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Metagenomic Capture as an Adjunct Diagnostic Strategy for Cutaneous Infectious Granulomas: A Prospective–Retrospective Cohort Study

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Abstract

Background

Diagnosing infectious granulomas remains challenging because conventional culture has limited sensitivity and histopathology is often nonspecific. Metagenomic capture (MetaCAP) may improve pathogen detection.

Objective

To evaluate the diagnostic performance of MetaCAP in cutaneous infectious granulomas (CIG).

Methods

We conducted a dual-center prospective–retrospective cohort study including 117 patients with suspected granulomatous skin lesions. Diagnostic performance of MetaCAP was assessed against a prespecified composite clinical reference standard established by blinded adjudication independent of MetaCAP results.

Results

MetaCAP demonstrated higher sensitivity than conventional culture (86.6% vs 49.3%) and showed good agreement with the reference standard (κ = 0.78). Its principal incremental value was observed in conventionally negative cases, in which additional pathogens were identified in 94.1% of cases. MetaCAP also showed high specificity for noninfectious granulomas (92.0%). Fresh tissue yielded a numerically higher detection rate and significantly higher pathogen relative abundance than paraffin-embedded specimens.

Conclusion

MetaCAP provides incremental diagnostic value in CIG, particularly in conventionally negative cases, and may serve as a complementary diagnostic strategy when conventional methods are inconclusive.

Introduction

Granulomatous inflammation represents a typical histopathologic pattern that may be associated with infectious or noninfectious etiologies. Further studies are often necessary to determine the underlying cause. Histologically, granulomas are characterized by organized aggregates of macrophages, epithelioid histiocytes, and multinucleated giant cells.(1, 2) Clinically, CIG presents as skin nodules or

plaque-like lesions at the site of infection. With disease progression, these lesions may grow and form abscesses or ulcers. In immunocompromised patients, CIG may spread along lymphatic vessels with a linear pattern.(2, 3) These features can resemble noninfectious granulomatous disorders such as sarcoidosis or granuloma annulare.(4, 5)

The primary diagnostic approaches for identifying pathogens in CIG include conventional culture, histopathological examination, polymerase chain reaction (PCR) testing, and metagenomic sequencing (mNGS),(3, 6–8) However, each of these methods has certain limitations. Conventional culture is time-consuming and labor-intensive and offers a relatively low detection rate.(5, 9, 10) Although histopathological examination combined with specific staining methods can serve as the initial screening method, they cannot always detect specific pathogens.(11) Despite offering high sensitivity, PCR depends on pre-designed primers, limiting its ability to detect unknown pathogens.(12, 13) Conventional metagenomic sequencing may also suffer from reduced sensitivity due to host nucleic acid interference or low pathogen load.(14) Given the limitations of current diagnostic methods, there is a pressing need for an early and precise strategy for pathogen diagnosis.

MetaCAP enriches target sequences through specific probe hybridization, enabling direct detection of multiple microorganisms, antimicrobial resistance genes, and virulence factors in clinical samples. Compared with conventional metagenomic sequencing, this probe capture–based approach may provide improved sensitivity for low-biomass samples, reduced interference from host DNA, and more stable performance in the setting of prior antibiotic exposure.(15) This technology offers several advantages, including high throughput, broad coverage, and excellent sensitivity. It significantly improves pathogen detection rates and provides valuable insights for early and precise drug use in clinical settings.(16) Comprehensive studies comparing MetaCAP with conventional culture are currently limited. Conventional culture was selected as the primary comparator because it remains the routine microbiologic benchmark for pathogen-directed diagnosis and antimicrobial susceptibility assessment in clinical practice. Although PCR may be valuable for selected slow-growing organisms, it was not uniformly performed across all patients; therefore, PCR results were incorporated into the composite clinical reference standard when available rather than used as a primary comparator.In this study, we included patients with suspected CIG from two large tertiary hospitals to investigate the potential of MetaCAP in improving pathogen detection and diagnostic accuracy, providing experimental evidence to support early diagnosis of this condition.

