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Retrospective clinical and microbiologic analysis of metagenomic next-generation sequencing in the microbiological diagnosis of cutaneous infectious granulomas

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Abstract

Background Cutaneous infectious granulomas (CIG) are localized and chronic skin infection caused by a variety of pathogens such as protozoans, bacteria, worms, viruses and fungi. The diagnosis of CIG is difficult because microbiological examination shows low sensitivity and the histomorphological findings of CIG caused by different pathogens are commonly difficult to be distinguished.

Objective The objective of this study is to explore the application of mNGS in tissue sample testing for CIG cases, and to compare mNGS with traditional microbiological methods by evaluating sensitivity and specificity.

Methods We conducted a retrospective study at the Department of Dermatology of Sun Yat-sen Memorial Hospital, Sun Yat-sen University from January 1st, 2020, to May 31st, 2024. Specimens from CIG patients with a clinical presentation of cutaneous infection that was supported by histological examination were retrospectively enrolled. Specimens were delivered to be tested for microbiological examinations and mNGS.

Results Our data show that mNGS detected Non-tuberculosis mycobacteria, Mycobacterium tuberculosis, fungi and bacteria in CIG. Compared to culture, mNGS showed a higher positive rate (80.77% vs. 57.7%) with high sensitivity rate (100%) and negative predictive value (100%). In addition, mNGS can detect more pathogens in one sample and can be used to detect variable samples including the samples of paraffin-embedded tissue with shorter detective time. Of the 21 patients who showed clinical improvement within a 30-day follow-up, eighteen had their treatments adjusted, including fifteen who continued treatment based on the results of mNGS.

Conclusions mNGS could provide a potentially rapid and effective alternative detection method for diagnosis of cutaneous infectious granulomas and mNGS results may affect the clinical prognosis resulting from enabling the patients to initiate timely treatment.

Keywords Metagenomic next-generation sequencing, Cutaneous infectious granulomas, Non-tuberculosis mycobacteria, Tissue, Paraffin-embedded tissue

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Introduction

Granulomas are organized aggregates of immune cells with a wide variety of stimuli, like infectious agents and foreign bodies. And the development of granulomas shows that original phagocytes' efforts to clear the particles from the initial contact were unsuccessful. Generally, the granulomas could be divided into non-infectious and infectious granulomas [1]. According to the various etiologies of cutaneous infectious granulomas (CIG), the common presence of a granulomatous inflammatory infiltration in the dermis may vary [2]. So, most granulomatous inflammation can only be diagnosed when a pathologist observes with specific stains for pathogen under the microscope. However, it has been calculated that near to 36% of granulomas do not have a specific etiology [3], so that new methods are needed to be used for helping in identifying the cause.

Metagenomic next-generation sequencing (mNGS) test, as an emerged promising diagnostic technology, has been used for detecting pathogen on a variety of samples [4–7]. However, scarce reports have been paid to the implementation of mNGS in clinical practice for patients with CIG. Our study aims to assess the applicability of mNGS by testing tissue samples from CIG cases, and evaluating its sensitivity and specificity to compare with conventional microbiological methods.

Methods

Patients and methods

From January 1st, 2020, to May 31st, 2024, a retrospective study was conducted at the Department of Dermatology, Sun Yat-sen Memorial Hospital, Sun Yat-sen University. The study screened 89 patients with clinical presentations of cutaneous infections, histologically confirmed, ultimately investigating 26 cases (Fig. 1). The exclusion criteria were as follows: secondary infection due to rupture of Sebaceous cyst/Epidermal cyst rupture: 43 patients; No culture/NGS: 20 patients (Figure S1). Samples were collected from the infected skin tissues of all patients, encompassing pus, secretions and tissue swabs. Specimens were delivered to be tested for microbiological examinations, including bacterial culture, mycological tests (KOH examination and fungal culture), and mNGS. Microbiological examinations were done following strict procedures. At least two technicians, who were in charge of discriminating between contaminants and isolates, as well as two clinicians, who were in charge of separating infections from colonizations, evaluated the results. Records included details about the patient's demographics, medical history, complications, precipitating factors, infection parts and symptoms, lab tests, antibiotic therapies, and the outcome of those treatments.

mNGS sequencing and analysis

The entire pathogen detection pipeline and sequencing process was completed in the Cellular & Molecular Diagnostics Center of Sun Yat-sen memorial hospital, Sun Yat-sen University, Guangzhou, China. The mNGS methods made reference to earlier studies. The procedures were, in brief, as follows.

