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Review article

Diagnosing nontuberculous mycobacterial cervicofacial lymphadenitis in children: An updated systematic review

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ABSTRACT

Background: Nontuberculous mycobacterial (NTM) cervicofacial lymphadenitis is an emerging infectious disease that affects children. Regarding the correct diagnosis of NTM cervicofacial lymphadenitis, there is widespread controversy. This updated systematic review aimed to reevaluate the efficacy and safety of diagnostic methods for NTM cervicofacial lymphadenitis. **Method**: We systematically searched the databases PubMed, Web of Science, Embase, and Google Scholar (from March 3, 2017, through January 11, 2023) to detect relevant studies, using Mesh keywords. The inclusive criteria were i) NTM cervicofacial lymphadenitis studies, ii) Reports on patients under 18 years old, iii) Reports on diagnostic methods. Immuno-incompetent patients, studies that reported adults, and non-clinical studies (laboratory studies, technical notes, letters to editors, reviews) or case reports/series were excluded. **Results**: 512 patients between the ages of 0 and 18 are included in eight research studies. Diagnostic accuracy of acid-fast bacilli (AFB) staining, histology, culture, polymerase chain reaction (PCR), tuberculin skin test (TST), immunodiagnostic assays, enzyme-linked immunosorbent spot assays, and radiomic features were studied. Culture sensitivity was 67.2%, while PCR sensitivity was 92%, AFB staining specificity was between 80 and 100%, and sensitivity was between 46 and 85%. Using radiomic markers to differentiate benign from malignant lymphadenopathy has a specificity of 93% and sensitivity of 91%. **Conclusion**: This review indicates that isolation of the specimen by PCR or culture is still required for the precise identification of mycobacterial infections. While TST cannot distinguish between tuberculosis (TB) and NTM, interferon-gamma release assays (IGRAs) and radiomic analyses show promising sensitivity and specificity and reduce the need for invasive procedures. While TST is not able to distinguish between TB and NTM, IGRA appears to perform more efficiently. IGRA with PPD stimulation appears to be an effective approach for the diagnosis of NTM cervicofacial lymphadenitis.

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Introduction

Lymphadenopathy is the term used to describe an irregularity in the size and texture of lymph nodes, while lymphadenitis is the term used to describe the enlargement of lymph nodes followed by inflammatory and infectious disorders [1].

When lymphadenopathy lasts more than three weeks in children and does not resolve with different antibiotics, we should suspect a non-tuberculous bacterial infection [1]. Nontuberculous bacteria are ubiquitous organisms that tend to infect young and immunocompromised individuals. Children are affected by consuming contaminated substances and clinical infections caused by *Mycobacterium avium* [2,3]. A collection of 190 mycobacteria species other than *Mycobacterium tuberculosis* and *Mycobacterium lepra* are referred to as non-tuberculous mycobacteria (NTM) [4,5]. Children under the age of five are more susceptible to nontuberculous *Mycobacterium* lymphadenitis (NTML), a significant major cause of persistent head and neck disease [2].

The average age of this disease is three years, and it rarely occurs at the age of 12 [6]. This disease usually turns into chronic and unilateral lymphadenopathy with mass and single colonies or enlarged clusters, mainly in the area under the face and neck or before the ear. Purulent lymph nodes and skin discoloration occur. Finally, if the discharge occurs spontaneously, it can lead to a change in the shape of the skin around it[6].

Environmental factors, such as climate and food changes, may influence the epidemiology and spread of NTM in children [7]. NTM lymphadenitis is uncommon but becoming more common in children and adolescents. Timely disease diagnosis necessitates suspicion, and communication between doctors and laboratories is required for NTM diagnosis [8].

It has been reported that the annual incidence of non-tuberculous mycobacterial lymphadenitis has reached 3.7 new cases per 100,000 kids under the age of five [9]. Recent reports also show an increase in annual incidence in older children. Lymphadenopathy is usually unilateral, often painless, and involves the submandibular lymph nodes, and the spread is reported in more than 15% of patients who show spontaneous drainage through the sinus tract [9].

Although the yield reported in cultivation data is only about 50%, tuberculin skin testing and acid-fast staining may demonstrate *Mycobacterium* as the causative agent, which may then be confirmed by a blood test that evaluates the interferon-gamma release factor, polymerase chain reaction (PCR), and culture [2]. Children can get a variety of illnesses from non-tuberculous mycobacteria, such as lymphadenitis, infections of the soft tissue and skin, respiratory illnesses, etc. The ability to diagnose a condition requires a high index of clinical suspicion, the collection of adequate samples for culture in specialist settings, and the use of molecular tools [10].

In this systematic review, we decided to review all diagnoses of nontuberculous mycobacteria in children. The systematic review is an update on a previously published systematic review with the same title, which updates the search from March 2017 to January 2023.

Methods

A previous systematic review appraising the diagnostic methods of nontuberculous mycobacterial cervicofacial lymphadenitis in children [11] only included up to 10 studies. A large amount of information was discarded in data extraction. In recent years, many studies regarding diagnostic methods of nontuberculous mycobacterial cervicofacial lymphadenitis in children have been published, but still, the efficiency of these methods is still under debate. Therefore, we carried out the current updated systematic review to reevaluate the efficacy and safety of diagnostic methods of nontuberculous mycobacterial cervicofacial lymphadenitis in children.

Protocol and registration

The present systematic review was performed by the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) statement [12]. The study protocol is registered in Open Science Framework (https://osf.io/registries/drafts/636151ce11b333002009b8bc/review).