Methods

1.1 Study design and patients

This study included a prospective cohort and a retrospective cohort of patients with suspected cutaneous infectious granuloma (CIG) seen in the Departments of Dermatology at the Affiliated Hospital of Xuzhou Medical University and the First People's Hospital of Lianyungang between January 2020 and December 2025. The prospective cohort comprised 97 treatment-naive patients enrolled from January

2023 to December 2025, and the retrospective cohort included 20 patients seen from January 2020 to December 2022 with complete clinical records and archived specimens, for a total of 117 patients. Eligible patients were 18 years or older, had chronic cutaneous lesions with clinicopathologic features suggestive of granulomatous inflammation, and had complete clinical, specimen, and follow-up data. Patients were excluded for severe immunodeficiency, malignancy, long-term systemic corticosteroid or immunosuppressant use, inadequate specimens(defined as insufficient tissue quantity for parallel conventional testing and MetaCAP analysis, poor preservation, or extensive necrosis or contamination precluding reliable pathogen detection), incomplete testing data, loss to follow-up, or a confirmed diagnosis of another specific granulomatous skin disorder, such as sarcoidosis or foreign body granuloma. All procedures involving human participants were conducted in accordance with the Declaration of Helsinki. Written informed consent was obtained from participants in the prospective cohort; the requirement for informed consent was waived for the retrospective cohort. The study was approved by the Ethics Committee of the Affiliated Hospital of Xuzhou Medical University (XYFY2025-KL073-01).

1.2 MetaCAP analysis and specimen processing

MetaCAP is a probe hybridization capture–based sequencing platform for microbial metagenomic analysis. A customized probe panel targeting pathogen-specific genes, antimicrobial resistance genes, and virulence factors covers more than 4,000 clinically relevant microorganisms. This approach enriches microbial nucleic acids and reduces host background, thereby improving sensitivity for low-abundance pathogens. Pathogens were identified through high-throughput sequencing and bioinformatic analysis after exclusion of likely contaminants.

Fresh tissue specimens were preferentially collected from active lesions at initial evaluation and underwent parallel conventional testing and MetaCAP analysis. Conventional testing included microbial culture and histopathologic evaluation with special stains. Archived paraffin-embedded specimens were included for retrospective cases when fresh tissue was unavailable.

Because the probe panel is predefined, detection of highly divergent or novel pathogens may be limited. Low-abundance sequencing reads were interpreted with caution given the potential for contamination or detection of non-viable microbial DNA.(15) (Supplementary Materials).

1.3 Clinical adjudication and case classification

Patients were classified using a prespecified composite clinical reference standard established independently of MetaCAP results. Briefly, the reference standard incorporated clinical presentation, histopathologic findings, conventional microbiologic results (including culture and, when available, PCR), treatment response, and follow-up outcomes, with final diagnosis determined by consensus adjudication rather than any single test result.Initial case adjudication was performed by a blinded expert panel without access to MetaCAP findings, thereby avoiding circular reasoning. To further minimize potential incorporation bias, adjudication was completed and locked before any MetaCAP results were accessed, and all supporting evidence for conventionally negative cases was derived from non–MetaCAP sources.

MetaCAP results were reviewed only after adjudication as post hoc supportive evidence.Detailed classification criteria are provided in the Supplementary Materials. Ultimately, 67 patients were classified as having CIG and 50 as noninfectious granulomas (Fig. 1.eps). Among the adjudicated CIG cases, mycobacterial and fungal infections were the predominant etiologies(Supplementary Fig. 2.eps).