DNA extraction

The DNA is extracted from all samples, including fresh tissue, swabs, and paraffin sections. Soy bean-sized piece of the fresh tissues were placed in a 2.0 ml EP tube, and add the necessary amount of trypsin and let the tube sit in a metal bath heated at 37 °C for 30 min prior to extracting DNA from the tissue. Swabs should be dissolved in phosphate buffered saline prior to DNA extraction. For paraffin sections, about 10 wax rolls were obtained and dewaxed with xylene before DNA was extracted. The DNA from fresh tissue and swabs was extracted by using a nucleic acid extraction kit (MAGEN Guangzhou, CHINA), and the DNA from paraffin sections was extracted by using a nucleic acid extraction kit (QIAgen FFPE DNA kit, USA). Further, DNA was quantified by quantified using fluorometric quantification, Qubit 4.0 Fluorometer.

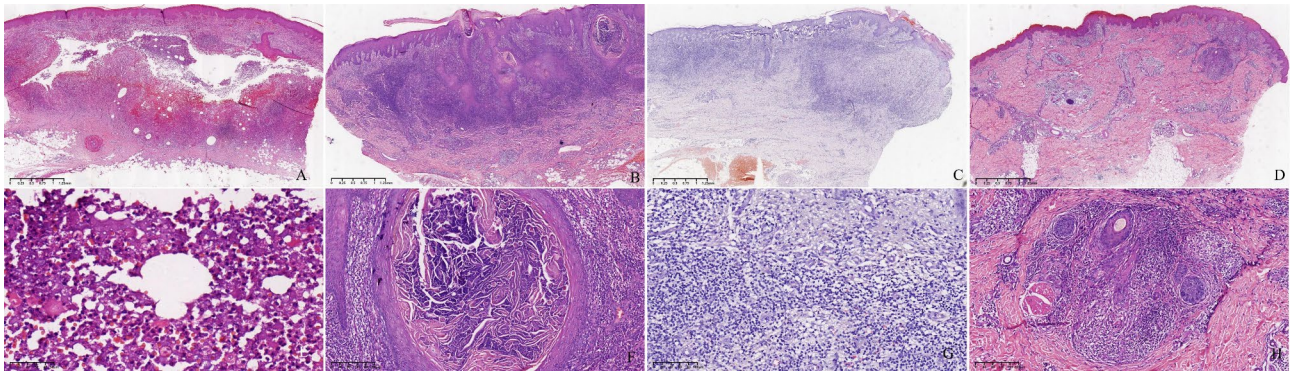

Fig. 1 Histological findings in CIG patients. **A-D**, H&E, Bar = 1.25 mm, **E**, H,&E, Bar = 50 μm, **F** and H, H&E, Bar = 200 μm.G, H&E, Bar = 100 μm. From left to right: Patient 2 (**A, E**), Patient 5 (**B, F**), Patient 14 (**C, G**) and Patient 7 (**D, H**)

Library construction and sequencing

The DNA library building kit (NextEra XT (Illumina, USA)) was used to prepare the DNA libraries. Using agarose gel electrophoresis and a Qubit 4.0 Fluorometer, the concentration of the DNA libraries was determined. The NextSeq 500/550 High Output v2 kit was then used to complete the sequencing (75 cycles).

Bioinformatics analysis

FastQC software was used to filter reads for low-quality and low-complexity sequences. The effective sequence, or clean reads, was the one that remained after the linker, low-quality base, and too-short sequence were removed. Next, clean reads were mapped to the human reference genome (GRCh38) in order to exclude human reads. The remaining information was matched with the database of microbial genomes. Burrows Wheeler Alignment (BWA, bowtie2) (http://bio-bwa.sourceforge.net/) was the comparison program used. Both FASTQC software and BWA use default parameters. More than 18,000 different types of microorganisms, including viruses, fungi, bacteria, and parasites that are clinically common, are listed in the microbiological database. Microbial genomes are mainly downloaded from the nucleotide and genome databases provided by the National Center for Biotechnology Information (NCBI), and the downloaded sequences need to be further selected and optimized to reduce redundancy and ensure the sequence quality of the reference genomes, and priority will be given to selecting the representative genomes.

