent forms have been approved by the Ethical committee of Huashan Hospital affiliated to Fudan University (protocol ID: KY2021-450). The investigator will objectively and comprehensively introduce the purpose, procedure, potential benefits and risks of the study to the subjects and

**Table 1** Inclusion and exclusion criteria

Inclusion criteria	<ul style="list-style-type: none"> <li>• Aged from 18 to 80 years old</li> <li>• Unlimited gender</li> <li>• Newly developed cough, expectoration, purulent sputum, or exacerbation of original respiratory diseases within 14 days</li> <li>• Meet at least one of the following items:               <ol style="list-style-type: none"> <li>(1) Fever (&gt; 38 °C)</li> <li>(2) Consolidation signs and/or wet rales on chest physical examination</li> <li>(3) Leucocyte &gt; 10*10<sup>9</sup>/l or &lt; 4*10<sup>9</sup>/l</li> <li>(4) Patchy infiltration or interstitial changes on chest radiology</li> </ol> </li> </ul>
Exclusion criteria	<ul style="list-style-type: none"> <li>• Highly-suspected of or confirmed with noninfectious lung diseases (tumor, autoimmune diseases, etc.)</li> <li>• The pathogen has been identified</li> <li>• Insufficient respiratory tract or blood specimen</li> <li>• Unable or refusing to cooperate due to physical or psychological factors</li> <li>• Participating in other clinical studies</li> <li>• Investigators or clinicians considers unsafe for the subject to participate</li> </ul>
Exit criteria	<ul style="list-style-type: none"> <li>• Participants request to withdraw from the interview</li> </ul>

**Table 2** ATS/IDSA criteria for severe CAP

**Minor criteria**

- Respiratory rate  $\geq 30$  breaths/min
- PaO<sub>2</sub>/FiO<sub>2</sub> ratio  $\leq 250$
- Multilobar infiltration
- Confusion/disorientation
- Uremia (BUN  $\geq 20$  mg/dL)
- Leukopenia (white blood cell count  $< 4 \times 10^9/L$ )
- Thrombocytopenia (platelet count  $< 100 \times 10^9/L$ )
- Hypothermia (core temperature  $< 36$  °C)
- Hypotension requiring aggressive fluid resuscitation

**Major criteria**

- Invasive mechanical ventilation
- Septic shock with the need for vasopressors

Respiratory rate, a need for noninvasive ventilation can substitute for a respiratory rate  $> 30$   
 PaO<sub>2</sub>/FiO<sub>2</sub>, arterial oxygen pressure/fraction of inspired oxygen; BUN, blood urea nitrogen; Leukopenia, as a result of infection alone