Search strategy

A systematic search was conducted in databases of PubMed, Web of Science, Embase, and Google Scholar (from March 3, 2017, through January 11, 2023) to detect the relevant studies.

The search strategy included a combination of keywords and Medical Subject Headings (Mesh)

terms related to “Lymphadenitis” AND “Nontuberculous mycobacteria” AND “Children”. The full search strategy used for all databases is summarized in Supplementary materials **(Table 1)**. We didn’t consider any language restrictions.

Eligibility criteria

Studies that matched the mentioned inclusion criteria were included: (1) studies reporting cases of Nontuberculous mycobacterial cervicofacial lymphadenitis; (2) studies reporting patients below the age of 18 years old; (3) Studies reporting diagnostic methods.

The exclusion criteria were as follows: (1) the studies included immuno-incompetent patients or children receiving immunosuppressive therapy; (2) studies that reported adults; (3) non-clinical studies (laboratory studies, technical notes, letters to editors, reviews) or case report/series. Furthermore, additional relevant studies were discovered by hand-searching the references of articles included in the primary search.

Study selection

After removing duplicate records, all titles and abstracts were screened by two reviewers (Narges Norouzkhani and Nasrin Mohammadi) independently. Discrepancies were resolved by consensus or using a third reviewer (Niloofar Deravi). When studies met the inclusion criteria, two authors (Sepide Mohit, Farzad Sheikhzadeh) obtained and independently analyzed the full texts. When there was a disagreement between reviewers, a third author (Niloofar Deravi) was consulted. Eventually, studies that did not match the inclusion criteria were excluded from participation. The screening process is shown by the PRISMA checklist in **Figure 1**.

Quality assessment

The evaluation of quality and risk of bias of all the studies that met the inclusion criteria was conducted by two reviewers independently using the Joanna Briggs Institute (JBI) Critical Appraisal tools (https://jbi.global/critical-appraisal-tools).

This instrument was used to assess the reporting or methodology of all types of studies. Ten questions make up the JBI tool for qualitative studies; each question has four possible answers: (1) yes, (2) no, (3) unclear, and (4) not applicable. Each "yes" response results in a score, and a study, if 70% of the questions were replied "yes," bias risk was assumed to be "low," if 50% to 69% of the questions were answered "yes," "moderate," and if less than 50% of the questions were answered "yes," "high risk," respectively. Conflicts were settled through consensus.

Data extraction

Two reviewers (Sepide Mohit, Farzad Sheikhzadeh) independently extracted data from articles using a preprepared standardized template. Authors extracted 1) patient demographics and study characteristics, 2) diagnostic method, 3) specificity, 4) sensitivity

If there was a disagreement between reviewers, a third author (Niloofar Deravi) was consulted.

Results

After searching in (PubMed, Embase, Google Scholar) databases total of 232 number articles were obtained and 60 duplicates were Removed. After reviewing the title & abstract screening, 81 studies remained.

This systematic review includes 8 articles with a total of 512 participants aged 0–18 years. The rest of the studies with unrelated data were deleted. Each of Canada [9], Korea [8], Australia [7], Finland [13], Spain [14], France [15], Cyprus [16], and Italy [17] has conducted one study. These studies included 4 retrospective articles [7-9, 15], 1 one case report [16], 1 one retrospective observational [13], one1 retrospective and prospective [14], and one1 prospective [17].

After conducting the quality assessment, all studies included in this systematic review were classified into the 'low' risk of bias group.

The follow-up period is between 8 months and 3.7 years. All patients included culture-confirmed cases of NTM lymph nodes [16]. All these studies were published between 2018 and 2022.

The employed diagnostic methods include Radiomic features [9], Culture [7, 16], TST [8, 14, 16], Modified interferon gamma release assay (IGRA) [13], IGRA [8, 14], Sonography, Acid-fast bacteria culture, RT-PCR Histopathology [8], PCR, Culture, Histology, PPD Clinical appearance of NTM lymphadenitis, Differential diagnoses exclusion [15], M. avium lysate IFN-γ ELISPOT Assay, M. avium lysate IL-2 ELISPOT, M. avium lysate IL-17 ELISPOT assay [17].

The results obtained from diagnostic tests include computed tomography (CT) -scan [9], fine needle aspiration (FNA) /biopsy [7], excisional biopsy [8,16], palpable induration and visible

erythema [8,14,16], and blood sampling [8,13,14,17].

These findings demonstrate that radiomics can classify NTM lymphadenitis with greater accuracy. Such a noninvasive, highly accurate diagnosis may lessen the necessity for invasive procedures in the pediatric population [9]. NTM cultures obtained by FNA compared with those obtained by biopsy were 45.5% vs. 36.4% (p = 0.07) [7]. After the adoption of the modified IGRA test as a diagnostic tool for NTM lymphadenitis, the need for biopsy decreased rapidly [13]. IGRAs are valuable tools in diagnosing the diagnosis of children with lymphadenopathy, especially when culture and polymerase chain reaction results are negative [14]. Children with non-tuberculous mycobacteria lymphadenitis can be distinguished from healthy children using immunospot assay based on *Mycobacterium avium lysate*, IL-2, and interferon gamma (INF-g) enzymes [17].

The entire data is shown in **Table 1.**

Table 1. The information of included studies.