Interpretation and reporting

The final results were obtained by annotating the microbial classification of the measured effective sequence against the microbial database. The following standards were used to mNGS positive results: When a pathogen or parasite species found by mNGS had readings per million (RPM) more than 1, the result was deemed positive. In the case of viruses and opportunistic infections, a result was deemed positive if the patient's sequence count exceeded five times the number of identified sequences. In addition to that, the judgment will be based on the sequence number, relative abundance, specificity of comparison, species sequence similarity, genus information, microbial pathogenicity class, and clinical manifestations of patients. For background bacteria, we judged based on the non-template control (NC) and laboratory background microbial lists. In addition, we will also choose NCBI BLAST (https://blast.ncbi.nlm.nih.gov) for the validation of the results to exclude some false positives from local database comparison.

Statistical analysis

For continuous data, the mean ± standard deviation value or the median with interquartile range were used.

Ethics

Prior to participation, participants gave their signed, informed consent. The Clinical Research Ethics Committee of Sun Yat-sen Memorial Hospital provided Ethical approval (Number: SYSKY-2024-600-01).

Results

Demographic characteristics

Totally, we retrospectively collected 26 patients who were diagnosed as CIG (Table 1). In this study, nine male patients made up the study's patient population, with an average age of 53.8 ± 16.3years. The areas of the granulomatous were mainly located on fingers and arms (19/26, 73.07%). Of the patients, eight patients denied any precipitating factor, and seventeen (17/26, 65.4%) had previous experiences of trauma with wounds that were infected. Six patients have a history of using glucocorticoid. Nodules were the most common type of rashes, followed by plaques and swelling (Fig. 2A-D). The average duration from beginning of symptoms to the collection of the specimen was 36 months.[Interquartile range (IQR) 3.25–10 months].

Consistency and variations between mNGS and culture results

21 distinct pathogen kinds were found by mNGS, while nine were found by culture (Table 1). The most common pathogen was Non-tuberculous Mycobacteria (NTM), followed by *Candida parapsilosis*, *Malassezia restricta*, *Staphylococcus aureus*, *Talaromyces marneffei*, *Fonsecaea monophora*, *Sporothrix globosa*, *Pneumocystis jirovecii*, *Streptococcus dysgalactiae*, *Pluralibacter gergoviae*, *Pseudomonas aeruginosa*, *Chryseobacterium gleum*, *Klebsiella pneumoniae*, *Enterobacter cloacae complex*, *Human gammaherpesviru* and *Torque teno virus* 0.13 cases had multiple pathogens detected by mNGS, but only 1 case had multiple pathogens detected by culture (Fig. 3A). Among the multiple pathogens infected cases detected by mNGS, a dual NTM infection have been found in 3 cases (Patient 2, Patient 12 and Patient 21), and 1 case has been detected a mixture of dual NTM and bacteria infection (Patient 5), and 3 cases have been detected a mixture of NTM and bacteria infection (Patient 3, Patient18 and Patient 25), and 1 case has been detected a mixture of NTM and virus infection (Patient 1), and 1 case has been detected a mixture of NTM and fungi infection (Patient 24), and 1 case has been detected a mixture of NTM, bacteria and fungi infection (Patient 26), and 1 case has been detected a fungi and bacteria infection (Patient 15), and 1 case has been detected a fungal and virus