1.4 Statistical analysis

Statistical analyses were performed using SPSS version 27.0. Continuous variables were compared using the independent-samples t test or Mann–Whitney U test, as appropriate, and categorical variables using the chi-square test or Fisher exact test. The primary endpoint was the diagnostic performance of MetaCAP against the prespecified composite clinical reference standard, including sensitivity, specificity, positive predictive value, negative predictive value, accuracy, and 95% CI; agreement was assessed using Cohen's kappa statistic. A key secondary endpoint was the paired difference in positive detection rate between MetaCAP and conventional culture among adjudicated cutaneous infectious granuloma cases, assessed using the McNemar test.

Sensitivity analyses excluding conventional-method-negative/MetaCAP-positive cases and other subgroup analyses were considered exploratory and interpreted with caution due to limited sample size.

Results

2.1 Primary Analysis: Diagnostic Performance and Concordance Against the Composite Clinical Reference Standard

Using the prespecified composite clinical reference standard, we evaluated the primary endpoint in 117 patients with histopathologically suspected cutaneous granulomatous lesions, including 67 adjudicated cutaneous infectious granulomas and 50 noninfectious granulomas. MetaCAP yielded 58 true-positive, 46 true-negative, 9 false-negative, and 4 false-positive results, whereas conventional culture yielded 33 true-positive, 50 true-negative, 34 false-negative, and no false-positive results (Table 1). MetaCAP showed substantially higher sensitivity (86.6% vs 49.3%), negative predictive value (83.6% vs 59.5%), and overall accuracy (88.9% vs 70.9%) than conventional culture, as well as stronger agreement with the reference standard ($\kappa = 0.78$ vs 0.45). In contrast, conventional culture showed higher specificity and positive predictive value (Table 1).

Table 1
Diagnostic performance of MetaCAP and conventional culture against the composite clinical reference standard

Measure(Classification against reference standard)	MetaCAP	Conventional culture
TP	58	33
FN	9	34
FP	4	0
TN	46	50
Sensitivity (95% CI)	86.57 (76.00-92.78)	49.25 (37.65–60.94)
Specificity (95% CI)	92 (81.16–96.85)	100 (92.87–100.00)
PPV (95% CI)	93.55 (84.55–97.46)	100 (89.57–100.00)
NPV (95% CI)	83.64 (71.74–91.14)	59.52 (48.83–69.38)
Accuracy (%)	88.89	70.94
Cohen's κ	0.78	0.45

Note: Values are presented as number of cases or percentage (95% confidence interval). Diagnostic performance measures were derived from classification against the prespecified composite clinical reference standard, which incorporated clinical presentation, histopathology, conventional microbiology, treatment response, and follow-up outcomes independent of MetaCAP results. Cohen's κ reflects agreement with the reference standard. PPV, positive predictive value; NPV, negative predictive value.

2.2 Sensitivity Analysis in a Restricted Cohort

In a sensitivity analysis excluding conventionally negative but MetaCAP-positive cases, 85 cases remained, including 35 adjudicated cutaneous infectious granulomas and 50 noninfectious granulomas. In this restricted cohort, MetaCAP sensitivity decreased to 74.3%, whereas conventional culture sensitivity increased to 94.3% (Table 2). The attenuation of MetaCAP's apparent superiority suggests that its principal diagnostic advantage in the primary analysis was largely driven by incremental detection in conventionally negative infections.

Table 2
Sensitivity Analysis Excluding Conventionally Negative/MetaCAP-Positive Cases

Measure(Classification against reference standard)	**MetaCAP**	**Conventional culture**
TP	26	33
FP	4	0
FN	9	2
TN	46	50
Sensitivity (95% CI)	74.29 (56.74–87.51)	94.29 (80.84–99.30)
Specificity (95% CI)	92.00 (80.77–97.78)	100.00 (92.89–100.00)
PPV (95% CI)	86.67 (69.28–96.24)	100.00 (89.43–100.00)
NPV (95% CI)	83.64 (71.20-92.24)	96.15 (86.79–99.53)
Accuracy (%)	84.71	97.65

Note: Sensitivity analysis was performed in the restricted cohort after excluding conventionally negative but MetaCAP-positive cases adjudicated as CIG (n = 85).