No.	Author (year) reference	Country	Type of Study	Population	Follow-up duration	Diagnostic test(s)	Diagnostic Test(s) result Derived from	Key Points
1.	Al Bulushi Y., *et al*. [9]	Canada	retrospective	180 pediatric patients aged 0–18 years who presented with lymphadenopathy to the Montreal Children's Hospital	3years	Radiomic features	CT-scan	• The model classifying nodes as pyogenic, NTM, reactive, or proliferative lymphadenopathy achieved an accuracy of 72%, a precision of 68%, and a recall of 70%. Between NTM and all other causes of lymphadenopathy, the model achieved an area under the curve (AUC) of 89%. Between NTM and pyogenic lymphadenitis, the model achieved an AUC of 90%. Between NTM and the reactive and proliferative lymphadenopathy groups, the model achieved an AUC of 93%. These results indicate that radiomics can achieve a high accuracy for the classification of NTM lymphadenitis. Such a non-invasive, highly accurate diagnostic approach has the potential to reduce the need for invasive procedures in the pediatric population.
2.	Olivas-Mazón R., *et al*. (2021) [7]	Australia	retrospective	41 children with nontuberculous mycobacterial lymphadenitis	8 months	Culture	FNA biopsy	• Sensitivity of NTM cultures obtained by FNA compared to biopsy was 45.5% vs. 36.4% (p = 0.07). • Two patients with previous skin alterations presented fistulas after FNA (4.9%); no other complications were described.
3.	Loizos, A.*et al*.(2018)[16]	Cyprus	Case series	22 children between 16 to 55 months with culture-positive lymphadenitis caused by NTM (50% female)	2 years	Culture TST	Excisional biopsy Palpable induration And visible erythema	• All patients have consisted of culture-confirmed cases of NTM lymphadenitis. • The tuberculin skin test revealed an induration in 81.0% of cases (diameter ranging from 4 – 17 mm) with a median diameter of 7 mm. The induration diameter was ≥ 10 mm in 33.3% of cases and ≥ 15 mm in one case (4.8%).

4.	Lyly, A. *et al*. (2019)[13]	Finland	Retrospective observational	52 children under 16 years with cervicofacial NTM lymphadenitis (63% female)	The median active follow-up was 8.2 months, and the median passive follow-up time was 3.7 years	Modified IGRA	Blood sampling	• 49 patients (94%) were tested with the modified IGRA test. All of them had a positive reaction to PPD stimulation and negative reactions to MTB-specific antigens indicative of NTM infection. • The diagnosis can be achieved in a few days, after which the treatment outlines can be discussed with the parents. • After the adoption of the modified IGRA test as a diagnostic tool for NTM lymphadenitis, the need for biopsies quickly diminished.
5.	Martínez-Planas, A.*et al*(2021)[14]	Spain	retrospective and prospective	78 children with confirmed (culture/PCR) MAC lymphadenitis (43.6% female) and 34 with confirmed (culture/molecular assay) TB lymphadenitis (52.9% female).	-	TST IGRA	Palpable induration and visible erythema Blood sampling	• Among MAC cases, 44 out of 74 (59.5%) had positive tuberculin skin test (TST) results at the 5-mm cut-off, compared with 32 out of 33 (97%) TB cases (P < .001); at the 10-mm cut-off TST results were positive in 23 out of 74 (31.1%) vs 26 out of 31 (83.9%), respectively (P < .001). • IGRA results were positive in only 1 out of 32 (3.1%) patients with MAC who had undergone IGRA testing, compared with 21 out of 23 (91.3%) TB cases (P < .001). • IGRAs had a sensitivity of 91.3% (95% CI 73.2%-98.4%), a specificity of 96.9% (95% CI 84.3%-99.8%), a positive predictive value of 95.4% (95% CI 78.2%-99.8%), and a negative predictive value of 93.9% (95% CI 80.4%-98.9%) for TB lymphadenitis. • In contrast to TST, IGRAs have high specificity, negative predictive value, and positive predictive value for TB lymphadenitis in children with subacute/chronic lymphadenopathy and consequently can help to discriminate between TB and MAC disease. Therefore, IGRAs are helpful tools in the diagnostic workup of children with lymphadenopathy, particularly when culture and polymerase chain reaction results are negative.
6.	Park SG *et al*. (2019)[8]	Korea	Retrospective	4 pediatrics confirmed with NTM lymphadenitis, 4 pediatrics with presumptive NTM lymphadenitis, 2 pediatrics with suspected NTM lymphadenitis.	18 months	TST IGRA Sonography Acid fast bacteria culture RT-PCR Histopathology	Excisional biopsy Palpable induration and visible erythema, Blood sampling	• TST was performed in six patients, where five had positive results with induration between 10–15 mm. • IGRA was done in nine patients, in which eight were negative while one reported indeterminate in multiple tests. • Ultrasonographic evaluation was done for eight patients, where none showed abscess formation, three showed perinodal fat swelling, two presented as cystic masses, and one had internal stippled calcification. • Tissue samples were obtained from nine patients via incision and drainage (I&D), FNA, or excisional biopsy, and none showed the presence of bacteria under AFB smear and fungus under GMS/PAS staining. • *M. haemophilum* was identified from two samples under AFB culture, both of which were positive after 48–50 days of growth. • Tuberculous (TB)-PCR was done on eight fresh tissue samples, and all were negative.