Table 1 Demographic characteristics of study participants

Patient NO.	Sex	Age	Infection part	Precipitating factor	Classifications of rash	Months from symptom onset to specimen collection	Sample classification	mNGS results	Relative abundance	Sequence number	Culture results	Culture and/or identification day	T-SPOT® results	Histopathology Special stains	
														Acid-fast	PAS-D
1	F	64	Left fingers and arm	Fishbone	Pink linear nodule	10	Tissue	*M. marinum*	28.5%	2	*M. marinum*	35	+	+	ND
								Human gammaherpesviru	100%	17					
2	F	68	Left arm	Soil	Red papule and linear nodule	5	Tissue	*M. ulcerans*	13.7%	4	*M. marinum*	18	+	-	-
								M. marinum	10.3%	3					
3	M	48	Right hand	Branch of trees	Nodule and cystis	3	Paraffin section	*Mycobacterium spp.*	15.7%	225	ND	ND	ND	ND	ND
								S. aureus	6.3%	91					
							Tissue	ND	ND	ND	-	-			
4	F	66	Right hand	Faucet	Red plaque	6	Paraffin section	-	-	-	ND	ND	ND	ND	ND
							Tissue	ND	ND	ND	-	-		-	ND
5	M	57	Right finger and opisthenar	Fishbone	Red plaque	24	Tissue	*Pluralibacter gergoviae*	92.73%	689	*Pluralibacter gergoviae*	2	-	-	-
								M.marinum	0.27%	2	*M. marinum*	23			
								M. ulcerans	0.13%	1					
6	M	78	Right leg	Soil	Giant plaque and swelling	312	Paraffin section	*Fonsecaea monophora*	78%	25	ND	ND	-	ND	ND
							Tissue	ND	ND	ND	*Fonsecaea monophora*	14			
7	M	35	Left knee and hand	Fall damage	Red plaque	24	Paraffin section	*M. abscessus*	0.14%	2	ND	ND	ND	ND	ND
							Tissue	ND	ND	ND	-	-		+	ND
8	F	55	Left leg	UNK	Swelling and ulceration	2	Pus	*M. abscessus*	30%	97	*M. abscessus*	25	-	+	-
9	M	65	Central face	UNK	Swelling and nodule	12	Paraffin section	-	-	-	ND	ND	-	ND	ND
							Tissue	ND	ND	ND	-	-		ND	+
10	M	19	Right forearm	Bruise	Linear nodule	10	Tissue	-	-	-	-	-	ND	-	-
11	F	57	Right midfinger and forearm	Fishbone	Swelling, ulceration and nodules	8	Tissue	-	-	-	-	-	+	-	-
12	M	30	Right index finger	Bruise	Swelling and nodule	12	Tissue	*M. marinum*	11%	1	*M. marinum*	32	-	-	-
								M. ulcerans	11%	1					
13	M	42	Left inner thigh and the dorsum of left foot	Fishbone	Verrucous plaques	360	Tissue	*S. aureus*	40.55%	8194	*M.tuberculosis*	38	+	-	-
14	F	65	Right hand and forearm	Branch of trees	Red plaque and nodules	12	Tissue	*M. gallinarum*	1.07%	1	-	-	ND	+	ND
15	M	60	Left forearm	UNK	Swelling and ulceration	1	Tissue	*Talaromyces marneffei*	59.58%	1349	*Talaromyces marneffei*	7	ND	ND	ND
								Chryseobacterium gleum	40.42%	915					
							Pus	*Talaromyces marneffei*	32.37%	414	ND	ND		ND	+
								Chryseobacterium gleum	67.63%	865					
16	F	52	Face	Cosmetic injection	Nodule and cystis	3	Tissue	-	-	-	-	-	ND	+	+
17	F	69	Right inner ankle	Nails	ulcers	4	Tissue	*C.parapsilosis*	98.1%	893,307	*C.parapsilosis*	6	-	-	-
18	M	18	Left upper limb	Branch of trees	Swelling, nodule	2	Tissue	*M.marinum*	4.8%	65	-	-	+	-	-
								Pseudomonas aeruginosa	7%	754					

Table 1 (continued)

Patient NO.	Sex	Age	Infection part	Precipitating factor	Classifications of rash	Months from symptom onset to specimen collection	Sample classification	mNGS results	Relative abundance	Sequence number	Culture results	Culture and/or identification day	T-SPOT® results	Histopathology Special stains	
19	M	29	Right hand	Fishbone	Erythema, Swelling	5	Tissue	*M.marinum*	3.8%	5	Positive	12	+	+	ND
20	F	55	Right middle finger	UNK	erythema	15	Tissue	*C.parapsilosis*	64.71%	11	-	-	+	ND	
21	M	50	Right wrist	UNK	erythema	12	Tissue	*M.marinum*	0.45%	2	-	-	+	-	-
								M.ulcerans	0.45%	2					
22	M	72	Left dorsal and medial ankle	Nails	Erythema, papules, nodules, ulcers	3	Tissue	*M.houstonense*	33.59%	43	Positive**	11	-	-	-
23	F	54	Right forearm	UNK	papules	60	Tissue	*Sporothrix globosa*	17%	3	*Sporothrix schenckii*	32	-	-	-
								M.restricta	43%	5					
								Torque teno virus	38%	7					
24	F	69	Right hand	UNK	plaque	36	Tissue	*M.marinum*	2.5%	3	*Lactobacillus plantarum*	3	-	-	-
								Pneumocystis jirovecii	80%	5					
25	M	52	Right elbow	UNK	erythema	12	Tissue	*M.marinum*	0.8%	1	Positive	19	ND	-	-
								Streptococcus dysgalactiae	1.1%	5					
26	M	65	Left thumb and left forearm	UNK	nodule	3	Tissue	*M.marinum*	0.7%	9	Positive	36	+	-	-
								C.parapsilosis	66.9%	60					
								M.restricta	21.4%	9					
								Klebsiella pneumoniae	5.9%	172					
								Enterobacter cloacae complex	1.3%	70					