2.3 Incremental Diagnostic Yield and Potential Clinical Utility of MetaCAP in Adjudicated CIG Cases

As a key secondary analysis, we compared paired positive detection rates between MetaCAP and conventional culture in 67 adjudicated CIG cases. MetaCAP showed a significantly higher positive detection rate than conventional culture (86.6% vs 49.3%, P<.001). Among 34 culture-negative cases, MetaCAP identified additional pathogens in 94.1% (32/34), highlighting its principal incremental diagnostic value in conventionally negative infections (Table 3).

Table 3
Paired comparison of incremental pathogen detection by MetaCAP versus conventional culture

Test methods		Conventional culture			*P* value
		positive	negative	Total	
MetaCAP	positive	26	32	58	<0.001
	negative	7	2	9	
	Total	33	34	67	

Note: McNemar’s paired chi-square test was used; P < 0.05 was considered to indicate statistical significance.

Among the 32 conventionally negative but MetaCAP-positive cases, pathogen-directed therapy modification was associated with clinical improvement in many patients; however, causality cannot be established. MetaCAP also shortened diagnostic turnaround compared with conventional culture (Supplementary Fig. 3.eps).

2.4 Comparison of MetaCAP Performance in Fresh Tissue Versus Paraffin-Embedded Specimens

MetaCAP was performed in 67 specimens, including 47 fresh tissue samples and 20 archived paraffin-embedded blocks. Fresh tissue showed a higher pathogen detection rate than paraffin-embedded specimens (91.5% [43/47] vs 75.0% [15/20]), although the difference was not statistically significant (P = 0.156). Among the 58 MetaCAP-positive specimens (43 fresh tissue and 15 paraffin-embedded blocks), pathogen relative abundance was significantly higher in fresh tissue (P = 0.002), whereas sequencing Counts and detection confidence were comparable between groups (P > 0.05)(Table 4) .

Table 4
Among MetaCAP-positive specimens

Sample Type	**Fresh Tissue specimens (n = 43)**	**Paraffin-embedded specimens (n = 15)**	***P*-value**
sequencing Counts (Median, IQR)	67(36,203)	50(15,203)	0.328
Confidence (Mean ± SD)	89.63 ± 14.35	77.53 ± 25.11	0.095
Relative Abundance (Median, IQR)	57.14(18.18,95.75)	5.13(0.26,54)	0.002
Note: Sequencing Count : The total sequence count is standardized to one million reads (RPM) to provide the number of high-quality sequences that align with the target pathogen.			
Relative Abundance: The relative proportion of sequences aligned to a particular pathogen within the respective categories (bacteria, fungi, viruses, and parasites).			
Confidence : The detection confidence of the pathogen is calculated using multiple alignment-based statistical indicators.			

2.5 Exploratory Analysis of Culture-Negative MetaCAP-Positive Cases

Among 58 MetaCAP-positive patients, 26 (44.8%) were culture positive and 32 (55.2%) were culture negative. Culture negativity was associated with longer disease duration, greater prior antibiotic exposure, and lower sequencing Counts, whereas detection confidence and relative abundance were similar between groups (Table 5). These findings may help explain the incremental diagnostic yield of MetaCAP in conventionally negative infections and suggest that MetaCAP performance may be less affected than culture by prior antibiotic exposure or chronic low-burden infection(Table 5).