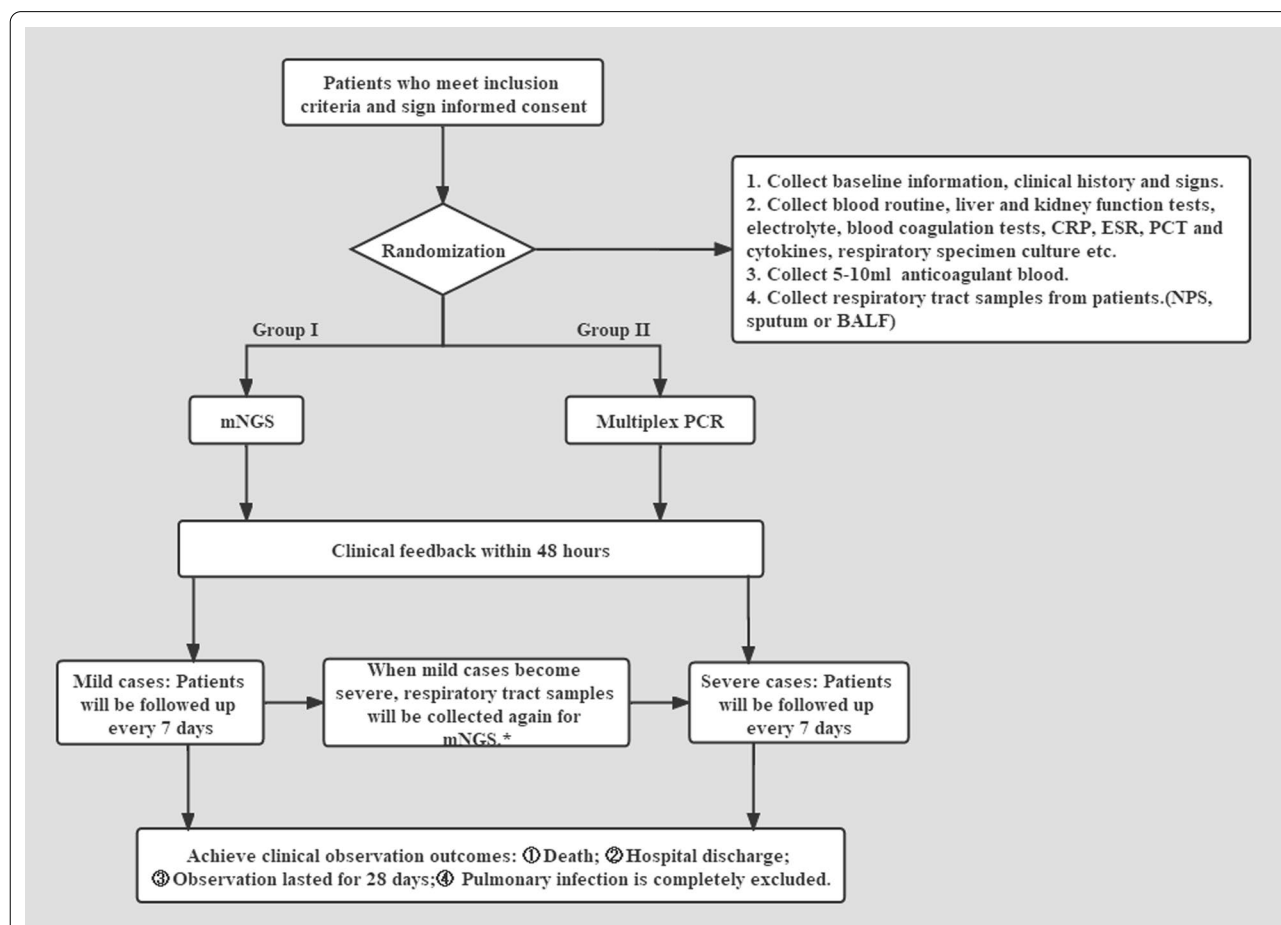
their legal representatives, and obtain informed consent through interview before registration. All patients will provide written informed consent.

