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Research Advances in Auxiliary Tests for Tinea Capitis

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Abstract

Tinea capitis is a common superficial fungal infection in China, often leading to inappropriate treatment due to misdiagnosis, which severely impacts patients' quality of life and mental health. Rapid and accurate diagnosis of tinea capitis has become a core challenge in its treatment. In recent years, significant progress has been made in auxiliary tests for tinea capitis. Wood's lamp examination and dermatoscopy-guided sampling can significantly improve the positive rate of fungal fluorescence microscopy. Combining fungal culture, histopathology, and molecular diagnostic techniques substantially shortens pathogen identification time. This review summarizes current auxiliary diagnostic methods for tinea capitis and their research advancements, aiming to provide references for establishing stratified, efficient diagnostic strategies in clinical practice. Particular attention is given to how different diagnostic techniques assist in pathogen species identification, thereby providing a basis for individualized treatment.

Keywords

Tinea Capitis, Auxiliary Tests

1. Introduction

Tinea capitis is a common superficial fungal infection in China. Its atypical clinical manifestations often lead to misdiagnosis as other scalp conditions, resulting in inappropriate treatment and potentially scarring alopecia, which severely impacts patients' quality of life and mental health. Studies indicate that among pediatric tinea capitis patients in China, males predominate (56.2%), often with a history of animal contact (57.4%), and the primary causative agent is zoophilic dermatophyte (73.5%). Adult tinea capitis patients are predominantly female (83.3%),

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often with prior fungal infections elsewhere on the body, with dermatophytes of human affinity being the primary causative agent (53.5%). The most common pathogen in China is the zoophilic Microsporum canis (65.2%), primarily distributed in eastern, western, and northeastern regions; followed by dermatophyte-like *Trichophyton rubrum* (13.6%), predominantly distributed in central regions [1]. Given pathogen variations across age groups, genders, and geographic areas, establishing rapid and precise diagnostic protocols holds critical clinical significance for personalized treatment. Therefore, in reviewing various auxiliary tests, this paper will focus on analyzing their capabilities in bacterial species identification and their significance in guiding treatment selection.

Traditional auxiliary tests for tinea capitis—including Wood's lamp examination, microscopic fungal examination, and fungal culture—have been continuously optimized in recent years. Using Wood's lamp and dermatoscopy to determine sampling sites and select appropriate fluorescent dyes can enhance the positive rate of microscopic examination. Combining molecular diagnostic techniques can shorten the identification time for fungal cultures. However, when faced with atypical clinical presentations and the need for rapid diagnosis, traditional methods still have limitations in sensitivity and timeliness. This has driven extensive research and the application of more efficient and precise new technologies. Dermoscopy provides crucial evidence for tinea capitis diagnosis by identifying characteristic hair shaft alterations such as "comma hair" and "spiral hair". Reflectance confocal microscopy (RCM) enables real-time observation of fungal structures at the cellular level, offering a "non-invasive biopsy" for differentiating tinea capitis from other scalp conditions. Molecular diagnostic techniques enable rapid, highly sensitive pathogen identification and typing, significantly aiding timely tinea capitis diagnosis. Despite the promising prospects of these new technologies, their high costs and lack of standardization remain major obstacles to clinical adoption, requiring a balance between diagnostic value and health economic benefits. This article summarizes auxiliary diagnostic methods for tinea capitis—from traditional to cutting-edge approaches—along with their research progress.

2. Clinical Rapid Screening Techniques

Robert Williams Wood invented the Wood's lamp for ultraviolet photography. Its ability to induce fluorescence in specific substances later led to widespread medical applications. Dermatophytes produce and accumulate specific fluorescent substances during growth and metabolism within hair shafts. Consequently, tinea capitis caused by different pathogens often exhibits distinct fluorescent colors under Wood's lamp. With evolving prevalent pathogens and deeper observations, the spectrum of fluorescent colors associated with various pathogens has significantly broadened beyond traditional understanding, marking the evolution of the Wood's lamp toward a multi-spectral fluorescence mode.