Table 5
Comparison of Clinical Features and Detection Results Between Culture-Positive and Culture-Negative Groups in MetaCAP-Positive Patients

Disease Duration	**MetaCAP (+) and culture (-) (n = 32)**	**MetaCAP (+) and culture (+) (n = 26)**	***P***
	6.5(5, 12)	**3(2, 12)**	**0.045**
Sample Distribution, n (%)			
Wax Block	6(18.75)	9(34.62)	0.170
Fresh Tissue	26(81.25)	17(65.38)	
Previous Antibiotic Use, n (%)	25(78.13)	10(38.46)	0.002
sequencing Counts (Median, IQR)	53.00(31.00,143.75)	123(55.25,726.00)	0.047
Confidence (Mean ± SD)	87.81 ± 15.22	84.88 ± 21.72	0.564
Relative Abundance (Mean ± SD)	46.39 ± 36.51	52.27 ± 43.35	0.577

Note: Exploratory subgroup analysis among MetaCAP-positive cases. Culture negativity was associated with longer disease duration, greater prior antibiotic exposure, and lower sequencing Counts, whereas detection confidence and relative abundance did not differ significantly.

Discussion

This study investigated the application of MetaCAP in the diagnosis of CIG. Skin tissue samples often present challenges due to limited sample size and low content of pathogen nucleic acid. These challenges decrease the sensitivity and specificity of traditional metagenomic sequencing.(17)Probe-capture enrichment may improve detection of low-abundance pathogens by reducing host nucleic acid interference.(16–18) conventional culture, histopathological examination, and PCR-based detection methods have inherent limitations, and no single method can serve as a "gold standard". This study supports the use of a composite reference standard, combining several diagnostic approaches to explore the strengths and weaknesses of new detection technologies compared to traditional methods and assess their value in diagnosing CIG.

Although conventional culture retained higher specificity, MetaCAP provided substantially greater sensitivity and diagnostic yield. Previous studies reported a sensitivity of 77.8% and specificity of 76.5% for MetaCAP in infectious pancreatic necrosis,(19) but its value in CIG has not been systematically

studied. Next-Generation Sequencing techniques such as mNGS and tNGS have recently been applied to pathogen detection in dermatology, with mNGS showing a positive detection rate of about 80% in CIG, higher than that of conventional culture.(20, 21) However, these methods remain limited by host nucleic acid interference, reduced detection of low-abundance pathogens, and lower sensitivity after antibiotic exposure.Using a composite clinical reference standard, we found that MetaCAP provided a higher overall detection yield than conventional culture in histopathologically suspected infectious granulomas, with its main incremental value observed in conventionally negative cases. Among culture-negative cases, MetaCAP identified additional pathogens in 94.1%, supporting improved detection of conventionally missed infections. These findings likely reflect probe-based enrichment of pathogen nucleic acids, which improves detection of low-abundance organisms while reducing host DNA interference.(22, 23) In contrast, culture depends on viable microbial growth and is readily affected by antibiotic exposure, disease duration, and pathogen-specific growth characteristics.(24–26) MetaCAP therefore appears to provide more informative pathogen data for early diagnosis and targeted therapy, particularly in low-abundance or difficult-to-culture infections.

Notably, in 32 cases with uniformly negative conventional results, MetaCAP provided the only actionable etiologic evidence and was associated with subsequent treatment modification and clinical improvement in many cases, although causality cannot be established, suggesting potential clinical value when conventional diagnostic approaches fail. MetaCAP also offered a substantially faster workflow than conventional culture,(13) with a mean turnaround time of 2.82 ± 1.27 days in our study, compared with culture turnaround times that may extend from several days to weeks for fungal and nontuberculous mycobacterial pathogens, supporting earlier diagnosis and treatment.In addition, because MetaCAP can simultaneously detect both DNA and RNA, it may offer advantages for detecting rare and atypical pathogens.(27) In the sensitivity analysis excluding the conventional-method-negative/MetaCAP-positive subgroup, the apparent superiority of MetaCAP was attenuated, indicating that its principal diagnostic advantage lies in conventionally negative cases, where it may provide actionable etiologic evidence when routine testing is unrevealing.