**Site selection**

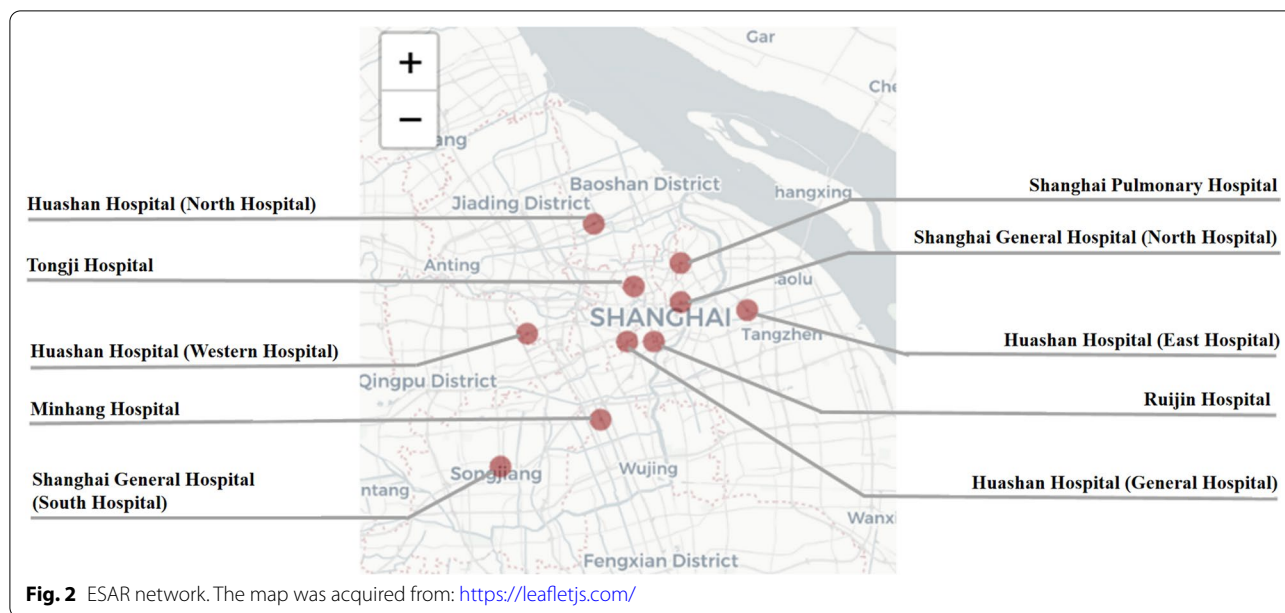
This trial is led by the Huashan Hospital affiliated to Fudan University and the recruiting-cooperative units were distributed over 5 points in Shanghai: Huashan Hospital, Ruijin Hospital, Tongji Hospital, Shanghai Pulmonary Hospital, Shanghai General Hospital, Minhang Hospital and their branches. Project leader will conduct the quality control every six months. ESAR network for early identification of acute respiratory infection in Shanghai is shown in Fig. 2 [16].

**Study flowchart and clinical evaluation of enrolled participants**

The following information will be collected at enrollment: baseline information, clinical history, physical examination, routine laboratory examinations (including



**Fig. 1** Study outline. \*Group I will be detected by mNGS for pathogens. Group II will first receive respiratory multiplex PCR, then mNGS in severe pneumonia if multiplex PCR results are negative or inconsistent with the clinical conditions. CRP C-reactive protein test, PCT procalcitonin, ESR erythrocyte sedimentation rate, NPS nasopharynx swab, BALF bronchoalveolar lavage fluid, mNGS metagenomic next-generation sequencing



blood routine, liver and kidney function test, electrolytes, blood coagulation tests, C reaction protein, erythrocyte sedimentation rate, procalcitonin, cytokines, etc.) and respiratory specimen culture. According to the above test results, the researchers evaluated the severity of pneumonia at the time of enrollment. 5–10ml EDTA anticoagulation and respiratory samples will be collected at enrollment (BALF  $\geq$  3 ml, or sputum  $\geq$  2 ml or more, or 2 nasopharyngeal swabs). After quality control, subjects will be randomly assigned to two groups according to the ratio of 1:1. Group I will be directly tested by mNGS, and group II will first receive multiplex PCR and subsequently mNGS according to multiplex PCR results and the severity of pneumonia. If mild pneumonia progressed to severity in subsequent follow-up, additional mNGS will be performed. Randomization was performed through an online central randomization system stratified by study site. Patients in both groups will be followed up for 28 days and a final diagnosis will be made by experienced physicians.

**Diagnostic methods**

In this study, we plan to detect pathogens in group I by mNGS, and in group II by respiratory multiplex PCR. Reports will be returned within 48 hours. The later will include 16 respiratory pathogens and 3 atypical respiratory pathogens, including coronavirus, influenza A virus, influenza B virus, mycoplasma pneumoniae, adenovirus, respiratory syncytial virus parainfluenza virus (I, II, III, IV), human metapneumovirus, haemophilus influenzae, klebsiella pneumoniae, staphylococcus aureus. mNGS assays can simultaneously cover a wide spectrum of

pathogens in a single sequence, including known pathogens and emerging unknown microorganisms.

**Duration of follow-up**

All participants will be followed up every 7 days for disease status and treatment conditions since enrollment, including symptoms, signs, routine laboratory examinations, along with respiratory specimen culture. Besides, SOFA score will be evaluated every 7 days.