								• RT-PCR for MTB/NTM was done on all (n = 9) FFPE specimens. • All samples were negative for MTB, and two samples were positive for NTM
7.	Gallois, Y.*et al*.(2019) [15]	France	Retrospective study	29 patients with a median age 2.40 ages (13 boys/16 girls) treated for NTM	Median follow-up was 8.00 months	PCR, Culture, Histology, PPD Clinical appearance of NTM lymphadenitis, Differential diagnoses exclusion	-	• According to the results of the different diagnostic methods, 3 diagnostic groups could be established: o The "proven diagnosis" group included all 17 patients with NTM found on culture and/or PCR. o The "highly probable" diagnosis group included 5 patients with caseous granulomas on histology, differential diagnoses having been excluded. o The "possible diagnosis" group corresponded to 7 patients with a typical clinical appearance of NTM lymphadenitis, differential diagnoses having been excluded. • Eighteen patients (62.07%) had a PPD test. Nine injections produced induration, the width varying from 4 to 20 mm, and 9 injections had no induration. Five cases (27.78% of PPD tests) were greater than 10 mm.
8.	Della Bella, C *et al*. (2019) [17]	Italy	Prospective	16 children with confirmed NTM infection (group 1), 30 probable NTM infected (group 2), 14 NTM uninfected (group 3)		M. avium lysate IFN-γ ELISPOT Assay, M. avium lysate IL-2 ELISPOT, M. avium lysate IL-17 ELISPOT assay	Blood sampling	• Statical analyses showed 87.5% sensitivity and 85.7% specificity in discriminating between group 1 and group 3 considering response to M. avium lysate by IL-2 ELISPOT assay, • IFN-γ ELISPOT assay performance was poorer. Sensitivity and specificity in discriminating between group 1 and group 3 were respectively 81.3%and 71.4%, • Even poorer was the performance of M. avium lysate IL-17 ELISPOT assay. ROC analyses demonstrated 50% sensitivity and 71.4% specificity. • *Mycobacterium avium lysate* IL-2 and INF-γ-based enzyme linked immunospot assays seem to be promising noninvasive diagnostic techniques for discriminating against children with nontuberculous mycobacteria lymphadenitis and noninfected subjects.

Figure 1. PRISMA 2020 flow diagram of the review process and included studies.

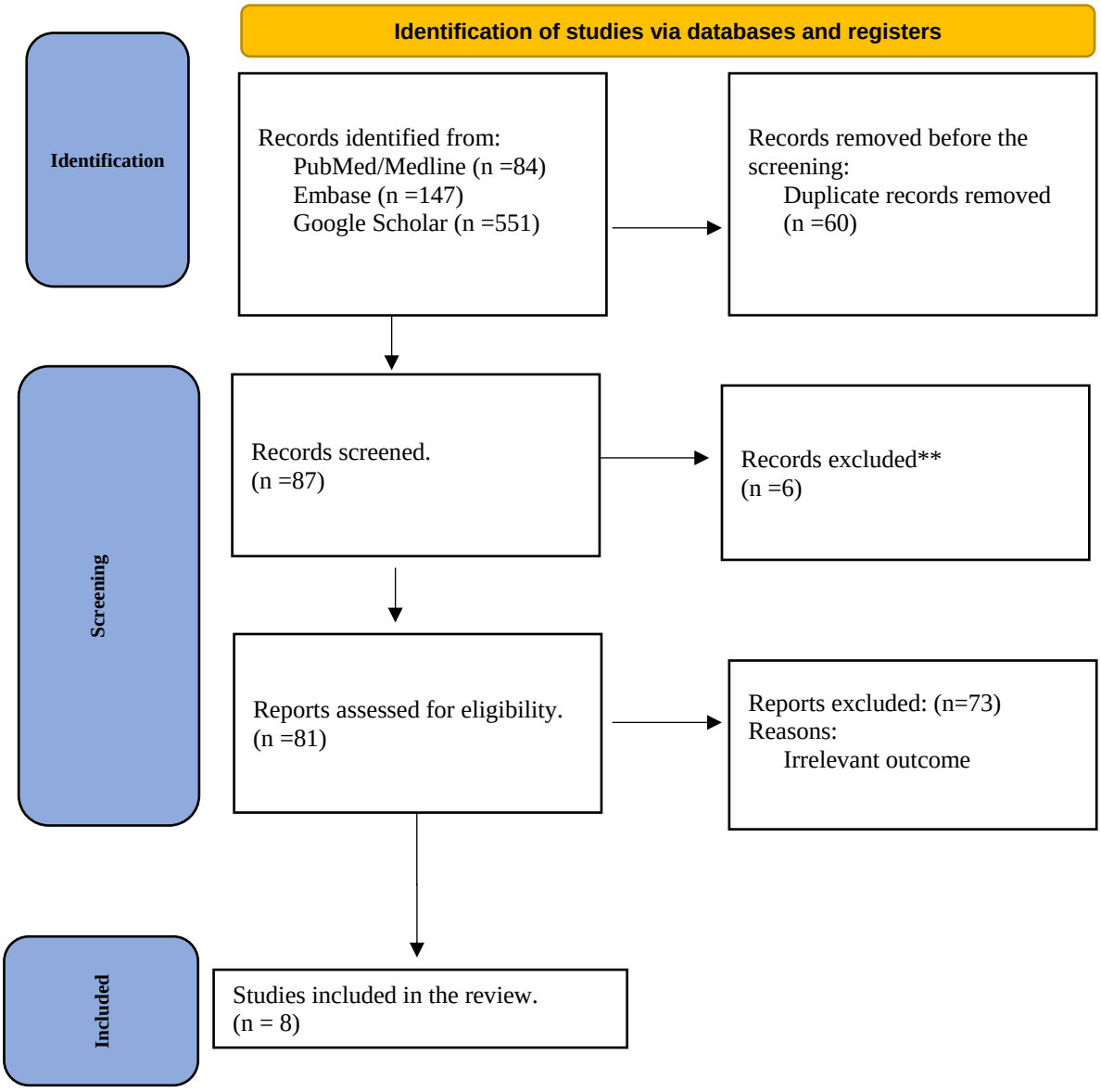
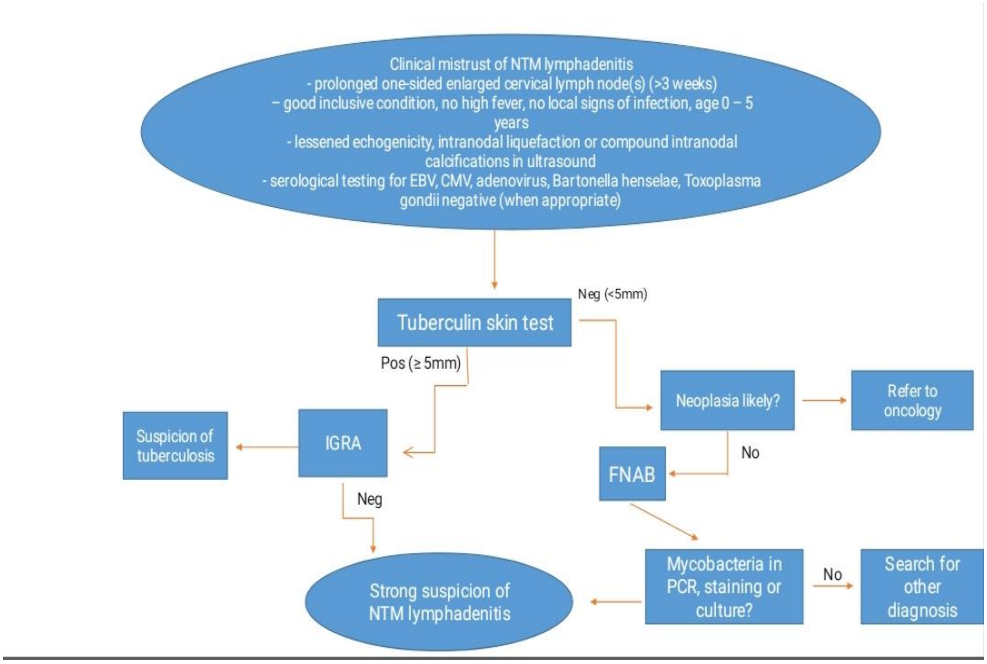