MetaCAP was primarily assessed against a prespecified composite clinical reference standard, whereas subgroup analyses were intended to clarify its incremental clinical value. In exploratory analyses, fresh tissue showed a numerically higher detection rate and significantly higher pathogen relative abundance than archived paraffin-embedded specimens, supporting its preferential use when feasible.(28, 29) Among MetaCAP-positive cases, sequencing reads were higher in culture-positive than culture-negative samples, whereas relative abundance and detection confidence were similar, suggesting that MetaCAP remains relatively stable across clinical settings. Because MetaCAP directly detects pathogen DNA and RNA, it may retain diagnostic value after antibiotic exposure or in chronic, low-burden infections. However, this characteristic may also introduce a potential limitation, as nucleic acids from nonviable organisms or prior unrelated infections may persist and be detected without representing active disease. Such residual signals could lead to clinically irrelevant findings or overinterpretation if sequencing results are interpreted in isolation. Therefore, MetaCAP findings should be integrated with

clinicopathologic features, exposure history, and treatment response to distinguish active infection from prior colonization or resolved infection.(15, 30, 31)

MetaCAP enables broad-spectrum sequencing with simultaneous detection of bacteria, fungi, mycobacteria, and other pathogens in a single assay.(32) This may accelerate diagnosis and support more targeted anti-infective therapy. MetaCAP detected several uncommon or difficult-to-culture organisms, supporting broader pathogen coverage. MetaCAP also showed high specificity for noninfectious granulomas, supporting its diagnostic value in distinguishing infectious from noninfectious lesions. However, false-negative results were still observed, likely because of low pathogen burden, degraded nucleic acids, or incomplete probe coverage of variable target regions.(15) Given the uncertain clinical relevance of some detected species, particularly uncommon mycobacteria such as *Mycobacterium duvalii*, results should be interpreted cautiously with clinicopathologic and microbiologic correlation. MetaCAP therefore should be considered part of a multimodal diagnostic strategy rather than a standalone test.

This study has several limitations. Although both prospective and retrospective cohorts were included, the retrospective component may have introduced selection and information bias. All patients were recruited from only 2 tertiary hospitals, which may limit generalizability, and several subgroup analyses were based on small sample sizes. Not all specimens underwent parallel PCR testing, and low read signals may have reflected false-positive findings rather than true pathogens. In addition, MetaCAP remains relatively costly and currently requires multidisciplinary expertise for interpretation because standardized interpretive guidelines are lacking. Larger multicenter prospective studies are warranted to further validate its diagnostic performance and support broader clinical application. These findings highlight the potential role of MetaCAP as a complementary diagnostic strategy in patients with suspected CIG when conventional methods are inconclusive, which may facilitate earlier etiologic diagnosis and more informed therapeutic decision-making.

Conclusion

Compared with conventional culture, MetaCAP provided additional pathogen detection in cutaneous infectious granuloma, particularly in conventionally negative cases and in infections involving low-abundance or uncommon organisms. It also showed high specificity for noninfectious granulomas and a substantially shorter turnaround time.

Abbreviations

Metagenomics capture
MetaCAP
Cutaneous Infectious Granulomas
CIG
Polymerase chain reaction

PCR
positive predictive value
PPV
negative predictive value
NPV

Declarations

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Patient Consent: The authors obtained consent for the publication of recognizable patient photographs or other identifiable material and included it at the time of article submission to the journal, stating that all patients gave consent with the understanding that this information may be publicly available.

IRB approval status: Reviewed and approved by the Medical Ethics Committee of the Affiliated Hospital of Xuzhou Medical University (approval number: XYFY2025-KL073-01).