**Sample size calculation**

PASS 11 software was applied to calculate the sample size. Assuming hazard ratio (HR) = 1.50,  $\alpha$  = 0.05,  $\beta$  = 0.10, and a 10% rate of participants in Group II receiving mNGS additionally, each group requires 200 subjects. Considering a 10% rate of lost to follow-up, each group needs  $200 \times 1.1 = 220$  subjects; therefore, the total sample size of the two groups combined is calculated to be 440 subjects.

**Data collection and quality management**

A series of quality assurance methods has been established and will be applied before and during ESAR project. All relevant personnel, including clinicians, laboratory personnel, and project management personnel will receive unified training on theoretical perspective and practical procedure.

On enrollment, clinicians are required to be familiar with the inclusion and exclusion criteria. Every case needs to be included continuously without selection or omission, and will be reviewed by the project leader from Huashan Hospital before official enrollment. On

data collection, clinicians will be trained with filling case report form (CRF) before official enrollment. Questions in CRF should be filled face-to-face by clinicians during enrollment and should not be hinted, induced or recalled afterwards. All relevant forms should be properly kept in a suitable place by a designated person to prevent burning, tearing, smearing, etc.

To ensure that each center conducts research in strict accordance with the plan, and the data is authentic and credible, quality control is carried out by the research team of Huashan Hospital Affiliated to Fudan University every six months. Supervisors will be assigned by the Huashan Hospital affiliated to Fudan University.

#### **Adverse event management**

Potential adverse events and their management will be evaluated during follow up. In this study, 8ml peripheral blood and respiratory tract samples will be collected along with the routine clinical examination, which will not cause additional operations, burdens and costs to the subjects. Potential adverse events include localized pain, bruising or needle infections.

If a patient has any psychological discomfort in the process, the clinician should provide timely comfort and notify the relevant personnel to evaluate and record within 2 h. If ineffective, a psychologist will be arranged to conduct psychological assessment and consultation within 12 h, which will be recorded by designated personnel. The subjects can receive corresponding compensation and treatment when an unexpected injury or loss related to the research come out. Any adverse events in this study will be recorded in detail by a dedicated person. Clinicians must carefully inquire and trace any adverse events during the trial, strictly record them in the CRF, and finally report them to the ethics committee and relevant departments.

#### **Assessment and analysis of outcomes**

The primary and secondary outcomes will be compared between group I and group II. Primary outcomes are time to start targeted antibiotic therapy. Secondary outcomes include incidence of clinically significant events (oxygen inhalation, ICU admission, tracheal intubation, tracheostomy, death, etc.), clinical remission rate (Meet all the following criteria for at least 24 h: (1) Heart rate < 100 bpm; (2) Blood pressure > 90 mmHg; (3) Body temperature < 38 °C; (4) Respiratory rate < 24/min; (5) Oxygen saturation at room temperature > 90%), hospitalization length within 28-day follow up.

SPSS 20 and Prism 8.0 will be applied for statistical analysis. Firstly, whether the enrolled cases met inclusion and exclusion criteria, and whether the actual number of enrolled, excluded, and dropped cases in each center

are counted will be reviewed at the end of the study. Second, demographics characteristics, baseline information, follow-up status and outcomes will be compared between two groups. Statistical methods including *t* test or Mann–Whitney test, Pearson's chi-square test or Fisher test, cox method, Kaplan–Meier survival curve, log-rank method, logistic regression models will be applied in the form of two-tailed test.  $P < 0.05$  was considered statistically significant.

#### **Confidentiality**

Participants' personal information is strictly restricted to outcome evaluation of study regimen. Paper documents containing participants information will be saved in a dedicated office in cooperative hospitals. Digital documents will be kept in password-protected files on website. The study documents can only be accessed by authorized personnel.