Figure 2. Proposed algorithm for diagnosing NTM lymphadenitis.



Discussion

The American Thoracic Society (ATS) has noted in its guidelines the uncertainty regarding the management of nontuberculous mycobacterial (NTM) infection in children [18]. In this study, we reviewed the existing evidence on NTM cervicofacial lymphadenitis diagnosis in children.

Clinical and Laboratory Findings

The NTM lymphadenitis typically manifests as painless unilateral lymphadenopathy, and the high anterior cervical lymph nodes and submandibular region are often involved [16,19,20].

Prodromal symptoms are often absent and have only been reported in less than 25% of cases [13,19,21,22].

Although the clinical presentations of most of the patients with NTM lymphadenitis are similar, due to the potential overlap with features of other disorders such as tuberculous lymphadenitis, proliferative lymphadenopathy, and pyogenic lymphadenitis, the diagnosis remains challenging for clinicians [23, 24].

On the other hand, inflammatory parameters such as C-reactive protein, erythrocyte sedimentation rate, and white blood cell counts are often in normal ranges or show modest elevation [25].

Hence, the lack of specific clinical markers has resulted in the utilization of other methods for diagnosing NTM lymphadenitis in current clinical practice [14,19,22,23,26,27].

Imaging Findings

Several potential imaging markers have been suggested for NTM lymphadenitis by previous studies. Most NTM lymphadenitis patients have been found to feature peripherally enhanced centrally cystic nodes, asymmetric lymphadenopathy, limited surrounding inflammation, and overlaying skin thickening [28, 29]. NTM lymphadenitis has also been reported to be related to hypoechogenicity and intranodal liquefaction on ultrasonography [30].

In a study by Della Bella *et al*., after comparing ultrasound findings between children with and without NTM infection, colliquative necrosis was significantly associated with NTM infection in children [31].

Another investigation revealed several recurrent NTM results, including central cystic alternations in 92% of patients, reduced nodal echogenicity in 100% of instances, and a majority of patients' involvement to be unilateral. However, this research did not compare these findings to other cervical lymphadenopathy sources, and these symptoms are not particular [30].

On CT scans, it has been demonstrated that in up to 90% of NTM cases, there is a relative lack of moderate or severe surrounding soft tissue stranding. Still, it can also present in other disorders such as proliferative lymphadenopathy or other infections [28, 29, 32].

Therefore, since imaging findings are not specific to NTM, diagnostic models have relied on other diagnostic methods in current practice [26, 28-30, 32-34].

Tissue Diagnosis

When there is diagnostic uncertainty, tissue diagnosis is essential for the clinical diagnosis confirmation, and a biopsy of the lymph node should be sent for AFB staining, histology, culture, or polymerase chain reaction (PCR) [35-37].

In histopathological studies, the presence of granuloma formation and caseation support the diagnosis, but it remains essential to exclude other disorders with similar histological findings, such as Tuberculosis (TB). So, to verify the type of bacteria and its antibiogram, NTM-specific microbiological studies such as PCR or culture are required [15, 35-38].

