Contemporary challenges in the diagnosis and treatment of nontuberculous mycobacterial cervicofacial lymphadenitis

Samuel Hendrik Willemse

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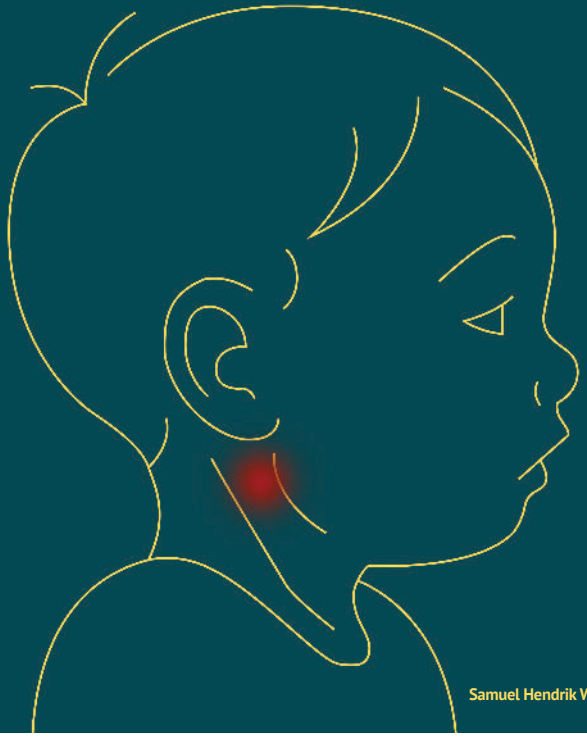

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General introduction

Willemse SH

Nontuberculous mycobacteria and a historical perspective

Mycobacterium is a bacterial genus, which currently consists of more than 190 species (1). This group contains several strict pathogens, including *M. leprae* and *M. tuberculosis.* The latter, a highly pathogenic species discovered by Robert Koch in 1882, causes the communicable disease tuberculosis, which is still one of the major causes of global mortality nowadays (2). It is, therefore, the most notorious species within the *Mycobacterium* genus. Nontuberculous mycobacteria (NTM) are, in contrast, opportunistic pathogens. They constitute the majority of the *Mycobacterium* genus, with approximately 140 pathogenic species and 60 potentially pathogenic species (3). *M. avium*, *M. intracellulare*, *M. kansasii* and *M. abscessus* are humans' primary causative agents of NTM disease (3).

Unlike the highly contagious *M. tuberculosis*, person-to-person or animal-to-human transmission of nontuberculous mycobacteria is generally believed not to occur (4, 5). However, a report from 2013 suggested indirect transmission of *M. abscessus* between cystic fibrosis patients (6).

NTM infections, although not recognized as such at the time, were already described in the medical literature in the 19th century. In 1868, Villemin and Crisp were the first to report on avian tuberculosis, a disease in birds resembling mammalian tuberculosis (7, 8). Approximately ten years after Koch discovered the etiologic agent of tuberculosis, two reports by Maffucci provided evidence of a separate cause of tuberculosis in birds. This newly discovered organism was a distinct variety of the tubercle bacillus and dissimilar in its cultural and pathogenic aspects to the organism responsible for tuberculosis in humans. These findings confirmed the earlier beliefs of Rivolta in 1889, which challenged Koch's contention of unity of the organism of tuberculosis regardless of its host (9-12). In 1884, a bacillus with the staining appearance of tubercle bacilli was found in human syphilitic chancres and gummae (13). Approximately one year later, Alvarez and Tavel found similar bacilli in syphilitic chancres and normal genital secretions (smegma) (14). This newly discovered organism, later named *M. smegmatis*, was the first mycobacterium to be identified after the tubercle bacillus and was later found to be nonpathogenic (15).