*UKN: Unknown. Positive: Positive antacid bacteria culture. ND: Not done

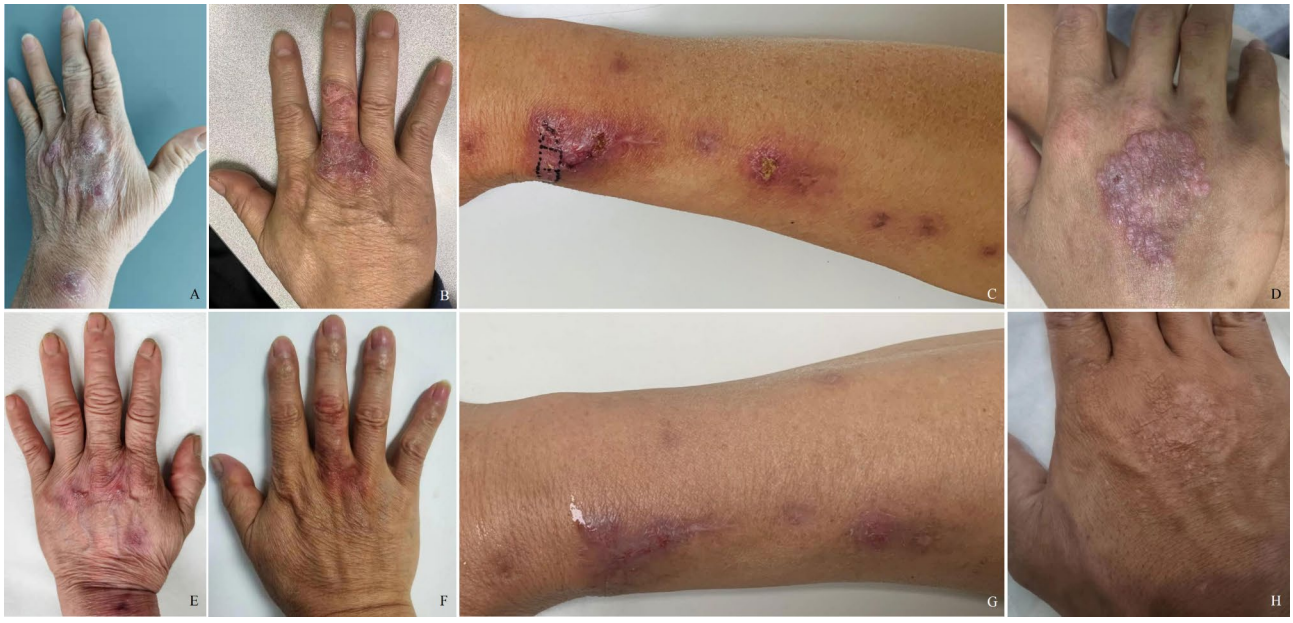

Fig. 2 Clinical manifestation of CIG patients. **A-D**, Before treatment. **E-F**, After treatment. From left to right: Patient 2, Patient 5, Patient 14 and Patient 7