Culture continues to be the gold standard for NTM diagnosis. Although it has a sensitivity of 67.2%, NTM proliferation typically takes 56 days on solid media and 42 days in liquid media, which delays diagnosis and the start of treatment [39].

PCR is the most sensitive (92%) laboratory test, and it can detect NTM infection more quickly, as results are available in 12–48 hours [40].

AFB staining has also been applied with a specificity of 80–100% and a sensitivity of 46–85% [41,42].

As another important factor, the quality of the specimen can also affect the performance of the mentioned tests. So, a standardized approach for collecting and processing microbiological specimens is crucial for achieving accurate diagnostic test results.

According to the current evidence, the isolation of mycobacterial specimens can be performed through three different methods; biopsy,

fine needle aspiration cytology (FNAC), or complete excision [41].

FNAC, frequently used in adults, is slowly gaining acceptance in pediatrics as a safe, simple, rapid, and cost-effective method [43,44].

FNAC can accelerate the diagnosis, avoiding unnecessary excisional biopsies in those patients where a prompt surgical approach is suggested [44-47].

In a study by Olivas-Mazón *et al*., FNAC provided rapid cytopathological information with a similar rate of positive cultures to an excisional biopsy [23]. In this study, cultures obtained using FNAC yielded NTB isolation in 45.5% of cases compared to 36.4% of cultures obtained using excisional biopsy [23].

In line with these findings, some publications have suggested yield for specimens obtained from FNAC is not necessarily lower [21,48]. For instance, NTM isolation from 93 of 115 cases (81%) using simply FNAC was described by Zeharia *et al*. [49].

Some previous studies have associated FNAC with an increased risk of fistula formation in NTM lymphadenitis [50,51]. Recent research, however, has not supported this association because there are few group variations in the rate of fistula formation [13,23].

Olivas-Mazón *et al*.. showed that FNAC could provide reliable material for cytopathologic investigations and even a higher sensitivity rate for culture than excisional biopsy in the study of suspected NTM lymphadenitis as an accurate and safe technique with no important complications in children [23]. They reported that out of 41 patients who underwent FNAC, fistula formation was reported in only two cases, and both of them had severe skin alternations before the FNAC, so they had a potential risk of spontaneous fistula formation even without FNAC [23].

In a different study by Lyly *et al*., there was a slight difference in the rate of fistula formation between the groups that underwent fine needle biopsy and those that did not (79% vs. 68%, respectively) [13]. The stated fistula formation rate in certain instances might have started many weeks after FNB [13]. Consequently, a direct link between fine needle biopsy and the development of fistulas could not be established [13]. If the FNAC is performed correctly, there is no important complication [52].

On the other hand, some investigations have suggested that sampled material obtained by FNAC is often insufficient for culture [51]. For this reason, FNAC was not performed in a study by Naselli *et al*.., and they preferred an excisional biopsy [21]. According to Park *et al*., *M. haemophilum* could only be detected in samples obtained through incision and drainage or excision, highlighting the significance of obtaining enough tissue to increase the NTM recovery yield [8].

However, the sensitivity of culture has been demonstrated to be around 41.8% after excisional biopsy, and despite 100% specificity, and sensitivity of PCR is reported to be 71.6% [41].

Recent research indicates that while FNAC is not required for the diagnosis of NTM lymphadenitis, it is a promising technology that aids in the process. Recent research seems to support the notion that FNAC is a straightforward, safe, and well-tolerated technique. If this approach is used by a qualified expert, it offers trustworthy samples for cytopathological analyses and adequate sensitivity for culture.

Skin testing

The tuberculin skin test (TST) is frequently used to diagnose latent TB infection and as an additional diagnostic tool when active TB is suspected. TST uses purified protein derivative (PPD) as the test reagent [40].

Various proteins from NTM, MTB, and *Mycobacterium bovis* BCG are found in PPD. In light of this, a positive antigen response to PPD in the test may indicate BCG vaccination, MTB infection, or NTM infection [13,40]. In children with NTM infection, positive TST results have been observed in 30% to 60% of cases [25,53-55].

In a study conducted by Gallois *et al*. [15], they found that among the 18 PPD tests performed in children with NTM, only 5 cases had induration >10 mm, four4 patients had induration <10mm, and 9 patients didn't have any reaction. The sensitivity for all mycobacterial infections is 32% to 44%, and only one-third of patients present with induration >10mm, which is consistent with the most recent data in the literature [36,56].

In another study by Loizos *et al*., the TST showed an induration in 81.0% of patients. In this study, the median induration measured by TST was 7 mm, although in one-third of patients, it was greater than 10 mm. Only one patient had M.

Kansasii-related induration that was greater than 15 mm in size. [15].

In one other study by Olivas-Mazón *et al*., positive TST (> 5 mm) was present in only 25.0% (7/28) of patients with NTM lymphadenitis [23]. Whereas in one other study, the induration diameters were more prominent, reaching ≥15 mm in more than half (59%) of the patients [57]. A TST was performed on six patients in a different study by Park *et al*., and five (83%) of them obtained positive results with induration between 10-15 mm [8].

These variations in induration diameter could be caused by the method used to measure skin induration or by different NTM species and subspecies that are unique to the region. So, it seems we cannot define a unique induration diameter cut-off for diagnosing NTM.