The first report of NTM disease in humans was published in 1893. An organism believed to be the same species earlier found in diseased birds was isolated from human sputum (16). Recognition of these mycobacteria other than tuberculosis marked the first step towards distinguishing the various types of mycobacteria (17). Early reports of probable NTM cervicofacial lymphadenitis, the subject of this thesis, date back to 1929 and 1935 (18, 19). However, due to the overwhelming impact of tuberculosis, little attention was paid to these less pathogenic bacilli at the time. These organisms resembling tuberculosis have been subject to many different designations, such as anonymous, atypical, nontuberculous, opportunistic, tuberculoid, and unclassified mycobacteria, paratubercle bacilli, pseudotubercle bacilli, and

non-epidemic agents of mycobacterial disease. 'Nontuberculous mycobacteria' appears to be the most appropriate designation, as suggested earlier by Wolinsky, and has become the most widely used term in medical literature. Around 1950, after culturing specimens became routine instead of merely performing smears for acid-fast mycobacteria, nontuberculous mycobacteria were more frequently isolated, and interest in these organisms increased (20, 21).

Mycobacteria are one of the few types of bacteria that can invade into macrophages, survive, grow, and replicate inside them. They possess a hydrophobic, lipid-rich outer membrane, which is an essential factor in aerosolization, biofilm formation, and disinfectant and antibiotic resistance (22). Pathogenic mycobacteria provoke a specific host response with the formation of granulomas. Starting as confluent masses of cells organized around a core of macrophages and epithelioid cells and surrounded by lymphocytes and occasional polymorphonuclear lymphocytes, these lesions increase in size. They may evolve to show areas of central necrosis, which later progress into large areas of amorphous acellular material showing a macroscopic cheesy (caseous) consistency (23).

Nontuberculous mycobacteria are ubiquitous organisms in environments shared with humans. They typically reside in natural water, drinking water systems, and soils (24). Reactivity to mycobacterial antigen skin tests has been used in epidemiologic studies as a surrogate marker for NTM exposure. Around 1970, surveys of skin test reactivity with a 2 mm cut-off among United States Navy recruits to purified protein derivative (PPD) from *M. avium* indicated that the frequency of exposure to this organism was approximately 31% (25). In Sweden, an epidemiologic study revealed that 43% of schoolchildren aged 8-9 years reacted to *M. avium* sensitin with a 3 mm cut-off (26). In another study by the same investigators, it was demonstrated that *M. avium* sensitin sensitivity (6 mm cut-off) was higher in children 8-9 years of age (25,4%) compared with children 4-5 years of age (7,8%) living in the same region, indicating that exposure appears to increase from childhood (27). These findings are corroborated by a study analyzing skin test reactivity in a population from Haiti, where *M. avium* sensitin sensitivity increased from early childhood to adulthood (28). This study also demonstrated that children between 0-4 years of age had already been sensitized by several mycobacteria (29).

Nontuberculous mycobacterial cervicofacial lymphadenitis

Respiratory infections, mainly in immunocompromised individuals, account for almost 90% of all NTM cases, while lymph node infections constitute the second largest group (30). 80% of lymph node infections due to NTM occur in the head and neck region (31). NTM cervicofacial lymphadenitis is a rare, non-contagious infectious disease (32). Unlike pulmonary NTM infections, it manifests almost exclusively in immunocompetent children, most commonly between 0 and 5 years old (31, 33, 34).

Based on a prospective nationwide surveillance study, the reported incidence varies between 0.6-4.5 per 100.000 children, and the annual incidence in The Netherlands is estimated to be 0.77 per 100.000 children. The highest incidence in this study, 2,3 per 100.000 children, was found in age 0-4 (35-39). A meta-analysis concluded that discontinuing mass BCG vaccination had led to an increased rate of NTM lymphadenitis and skin infections (40). The infection rate in Finland at the time of national BCG vaccination was 30 times lower than in Sweden, a country with similar environmental and epidemiological characteristics and without a BCG vaccination program (41). Moreover, an increase in NTM infections was observed in the Czech Republic and in Sweden after the discontinuation of national BCG vaccination programs (42, 43).