#### **Discussion**

Exploring the efficiency of different diagnosis strategies is of great significance for optimizing ARI early management and prognosis. Since multiplex PCR was first applied by Chamberlian et al. to screen missing locus on Duchenne muscular dystrophy in 1988 [17], it has been successfully applied in many fields. The sensitivity and specificity of respiratory multiplex PCR has been proved to be higher than 90% [18]. Shengchen et al. [19] reported that FA-RP can shorten the length of stay and reduce antibiotic administration in hospitalized adults with lower respiratory tract infection. Similarly, our previous study [20] found that 53.8% received antiviral therapy and 69.6% received antibiotics adjustment in multiplex PCR group, while 12.7% and 5.1% in traditional examination group, indicating a positive effect on outcomes. Simultaneously, FA-RP can also lower the risk of infectious disease transmission [20]. However, Brendish et al. [21] argued that multiplex PCR was not associated with a reduction in the overall duration of antibiotic use in hospitalized adults with acute respiratory illness. Besides, multiplex PCR cannot detect rare or unknown respiratory pathogens.

Compared with traditional culture, mNGS improves sensitivity and shortens time to identify various pathogens [22]. The sensitivity and specificity in BALF samples were 88.89% and 14.86% [23], and 34% of candidate pathogens can be detected by mNGS [24] to provide more accurate diagnostic information. Furthermore, it can screen rare pathogens [25] such as COVID-19 [26] and *Nocardia* [27], but subject to missing detection, expensive equipment and high personnel demand. There is few studied reporting impact of mNGS on ARI prognosis. Zhang et al. [28] performed repeated mNGS

testing in nine patients with suspected central nervous system infection (CNS) and found that mNGS semi-quantitative values can be used to dynamically monitor pathogen load and disease progression. Hongxia Duan et al. [29] suggested that 28-day mortality in mNGS-positive group was higher (9.0% vs 0%,  $P = 0.049$ ), but the average survival time (176.64 vs 150.96 days,  $P = 0.425$ ) was not statistically different, indicating that positive results were correlated with worse prognosis. However, these studies did not analyze the influence of antibiotics, which might lead to the lower detection rate by traditional methods.

Although mNGS can cover most pathogens, it requires longer time than respiratory multiplex PCR. Moreover, the expensive cost makes mNGS not a panacea for any respiratory infection, but a technology that can be used in specific situations. Therefore, based on previous research, ESAR is designed to compare a combination of respiratory multiplex PCR and mNGS with direct mNGS detection to optimize diagnosis pathway in ARI.

This study has several limitations. Firstly, although multiplex PCR and mNGS will be performed in the central laboratory, there existed certain heterogeneity for traditional respiratory specimen detection method between hospitals. In addition, all the methods above still cannot directly distinguish pathogenic organism and colonization. The reports will be interpreted by experienced clinicians and controversial cases will be discussed and diagnosed by experts.

#### Abbreviations

WHO: World Health Organization; LRTI: Lower respiratory tract infection; qPCR: Real-time quantitative PCR; RT-PCR: Reverse transcription PCR; dPCR: Digital PCR; ddPCR: Digital droplet PCR; FA-RP: Filmarray respiratory detection system; NPS: Nasopharyngeal swab; TB: Tuberculosis; MTB: Mycobacterium tuberculosis; RIF: Rifampicin; POCT: Real-time checkout; ARIs: Acute respiratory infections; mNGS: Next generation metagenomic sequencing; PCR: Polymerase chain reaction; CRF: Case report form; COVID-19: Novel coronavirus pneumonia; H1N1: Swine-origin influenza A virus; BALF: Bronchoalveolar fluid; HR: Hazard ratio; CNS: Nervous system infection.

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#### Author contributions

Conception or design of the work: JW, JWA, WHZ. Study revision: YHZ, JYS, TY, SL, MJL, KX, YYZ, JFX, RLW, EQM, ZQZ, CQ, NJ, H CZ, JXG, YZ, SW, XL, LJJ, LZ, FQZ, SSJ. Drafting the article: GMY, HYW, JWA. All authors read and approved the final manuscript.

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#### Availability of data and materials

Not applicable.

#### Declarations

##### Ethics approval and consent to participate

The study protocol and informed consent forms have been approved by the Huashan Hospital Ethical committee (protocol ID: KY2021-450). All patients will provide written informed consent.

##### Consent for publication

Not applicable.

##### Competing interests

The authors declare that they have no competing interests.

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