According to Martnez-Planas *et al*., MAC patients had smaller induration diameters and a reduced TST positivity rate; however, this test → could not was unable to distinguish between *Mycobacterium avium* (MAC) infection and TB infection [14]. These findings are concordant with previous studies suggesting that positive TST results can happen in both TB and NTM infections [13, 25, 53-55,58]; hence, the tuberculin PPD test alone is not an excellent a good positive diagnostic method, as it lacks sensitivity. Surprisingly, in another study, Delle Bella *et al*.. reported that 60% of negative TSTs were in confirmed NTM-infected children [31]. Regarding this issue, to improve the sensitivity of TST, some studies have recommended using antigens derived from NTM species. Still, several studies reported a high percentage (6–32%) of false positive results [59,60]. Moreover, most PPDs prepared from NTMs, such as *M. avium* skin tests, are not commercially available.

Hence, due to the lack of specificity and sensitivity of optimum and the unavailability of commercial PPDs prepared from NTMs, it seems that TST alone is ineffective in distinguishing between NTB and TB infection. Additionally, this condition may be more complicated if the patient with NTM also has a previous history of TB.

Immunodiagnostic assays

Interferon-γ release assays

Interferon-γ release assays (IGRAs) are immune-based tests for diagnosing TB infection [40].

The foundation of IGRAs is detecting the detection of interferon-gamma responses to antigens that are generally MTB-specific and encoded in the region of difference 1 (RD-1). Although there are significant exceptions, such as *Mycobacterium marinum*, *Mycobacterium szulgai*, and *Mycobacterium kansasii*, RD-1 is lacking in most the majority of the NTM species, including *Mycobacterium avium* (MAC) [61]. As a result, IGRA thought to be able to distinguish IGRA ought to be able to distinguish between NTM and TB by nature [25,62,63].

Only 3 (3.8%) of the 78 children in a study by Martinez-Planas *et al*. (33), who had proven NTM infection, had positive IGRA results. In two of those patients, cutaneous M marinum infection—an NTM species that expresses RD-1 antigens—was present, and the outcomes were as expected favorable. The remaining case developed MAC lymphadenitis and likely had a concurrent TB infection as a result of earlier TB interaction. These findings confirm the value of IGRA in differentiating TB from MAC infections in pediatric patients with peripheral lymphadenitis [14].

These findings align with a previous study by Hermansen *et al*. that evaluated IGRAs in NTM patients. They demonstrated that the IGRA holds the potential to differentiate between MTB and NTM infection. They reported no positive IGRA test results among children with NTM, resulting in a 100% specificity [63].

An advantage of immunodiagnostic tests is their noninvasive nature, as there is no need for invasive procedures such as FNAC or excisional biopsies.

In this line, in a study by Lyly *et al*., after the adoption of the IGRA with PPD stimulation as a diagnostic method for NTM lymphadenitis, the need for biopsies rapidly declined, and the diagnosis was achieved in a few days [13].

Another advantage, commercial IGRAs are not confounded by previous BCG immunization, despite TST, which can produce false-positive results in BCG-immunized individuals [64].

These findings are concordant with previous studies reporting that IGRA with PPD stimulation seems promising in diagnosing NTM cervicofacial lymphadenitis pre-operatively [62,65,66].

It seems that IGRAs in a child presenting with cervical lymphadenitis can be helpful in discriminating TB from NTM lymphadenitis. However, more prospective studies with sufficient

enrolled pediatrics should be performed to validate these findings.

Enzyme-linked immunosorbent spot assay

As a further immune-based test, a recent study by Della Bella *et al*. showed that MAC lysate IFN-γ and IL-2 enzyme-linked immunosorbent spot (ELISPOT) could be helpful for usefulhelpful for diagnosis of NTM in pediatrics with lymphadenopathies [31].

In their investigation, the MAC lysate IL-2 ELISPOT sensitivity in differentiating children with NTM infection and uninfected children was 87.5%, with a specificity of 85.7% [31].

This study also demonstrated that anti-mycobacterial therapy did not impact the IFN-γ and IL-2 ELISPOT assay performance. Finally, interestingly, they found that the number of detected spots by IL-2 ELISPOT assay was elevated in children with lymphadenopathies onset more than three months before. Conversely, the number of detected spots in IFN-γ ELISPOT was elevated in patients with more recent lymphadenopathies [31], which may help estimate the onset of infection.

It seems that IFN-γ and IL-2 -based ELISPOT assays can help discriminate between children with and without NTM as well as TM infection and could be used in early diagnosis to address pediatrics to prompt treatment [17,31].

However, these findings are preliminary and should be supported by further studies.

Radiomics

Radiomics is the study of using medical images as data to be mined and high-throughput quantitative feature extraction from such images for analysis and clinical decision support [67,68].

Using these extracted properties, machine learning can be an excellent a great tool for creating prediction algorithms [69,70].

Radiomics seeks to examine and extract several complicated quantitative aspects, many of which might not be apparent, obvious, or helpful based just on qualitative image analysis [67,71-76].

Radiomic markers have been found to have high accuracy in discriminating the causes of lymphadenopathy, with a specificity of 93% and sensitivity of 91% for identifying benign versus malignant lymphadenopathy [77].

In order to distinguish non-tuberculous mycobacterial lymphadenitis from other types of lymphadenopathy in children, Al Bulushi *et al*. carried out a study to develop and evaluate a radionics-based machine learning classifier. They reported an accuracy of 82% in the ability of radiomic characteristics to distinguish NTM lymphadenitis from other lymphadenopathies. The thorough research also showed that NTM can be distinguished from the reactive and proliferative causes of pediatric lymphadenopathy with 85% accuracy. Despite being preliminary, these results are quite encouraging and point to a potential use of radiomics in evaluating NTM in children. As an advantage, this method is a noninvasive approach and diminishes the need for specimen isolation or/and invasive procedures [27].