Recent studies have reported that infections caused by *Trichophyton rubrum*

may exhibit pale blue, dark blue, or dark gray-green fluorescence; infections caused by *Microsporum canis*, *Trichophyton rubrum*, and *Trichophyton audouinii* may show blue-green, bright green, or yellow-green fluorescence; while infections with *Trichophyton violaceum* and *Trichophyton rubrum* show no fluorescence [2]-[5].

In a study of 73 patients, Johansen *et al.* found that Wood's lamp had 100% sensitivity, 86% specificity, 100% negative predictive value, and 53% positive predictive value. Thus, a negative result cannot rule out tinea capitis, requiring further mycological examination for definitive diagnosis. Given that *Trichophyton rubrum* is currently one of the common pathogens in China, its lack of fluorescence under Wood's lamp has led to a decrease in the overall sensitivity of Wood's lamp compared to previous studies. Johansen *et al.* also noted that Wood's lamp can assist physicians in accurately locating sampling areas, and combining it with fungal microscopy or fungal culture can improve the positive detection rate [6]. Jing Zeng *et al.* found that in assessing treatment efficacy, fluorescence disappearance under Wood's lamp occurred earlier than negative fungal microscopy results, suggesting potential superior sensitivity for evaluating treatment response. However, limited data currently exist to support this, and fluorescence disappearance cannot yet be considered a cure marker; definitive diagnosis still requires fungal examination [7].

Therefore, Wood's lamp is currently primarily used for rapid screening of tinea capitis and for guiding specimen collection for fungal fluorescence microscopy. Its dynamic fluorescence changes may offer new insights for future exploration of non-invasive efficacy monitoring indicators, but further studies are needed to validate its reliability. However, the Wood lamp cannot distinguish specific bacterial species, and its screening value is limited by the fluorescent characteristics of prevalent bacteria in the region.

3. Gold Standard for Pathogenic Diagnosis

3.1. Microscopic Fungal Examination

Traditional microscopic fungal examination involves applying KOH solution to tinea capitis specimens for direct microscopic observation. This method has been gradually replaced by more sensitive and rapid fluorescent microscopic examination due to its suboptimal imaging quality. The rise of fungal fluorescence microscopy stems from the widespread adoption of novel fluorescent dyes. Commonly used clinical dyes include Calcofluor White (CFW), Trypan Blue (TB), Rhodamine, and Propamidine Orange. Izabella *et al.* demonstrated that TB exhibits significantly longer fluorescence persistence than CFW, indicating superior stability. However, excessive TB concentration may cause precipitation, while insufficient concentration may prevent fungal structures from fluorescing under brightfield illumination. Thus, selecting appropriate types and concentrations of fluorescent agents is critical for fungal fluorescence microscopy [8].

Fungal fluorescence microscopy serves as one of the gold standards for diag-

nosing tinea capitis, offering advantages of simplicity and low cost. However, results are susceptible to sample quality and disease severity, with false-negative rates reaching up to 35% [9] [10]. Inadequate disinfection, inaccurate sampling sites, collection of non-active infected hairs, insufficient scale scraping, or prior antifungal treatment can all lead to false negatives and should be avoided during procedures. Multiple studies indicate that first identifying affected areas with a Wood's lamp, then using a dermatoscope to locate characteristic lesions such as Morse code-like, spiral, or comma-shaped hairs for sampling, significantly improves the positive rate of fungal fluorescence microscopy [6] [11] [12]. Shemer *et al.* demonstrated that non-inflammatory tinea capitis exhibits the highest positive rate in mycological examination, followed by mildly inflammatory cases, with severely inflammatory lesions showing the lowest rate. Antifungal treatment can enhance the sensitivity of mycological examination in severe inflammatory tinea capitis, likely because intense inflammatory reactions combined with bacterial infections may cause false-negative results [13]. Additionally, fungal fluorescence microscopy can only confirm the presence of a fungal infection; it cannot identify the specific pathogen. Since different fungal species exhibit significant variations in their susceptibility to antifungal drugs, this limitation prevents fungal fluorescence microscopy from directly guiding precise drug selection.