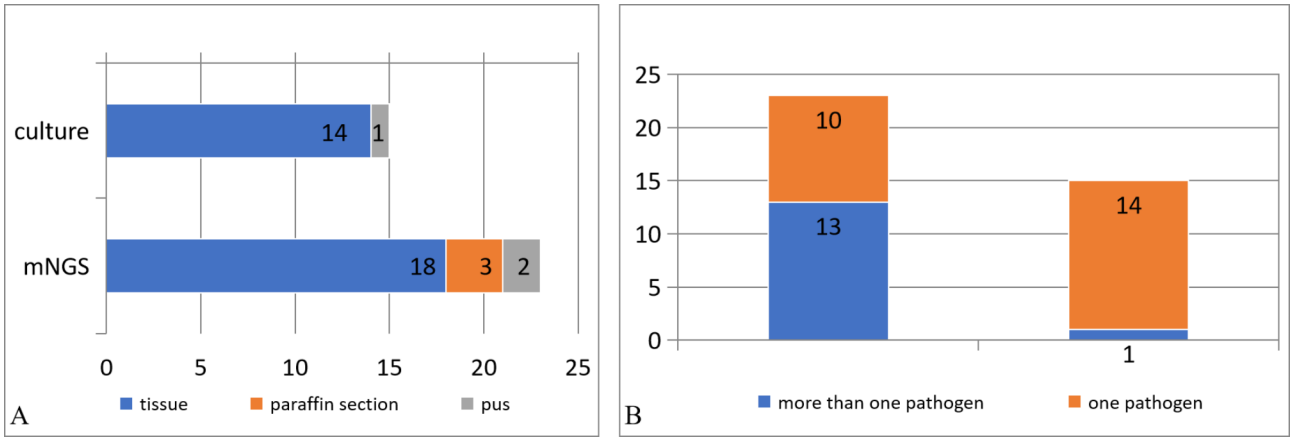

Fig. 3 **A**, Bar graph illustrating the relative amounts of multiple pathogens in cases. 13 cases had multiple pathogens detected by mNGS, but only 1 case had multiple pathogens detected by culture. **B**, Bar graph illustrating the positive results of mNGS and culture for different sample types. For samples tested by culture, 2 samples were extracted from paraffin section and 7 samples were extracted from fresh tissue. For samples tested by mNGS, 3 samples were extracted from paraffin section and 2 samples were extracted from pus, and the remaining 18 samples were extracted from fresh tissue (Patient 15 got positive results both in fresh tissue and pus)

infection (Patient 23). 80.77% (21/26) and 57.7% (15/26) of CIG patients had positive mNGS results and cultures, respectively (Table S1). The sensitivity rates and specificity rates of mNGS were 100% and 54.5%, respectively, in contrast to traditional culture. Furthermore, the positive predictive value and negative predictive value were 75% and 100%, respectively. NGS sample identification seemed to have no effects on the test results, 3 samples were extracted from paraffin section and 2 samples were extracted from pus, and the remaining 18 samples were extracted from fresh tissue (Patient 15 got positive results both in fresh tissue and pus) (Fig. 3B). The median time needed for mNGS was around 2 days, while for culture it was around 19.6±12.4 days. So the time needed for NGS was significantly shorter than culture. Eleven of patients have been misdiagnosed and five of them have been treated with anti-fungal medicine showed no response or worsening (Table 2). Four patients returned to rural area or refused to take medication and as a result of losing follow-up. One patient with SLE had serious complications and died of *pneumocystis carinii* infection. Notably, at the 30-day follow-up, 21 patients exhibit clinical improvement. Eighteen of them received improved response after adjusted therapy with appropriate anti-microbial agent (fifteen of them are mNGS-positive cases) (Fig. 2E-H). In addition, two patients showed clinical improvement with