It seems that machine learning and radiomics approaches can provide high accuracy for differentiating NTM lymphadenitis from other causes of lymphadenopathy in children and, as a safe method, diminish the need for invasive procedures. However, still, larger studies for validating this approach are still needed.

General Discussion

A strength of the present study is that we systematically gathered the available evidence in the current literature following the PRISMA guideline. The design and baseline features of the existing literature addressing the diagnosis of NTM cervicofacial lymphadenitis in pediatrics varied widely, therefore, the findings of our study should be regarded with caution. The weakness of our study is the retrospective design of the included studies in this systematic review; data are frequently biased in retrospective studies. Moreover, evaluating the clinical criteria of an unfavorable and favorable outcome is challenging retrospectively. Thus, more prospective assessments of NTM patients need to be done using standardized measures. Moreover, because NTM cervicofacial lymphadenitis in children is a rare illness, it may be challenging to recruit a sufficient number of cases. The considerable clinical variability of the results precluded us from completing a meta-analysis or computing overall diagnostic accuracy values. To validate the findings of our systematic review, additional research with excellent methodological standards is required.

Implications for practice

The current systematic review demonstrates that there are few studies with excellent methodological quality on the diagnostic efficacy of described methods and that these methods' sensitivity is still insufficient. As a result, a stepwise approach is still advised for making a

diagnosis of NTM cervicofacial lymphadenitis because no one diagnostic technique can offer the best possible specificity and sensitivity.

In clinical practice, excluding other causes for cervicofacial lymphadenitis is essential at first.

It seems that TST cannot discriminate between TB and NTM; instead, IGRA is more efficient.

IGRA with PPD stimulation appears to be a promising approach to diagnosing NTM cervicofacial lymphadenitis.

A mycobacterial specimen must still be isolated for a final diagnosis, which can be accomplished via PCR and culture. PCR has higher sensitivity, and the result will be determined in a shorter time, which can prevent treatment delay. It is crucial to understand that PCR and culture may not always yield 100% sensitivity, partly due to the quality of the sample. However, it seems that FNA is no less efficient than an excisional biopsy. It can perform as a simple and safe approach for providing material collection without any important complications for children.

Implications for future research

To reduce this lack of diagnosis, we should consider expanding our diagnostic procedures. In order to improve the detection rate and the negative predictive value, future advances should attempt to introduce NTM diagnostic approaches with improved sensitivity.

Recently, the 16S ribosomal RNA sequencing has been suggested as a novel technique that can improve the sensitivity and expedite the isolation from clinical specimens [78].

Novel non-invasive approaches such as radiomics and ELISPOT seem safe, operator-independent, and fast, with high sensitivity and specificity, which can permit considering a smear-independent algorithm for NTM diagnosis. However, the accuracy of these tests should be validated by further studies.

Our proposed algorithm for diagnosing this disease is shown in **Figure 2**.

Conclusion

In conclusion, this study revealed the crucial role of employing reliable and accurate diagnostic approaches for the identification of NTM cervicofacial lymphadenitis in children. While PCR and culture remain essential for precise diagnosis, PCR offers more rapid results. FNA is increasingly recognized as a safe and efficient diagnostic tool. However, excisional biopsy may still be necessary in some cases for adequate tissue sampling. Recently, emerging diagnostic approaches, such as IGRA and radiomic analyses, have demonstrated significant potential to enhance diagnostic precision and reduce the reliance on invasive procedures. Nevertheless, further validation and standardization of these advanced diagnostic techniques are essential to optimize clinical practice and improve patient outcomes. Environmental factors, such as climate and food changes, may influence the epidemiology and spread of NTM in children [7]. NTM lymphadenitis is uncommon but becoming more common in children and adolescents. Timely disease diagnosis necessitates suspicion, and communication between doctors and laboratories is required for NTM diagnosis [8].

IGRA is a helpful tool for diagnosing lymphadenitis in children, especially when culture and polymerase chain reaction results are negative [14]. Surgical removal of diseased lymph nodes to NTM is an efficient diagnostic and therapeutic method. When complete surgical removal of the infected gland is not possible, combined treatment, which involves surgical removal of a portion of the gland along with a special drug regimen containing safe clarithromycin, produces milder and fewer side effects than complete surgery [16]. In order to choose a therapy that has the fewest adverse effects, it is important to take into account the treatment's results in terms of any residual scars and lesions on the facial nerve [15].

Data Availability Statement

The study's original contributions are included in the article/supplementary material; further questions can be directed to the corresponding author/s.

Author Contributions

The search was carried out by Motahare Rouhparvarzamin and Farzad Sheikhzadeh. Narges Norouzkhani and Nasrin Mohammadi did the initial screening. Sepide Mohit and Farzad Sheikhzadeh carried out the second screening. Nasrin Mohammadi and Motahare Rouhparvarzamin assessed the possibility of bias. The manuscript was written by Narges Norouzkhani and Sepide Mohit. The manuscript was revised by Farzad Sheikhzadeh and Nasrin Mohammadi. The article was designed, critically revised, and supervised by Niloofar

Deravi. The article was written by all of the authors, and the final version was approved by all of them.

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The protocol of this systematic review is available at https://osf.io/registries/drafts/636151ce11b333002009b8bc/review.

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