M. avium, *M. intracellulare*, and *M. scrofulaceum* account for 68% of NTM cervicofacial lymphadenitis cases worldwide. The next most frequent etiologic agent is *M. haemophilum*, which is isolated in 6% of cases and has only been reported in two countries: The Netherlands and Israel (44). *M. avium* and *M. intracellulare* are NTM species belonging to the *Mycobacterium avium* complex (MAC), and *M. avium* is differentiated into four subtypes: *M. avium* subsp. *avium, M. avium* subsp. *hominissuis, M. avium* subsp. *paratuberculosis* and *M. avium* subsp. *silvaticum* (45).

NTM cervicofacial lymphadenitis is usually unilaterally located in the submandibular, pre-auricular, or submental region. Despite the appearance of a single infected lymph node at clinical examination, multiple infected lymph nodes are encountered during surgery in 34% of cases (46). In addition, macroscopically normal appearing lymph nodes surrounding the infected tissue may also show microscopic foci of infection on histopathologic examination (47).

Generalized symptoms are typically absent, although fatigue, fever, and weight loss may occur (48, 49). Over the course of weeks to months, infected lymph nodes may fluctuate, and the skin color changes from erythematous to purplish-red, also referred to as violaceous (50). Eventually, the infection breaks through the skin, resulting in the spontaneous discharge of purulent material in 73-91% of cases (51, 52). In an observation-only study by Zeharia et al., fistulas with discharge of purulent material persisted 3-8 weeks in most cases. Total resolution was observed in 71% of cases within 6 months and in 9-12 months in the remainder of cases (51). Lyly et al. reported intermittent drainage for more than 8 weeks in 38% of cases (52). Generally, dissemination of the infection does not occur, but complete healing may take years (53, 54). A system to classify the disease stage, as shown in Table 1, was proposed by Penn and colleagues and is referred to as The Georgetown classification (55). Few children are referred to a secondary or tertiary reference center before the appearance of skin discoloration (56). Clinical examples of different disease stages are represented in Figure 1a-1d.

Table 1 Georgetown disease stage classification system

Clinical stage	Disease characteristics
Stage I	Painless, firm swelling, adherent to overlying skin, increased vascularity
Stage II	Liquefaction of the affected lymph nodes with fluctuation by palpation
Stage III	Skin changes – violaceous coloration, thinning of skin, parchment-like changes, shiny appearance
Stage IV	Fistulization