Table 2 Changing antibiotic treatment according to mNGS results

Patient NO.	Antibiotics using before specimen collection	Immunosuppressive agent usage	Conversion of targeted antibiotics treatment	Outcome during 30-day follow-up
1	NO	Leflunomide	Clarithromycin, Sulfamethoxazole	Refuse to take medication
2	Itraconazole	NO	Rifampicin, Isoniazide, Clindamycin	Improve
3	Rifampicin, Isoniazide, Clarithromycin	NO	NO	Improve
4	Levofloxacin, Itraconazole	NO	NO	Improve
5	NO	NO	Rifampicin, Isoniazide, Clindamycin	Improve
6	NO	NO	Itraconazole, Terbinafine, Amphotericin B (local injection)	Improve
7	Clarithromycin, Rifampicin, Isoniazide	NO	NO	Improve
8	NO	NO	Clarithromycin	Died of pneumocystis carinii infection
9	NO	Triamcinolone	Amphotericin B	Improve
10	NO	Prednisone and thalidomide	NO	Lose track
11	Clindamycin, Rifampicin, Roxithromycin	NO	Rifampicin, Isoniazide	Improve
12	Itraconazole	NO	Rifampicin, Clarithromycin	Improve
13	NO	NO	Salbutamol, Rifampicin, Isoniazide	Improve
14	Itraconazole	NO	Rifampicin, Isoniazide, Clarithromycin	Improve
15	NO	Methylprednisolone	Itraconazole	Improve
16	NO	NO	Clarithromycin, Levofloxacin	Improve
17	Cefoperazone sulbactam	NO	Clarithromycin, levofloxacin	Improve
18	NO	NO	Clarithromycin, rifampicin	Improve
19	NO	NO	rifampicin	Improve
20	NO	NO	methyldopa	Lose track
21	NO	NO	Levofloxacin, Rifampicin	Improve
22	Cefoperazone sulbactam	NO	Rifampicin, Moxifloxacin	Improve
23	NO	Cyclosporin	Linezolid, itraconazole	Improve
24	Itraconazole	NO	Sulfamethoxazole, Rifampicin	Improve
25	NO	NO	Clarithromycin	Improve
26	NO	NO	Rifampicin	Lose track

continuous treatment due to mNGS-positive results at 30 days follow-up.

Discussion

Commonly, cutaneous infectious granulomas (CIG) are localized and chronic skin infection caused by a variety of pathogens such as protozoans, bacteria, worms, viruses and fungi. The diagnosis of CIG depends mainly on the clinical examination of the skin, microbiological and histopathological examination. However, microbiological examination, including direct smear and culture, shows low sensitivity. Moreover, the histomorphological findings of CIG caused by different pathogens are commonly very similar so the exact identity of the infectious graulomas is hardly to work out [8]. Although pathogen detection is important, histopathology even with special staining can rarely identify pathogens to species level [3]. As a result, the CIG usually remains undiagnosed for several years and resulting in delayed or inadequate treatment, prolonged stays, readmissions. According to our findings, it took an average of 36 months from the start of symptoms until the collection of the samples. [Interquartile range (IQR) 3.25–10 months], so our study consistent with those conclusions. The epidemiological pathogens of CIG vary depending on geographical regions throughout the world [2]. In general, fungi and mycobacteria are the two most common types of CIG, our study consistent with those conclusions. In the present study, the most common pathogen was Non-tuberculous Mycobacteria (NTM), while positive result of fungi are six (*C. parapsilosis*, *M.restricta*, *S. globosa*, *P. jirovecii*, *Talaromyces marneffei*, *Fonsecaea monophora*). The main reason

is that fungi generally bigger in diameter, so that fungi structure are more easily to be found through the examination of histomophology and direct smear. Although some of the studies stated that fungi cell wall is so thick that mNGS with lower ratio of positive results [9], mNGS is able to detect the fungi of *Fonsecaea spp.*, which presented as muriform cells with thick cell wall in tissue.

Secondly, our study's conclusion is that, in the following areas, mNGS produced higher-quality detection than traditional examination.1) mNGS results have a greater positive rate than culture (80.77% vs. 57.7%). The positive rate of mNGS ranged from 41.3 to 82.14% in earlier research, which was often greater than the culture rate [10–12]. 2) Multiple pathogens can be simultaneously detected by mNGS in a single specimen. Generally, it needs to collect multiple samples for different examination, including bacteria, fungi, mycobacteria, etc. Therefore, mNGS provides an effective alternative to optimize microbiological detection. 3) mNGS not only can be used to detect the sample of fresh tissue and pus, but also the sample of paraffin-embedded tissues. In many cases, sample of tissue is not sent for cultures since gurnulomatous inflammation is not found until microscopy is performed. Also, a sample may have been sent for culture but without positive results neither in bacterial culture nor fungal culture. Thus, mNGS provides an effective alternative to test with the samples of paraffin-embedded tissue, which are the only material available for testing. 4) The total time for detection by using mNGS is much shorter than by culture. As we know, the culture-based method for microbiological detection is time-concuming. mNGS could be done within two days, while median time for culture is 19.6±12.4 days. Totally, 21 patients showed clinical improvement by the 30-day follow up. Eighteen of them were adjusted treatment and fifteen of them continuous treatment based on the results of mNGS. So, it showed that mNGS results may affect the clinical prognosis resulting from enabling the patients to initiate timely treatment.