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Figures

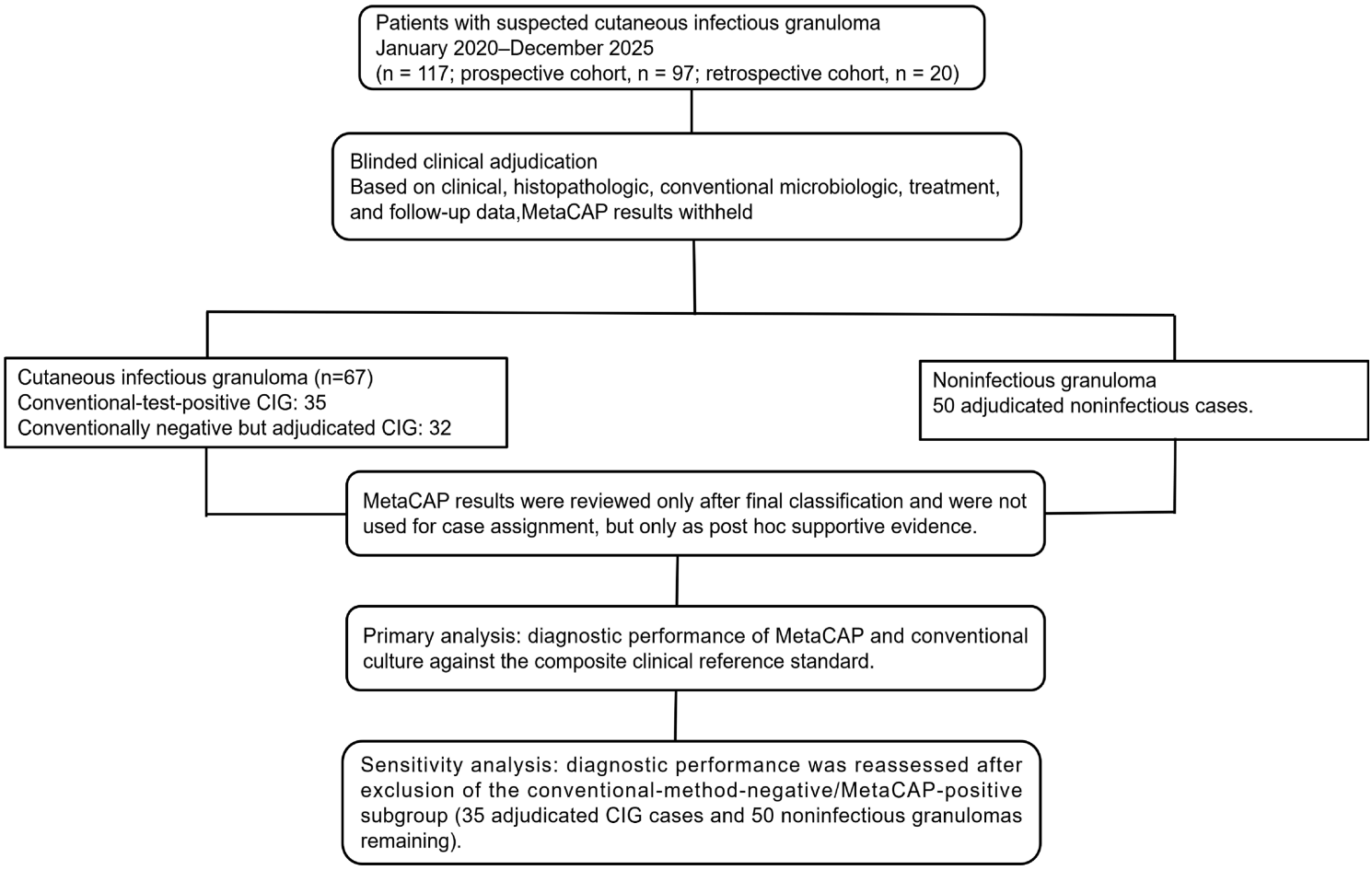


Figure 1

Study flow diagram showing patient inclusion, blinded adjudication using a prespecified composite clinical reference standard, final classification, and post hoc role of MetaCAP in conventionally negative cases.

Supplementary Files

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- Supplementarymaterials.doc
- coverletter.docx