Therefore, fungal fluorescence microscopy is currently primarily used for rapid clinical diagnosis. Combining it with Wood's lamp examination and dermatoscopy can enhance sensitivity. However, its limitations in guiding individualized treatment necessitate supplementation with fungal culture or molecular diagnostic techniques.

3.2. Fungal Culture

Fungal culture involves inoculating affected skin or scalp scales onto Sabouraud agar to obtain fungal colonies. After 2 - 4 weeks of incubation, species identification is performed based on colony morphology and microscopic structure. Traditional fungal culture suffers from drawbacks such as lengthy processing times and high technical demands on laboratory personnel. Consequently, it has increasingly been integrated with molecular diagnostic techniques like DNA sequencing and Matrix-Assisted Laser Desorption/Ionization Time-of-Flight Mass Spectrometry (MALDI-TOF MS) in recent years. The advantage of MALDI-TOF MS lies in its ability to rapidly perform high-throughput proteomic profiling of pure colonies obtained from cultures. However, it should be noted that this method typically cannot be directly applied to the analysis of raw clinical specimens, unlike PCR-based approaches. This combined approach significantly reduces the time required to identify dermatophyte pathogens, overcomes the subjectivity of morphological identification, and offers superior discrimination between closely related, morphologically similar species. It facilitates standardized laboratory procedures and high-throughput sample processing, substantially improving workflow efficiency. Concurrently, fungal culture's irreplaceable value in obtaining

pure strains for molecular epidemiological studies, antimicrobial resistance surveillance, and novel drug development has become increasingly prominent.

Thus, fungal culture remains essential for confirming pathogenic species today. Future developments may establish an integrated, efficient workflow combining "culture-based identification-antimicrobial susceptibility testing", enabling it to play a more central role in addressing complex infections and public health challenges.

4. The Gold Standard of Morphological Diagnosis

Histopathology involves microscopic examination of fungal morphology and distribution within scalp biopsy specimens. While long regarded as the morphological "gold standard" for diagnosing tinea capitis, its invasive nature limits routine use, reserving it primarily for confirming challenging cases.

Histopathology does not always directly reveal fungal elements, sometimes presenting solely as folliculitis. Elmas *et al.* found that fungal elements are difficult to observe in histopathological examinations of pyruvata infections, likely due to dense inflammatory infiltration obscuring the pathogens. Guiding biopsy site selection with dermatoscopy—specifically, seeking characteristic dermatoscopic features like "comma hair" at the periphery of inflammatory areas—significantly improves fungal detection rates. Additionally, observing the autofluorescence of fungal components under fluorescence microscopy on periodic acid-Schiff (PAS)-stained sections offers a novel technical approach to enhance detection rates [14].

Traditional histopathology may not reliably distinguish all fungal species. When species identification is uncertain, polymerase chain reaction (PCR) and in situ hybridization can supplement diagnosis [14]. Eckert *et al.* found that PCR targeting the fungal ITS2 region demonstrated significantly higher sensitivity (95%) in tissue sections compared to conventional PAS staining (74%) [15].

Therefore, histopathology has evolved from a static "gold standard" into a dynamic, integrated diagnostic analysis node. Through integration with molecular techniques, it not only confirms tinea capitis but also enables in situ fungal species identification within tissue samples. Future efforts will focus on addressing clinical challenges, such as diagnosing severe inflammatory tinea capitis and differentiating deep fungal infections.