In other hand, although we demonstratd some merits of mNGS, there are still some limitations as follows: it is needed to combine with comprehensive assessment of cases, including patients' information, clinical manifestation, location of specimen collection and microbiological characteristics, for clinical interpretation of positive results of mNGS. In addition, there are still some samples got negative mNGS, but positive in special straining of histopathology (Patient 16) or T-SPOT®. TB test (Patient 11). Except for the patient 11, there are totally nine patients, including one patient infected by *M. tuberculosis* and six patients infected by *M. marinum*, with positive T-SPOT®. TB test (9/18, 50%). T-SPOT®. TB test used to diagnose latent tuberculosis, pulmonary tuberculosis, or extrapulmonary tuberculosis is the interferon-γ (IFN-γ) release assay (IGRA), which has a high sensitivity for the specific detection of these conditions. But some NTMs, like *M. marinum*, could show false positive results in IGRA test [13]. Moreover, GeneXpert is frequently employed for tuberculosis detection. Findings from a limited number of current studies indicate that GeneXpert and mNGS have comparable specificity. However, in terms of sensitivity, mNGS appears to be marginally superior to GeneXpert [14, 15]. Nevertheless, we believe further research is necessary before drawing definitive conclusions. So, more detection methods should be used spontaneously to confirm the findings and make proper diagnosis.

In summary, our data show that mNGS detected NTM, *M. tuberculosis*, fungi and bacteria in cutaneous infectious granulomas. Compared to culture, mNGS showed a higher positive rate with high sensitivity rate and negative predictive value. In addition, mNGS can detect more pathogens in one sample and can be used to detect variable samples including the samples of paraffin-embedded tissue. However, our study had some limitations: Our limited study population and the risk of selection bias affect the representativeness and generalizability of the experimental results. Nonetheless, this study reveals preliminary trends and provides direction for subsequent exploration. Future studies should aim to expand the sample size and provide more scientific evidence for the application of mNGS in the prevention and treatment of CIG. In addition, mNGS has an extremely broad detection range of microorganisms, a feature that brings both advantages and challenges: It is difficult to accurately distinguish between colonization or contamination during the interpretation of results of mNGS. For the samples used in this study, the colonizing bacteria usually originated from the skin surface, such as *Cutibacterium acnes*, *Staphylococcus epidermidis*, and *Malassezia globosa*, and were relatively easy to distinguish. On the other hand, contamination is related to originate from the reagents themselves, the experimental environment, and cross-contamination between different samples. These sources of contamination may lead to misinterpretation of mNGS results. In recognition of this, researchers should fully understand and consider the microbial community characteristics in the study background when interpreting mNGS results, which is the key to improving the accuracy of mNGS results interpretation and guiding clinical decision-making, as well as a direction worthy of continuous efforts in our future research and practice.

In conclusion, although mNGS provides a potentially rapid and effective alternative test for the diagnosis of cutaneous infectious granulomas, a comprehensive judgment must be made in conjunction with clinical evaluation, microbial culture results, and histopathological

examination to ensure the accuracy and reliability of the diagnosis in practical application.

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s12941-024-00744-w.

Supplementary Material 1

Supplementary Material 2: **Figure S1** Flow diagram of patient recruitment. A total of 89 CIG patients were screened, and 26 of them were ultimately studied in this study, having a clinical presentation of cutaneous infection verified by histological testing. The exclusion criteria were as follows: secondary infection due to rupture of Sebaceous cyst/Epidermal cyst rupture: 43 patients; No culture/NGS: 20 patients.

Author contributions

SL contributed to the study conception and design. Material preparation, data collection and analysis were performed by HL and QR. JZ, XL, JM and JZ contributed to collect the patients. NT contributed to mNGS sequencing and analysis. The first draft of the manuscript was written by SL and QR. JZ and XL revised the manuscript. Funding acquisition by LX and SL. All authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

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Data availability

No datasets were generated or analysed during the current study.

Declarations

Consent to participate

Informed consent was obtained from all individual participants included in the study.

Consent to publish

The participants have consented to the submission of the cases reports to the journal.

Competing interests

The authors declare no competing interests.

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