5. Advanced Application of Imaging Technologies

5.1. Dermoscopy

Dermoscopy serves as a non-invasive, visual diagnostic tool for examining the epidermis and reticular dermis, often referred to as the dermatologist's "stethoscope". Its principle combines polarized light imaging and optical magnification to eliminate surface reflections and magnify skin details. Numerous clinical studies have identified diagnostic dermatoscopic features of tinea capitis, including comma-shaped hair, spiral hair, Morse code-like hair, serrated hair, curved hair, clubbed hair, and type I hair. These characteristics aid in distinguishing tinea capi-

tis from other scalp conditions such as seborrheic dermatitis and alopecia areata. Other common but non-diagnostic dermatoscopic features include broken hairs, black dots, perifollicular scaling, and diffuse scaling. These characteristics may also appear in conditions like discoid lupus erythematosus or psoriasis and cannot serve as specific diagnostic criteria for tinea capitis [16] [17]. Additionally, the single hair shaft indicator may suggest tinea capitis but lacks diagnostic specificity, necessitating integration with clinical presentation and other ancillary tests [16] [18]. However, dermoscopy has the limitation of being susceptible to treatment effects. Any oral or topical therapy may obscure characteristic microscopic images, complicating interpretation. Thus, a negative result cannot rule out tinea capitis; comprehensive evaluation through fungal microscopy and fungal culture remains essential. Studies indicate that experienced dermatologists achieve 94% sensitivity and 83% specificity in diagnosing tinea capitis using dermatoscopy. Even dermatology residents in training demonstrate 89% sensitivity and 77% specificity [19].

Dermoscopy can also preliminarily distinguish between microsporum and *trichophyton*-induced tinea capitis. Anna *et al.* found that Morse code-like hair, serrated hair, curved hair, and diffuse scaling occur exclusively in microsporum-induced tinea capitis, while spiral hair was significantly more prevalent in *Trichophyton*-induced tinea capitis. The detection rates of broken hair and the black dot sign showed no significant difference between *Trichophyton* and Microsporum infections [16]. Current findings regarding which type of tinea capitis more commonly exhibits comma-shaped hair are inconsistent and controversial. Anna *et al.* reported no significant difference in the detection rate of comma-shaped hair between *Trichophyton* and *Microsporum* tinea capitis. Studies by Dhaille, Meghwal *et al.* indicated that comma-shaped hairs are more common in *Trichophyton*-induced tinea capitis [19] [20]. Meneses *et al.* found that the curved single-morphology pattern (comma-shaped and spiral hairs) demonstrated 94.9% specificity and 96.4% positive predictive value for *Trichophyton rubrum* infections, while the linear single-morphology pattern (barcode-like hair, serrated hair) showed 85.4% specificity and 82.9% negative predictive value for *Microsporum canis* infections [21]. These discrepancies may stem from various factors, including sample size differences, regional variations in prevalent fungal species, and differing disease severity. The preliminary differentiation of dermatophyte species using dermatoscopy provides important guidance for developing individualized treatment plans. Currently, terbinafine is the first-line treatment for *Trichophyton*-induced tinea capitis, while griseofulvin is the first-line treatment for *Microsporum*-induced tinea capitis [19]. Research by Su Chenlin *et al.* demonstrated that dermatoscopy exhibits higher sensitivity than microscopic fungal examination during mid-to-late treatment phases, offering greater value for monitoring clinical efficacy [12].

Consequently, dermatoscopy is primarily used today for tinea capitis screening, preliminary classification of tinea types, and guiding specimen collection for fun-

gal fluorescence microscopy. Further confirmation of the causative pathogen requires additional tests, such as fungal culture.

5.2. Reflective Confocal Microscopy (RCM)

As a non-invasive, real-time, in-vivo imaging technique, RCM enables a "non-invasive biopsy" of scalp tissue at the cellular level. Its principle involves emitting monochromatic coherent light from a near-infrared low-power laser onto the skin, generating images from the reflected light. Compared to dermatoscopy, RCM achieves a leap from the tissue level to the cellular level. Traditionally, RCM provides intuitive diagnostic evidence for tinea capitis by directly visualizing fungal hyphae and spores within hair shafts and the stratum corneum. However, recent research has expanded its role beyond pathogen observation to include differential diagnosis of inflammatory scalp disorders and treatment monitoring. It plays a crucial role in managing challenging cases with atypical clinical presentations or negative mycological results.

RCM identifies inflammatory response patterns, yielding results highly consistent with histopathology, thereby bridging clinical and pathological findings. It also allows observation of follicular distribution and density, hair shaft integrity, and length, providing objective metrics for pre-treatment baseline assessment and treatment efficacy evaluation during therapy [22]. Furthermore, correlating and cross-referencing dermatoscopy with RCM creates a non-invasive diagnostic loop from macro- to micro-levels, enhancing diagnostic accuracy. Applying RCM to evaluate efficacy endpoints in clinical trials of novel antifungal drugs or to monitor treatment responses in refractory cases represents a cutting-edge area of exploration.

Currently, RCM still lacks large-scale studies to establish diagnostic criteria for different diseases. The future development of standardized diagnostic consensus for RCM and the construction of image databases will further unlock its immense potential in precision dermatology.

6. Molecular Diagnostic Technologies

Methods such as Wood's lamp examination, fungal fluorescence microscopy, and fungal culture each have distinct limitations, driving the development of molecular diagnostic technologies with higher sensitivity, specificity, and speed. Molecular diagnostics centered on nucleic acid amplification and sequencing have propelled tinea capitis diagnosis from reliance on phenotypic observation into an era of precision based on genotypic identification. The most prevalent molecular diagnostic techniques currently include various PCR methods and MALDI-TOF MS.

Conventional PCR employs universal primers to amplify the fungal rDNA ITS region followed by sequencing. However, this method demands high template DNA quality and quantity while being prone to non-specific binding. Nested PCR represents an improvement over conventional PCR, primarily in-

volving secondary amplification of fragments amplified by conventional PCR. This reduces non-specific binding of the initial PCR products, enhancing specificity and sensitivity. However, the secondary amplification process carries a higher risk of contamination and prolongs diagnostic time [23]. Multiplex PCR enables simultaneous detection of multiple targets within a single reaction, completing the process from DNA extraction to result interpretation within hours. It is suitable for scenarios such as negative mycological test results, contaminated cultures, and clinical mixed infections. However, a drawback is that primers may form dimers, potentially affecting results [9]. Real-time PCR employs specific probes to minimize contamination risks, eliminating the need for post-PCR detection and gel electrophoresis analysis. It also provides quantitative data on microbial load in samples, facilitating disease severity assessment. Sebastian *et al.* demonstrated that real-time PCR achieves an identification rate of 82.9% for dermatophytes [24].

MALDI-TOF MS identifies fungi by analyzing fingerprint spectra of protein extracts. It should be clarified that MALDI-TOF MS is typically used for rapid identification of pure colonies obtained from culture, rather than for direct detection of clinical specimens. This positions it in the diagnostic workflow after the culture step. Sebastian *et al.* reported a dermatophyte identification rate of 97.2% for MALDI-TOF MS [24]. Current limitations of MALDI-TOF MS primarily stem from insufficient representation of dermatophyte species in the reference spectral library of the identification system, coupled with the lack of a comprehensive database for rapid consultation [9]. Future research is needed to expand the reference spectral library.

While the aforementioned molecular diagnostic techniques significantly enhance the sensitivity, specificity, and timeliness of tinea capitis diagnosis, their drawback lies in demanding high laboratory standards, making widespread adoption in primary care hospitals challenging. Extending molecular testing beyond laboratories to primary care settings is crucial for improving diagnostic accessibility, a gap effectively addressed by isothermal amplification technology. Isothermal amplification techniques enable nucleic acid amplification using portable devices at constant temperatures, yielding results within 30 - 60 minutes. Examples include recombinase polymerase amplification (RPA) and loop-mediated isothermal amplification (LAMP). Currently, loop-mediated isothermal amplification (LAMP) kits targeting specific pathogens such as *Trichophyton rubrum* and Candida species are available. Developing point-of-care isothermal amplification detection kits for common pathogens that cause tinea capitis is a key direction for advancing molecular diagnostics at the primary care level.

Although molecular diagnostics carry higher costs, from a health economics perspective, they may offer long-term cost-effectiveness advantages by enabling rapid and precise pathogen identification. This approach helps prevent prolonged illness, recurrence, transmission, and additional medical expenses resulting from misdiagnosis or inappropriate empirical treatment.

7. Limitations of This Review

This systematic review of research progress in auxiliary tests for tinea capitis nevertheless has certain limitations. First, the cited studies may exhibit publication bias. Second, the review primarily references data from Chinese populations, meaning conclusions may be more applicable to regions with similar epidemiological contexts, and their global applicability requires further validation. Future work necessitates more multicenter, large-sample prospective studies to validate and optimize the diagnostic strategies proposed herein.

8. Clinical Diagnostic Pathway Recommendations

Based on the advantages and limitations of the aforementioned techniques, we propose a preliminary tiered diagnostic workflow for clinical application as a reference: 1) For patients suspected of having tinea capitis, prioritize rapid screening with Wood's lamp to guide specimen collection; 2) Combine with dermatoscopy to identify characteristic hair changes, and perform fungal fluorescent microscopy on active lesions to achieve rapid diagnosis; 3) If fungal microscopy is positive but clinical presentation is atypical, treatment response is poor, or precise medication is required, proceed with fungal culture; 4) Following positive culture, select MALDI-TOF MS or PCR sequencing for species identification based on laboratory capabilities; 5) For challenging cases with severe inflammation or diagnostic uncertainty, consider dermoscopy-guided biopsy for histopathological examination combined with molecular techniques like PCR for identification; 6) Utilize RCM as a complementary tool for non-invasive differential diagnosis and treatment monitoring. This workflow aims to balance diagnostic speed, accuracy, and cost to achieve personalized diagnosis and treatment.

9. Conclusion

In summary, tinea capitis diagnosis has evolved from reliance on clinical experience and single tests to a tiered, efficient diagnostic system combining multiple techniques. Wood's lamp sensitivity remains unsatisfactory and is generally used only as a rapid screening tool. Fluorescent microscopy, guided by Wood's lamp or dermatoscopy to optimize sampling sites, improves fungal positivity rates and serves as a common rapid clinical diagnostic method. Combining fungal culture with molecular diagnostics shortens identification time but remains limited by high laboratory requirements. Dermoscopy and RCM enable non-invasive microscopic examination, yet standardized diagnostic criteria supported by sufficient data remain lacking. Molecular diagnostics achieve rapid, precise pathogen identification and resolution of mixed infections, but high costs limit their adoption. Future research should focus on: 1) Developing AI-assisted automated dermatoscopic image recognition systems to enhance diagnostic objectivity and efficiency; 2) Expanding dermatophyte protein profiling databases using technologies like MALDI-TOF MS to improve species identification coverage; 3) Developing low-cost, portable point-of-care isothermal amplification devices for common scalp

ringworm pathogens to advance molecular diagnostics at primary care levels; 4) Conducting cost-effectiveness studies grounded in health economics to provide evidence-based support for optimizing diagnostic strategies across healthcare tiers. Through technological innovation and process optimization, a more accessible and precise diagnostic system for scalp ringworm can be established.

Authors' Contributions

Conceptualization: Faqing Huang; Writing—original draft preparation: Qianqian Wu; Manuscript critical revision: Shan Su; Writing—review and editing: Faqing Huang; Supervision: Faqing Huang.

Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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