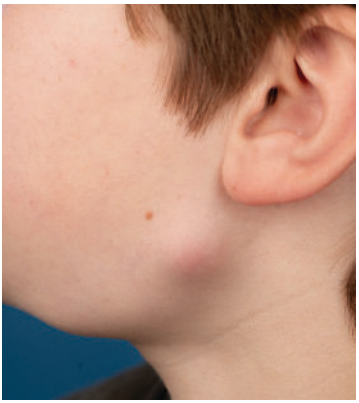

Figure 1a Clinical example of Stage I NTM cervicofacial lymphadenitis

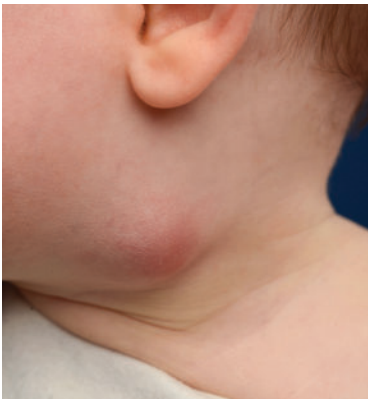

Figure 1b Clinical example of Stage II NTM cervicofacial lymphadenitis

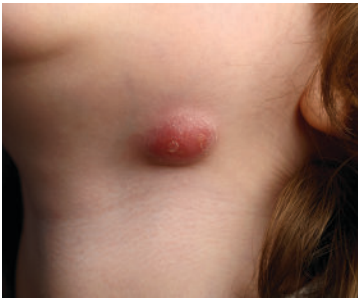

Figure 1c Clinical example of Stage III NTM cervicofacial lymphadenitis

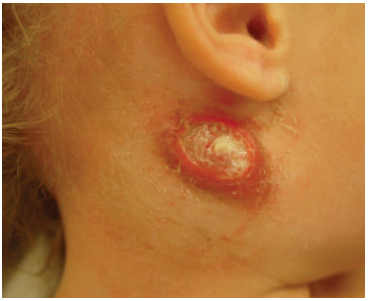

Figure 1d Clinical example of Stage IV NTM cervicofacial lymphadenitis

Pathophysiology

It is generally believed that the environment is the source of infection in patients with NTM disease. *M. avium* complex organisms are found in water and soil, and *M. haemophilum* in water (57-61). Both *M. avium* and *M. haemophilum* have also been isolated from raw milk (62). In addition, *M. avium* complex organisms have been isolated from oysters, house dust, and cigarettes, and one study described the presence of *M. haemophilum* on the surface of hospital cockroaches in Taiwan (63-67). *M. chelonae, M. fortuitum, M. kansasii* and *M. gordonae*, other NTM species that cause NTM lymphadenitis, have been isolated from pasteurized milk. Finally, NTM species causing lymphadenitis have also been found on several fruits and vegetables, including sprouts (*M. avium, M. abscessus, M. gordonae)*, rocket (*M. gordonae, M. fortuitum*), blueberries (*M. lentiflavum*) and ready-to-eat salads (*M. chelonae, M. fortuitum*) (68, 69).

MAC organisms are the causative pathogens in 78% of all disease cases due to NTM in Australia, 73% in Canada, and 62% in the United States (30, 70). The recovery of *M. avium* species worldwide in water and soils in contrast to the regionally varying presence of other NTM, reflects *M. avium's* adaptive abilities (71).

It has been suggested that children serve as sentinels for the presence of mycobacteria in water (72). Since approximately 1980, *M. avium* has become the predominant species that causes NTM lymphadenitis in children, whereas *M. scrofulaceum* was isolated most frequently before (73). This shift may have reflected a changing prevalence of *M. scrofulaceum* and *M. avium* in the water. The numbers and frequency of *M. scrofulaceum* isolations from southeastern U.S. water samples decreased over a period of 15 years from 1975 after the implementation of Clean Water Acts, increasing chlorination rates (74, 75). Since *M. scrofulaceum* is sensitive to chlorine in contrast to *M. avium*, this may have induced a substantial reduction of *M. scrofulaceum* in water (72).

MAC can also cause a wide array of infections in animals, including cervids, rabbits, and although rarely encountered, in sheep, goats, and dogs (76, 77). *M. avium* infections are common in poultry and swine (78). Clinical signs of infection in birds consist of emaciation, depression, diarrhea, and atrophy of breast muscle, whereas pig infections present as localized mesenteric or head and neck lymphadenitis (79). The importance of *M. avium* infections in swine was already noted in 1925 by van Es and Martin. They demonstrated that 89% of swine tuberculosis in Nebraska was caused by the 'avian variant' instead of the 'mammalian variant' of tuberculosis (80). The *M. avium* subtype, which is found in birds (*M. avium* subsp. *avium*), is different from *M. avium* subtypes isolated from humans and pigs (*M. avium* subsp. *hominissuis*) (81). Although a previous Danish study found different MAC strains in humans and animals, a later Danish study demonstrated that several MAC strains found in pigs and peat were identical to MAC strains isolated from humans. It was suggested that peat

is a common source of *M. avium* infection for humans and animals or that animals and peat are potential sources of infection for humans (82, 83). A Dutch study, carried out after a significant increase in the incidence of caseous lesions in the lymph nodes of slaughtered pigs, corroborates these findings. Restriction fragment length polymorphisms of 61% of human and 59% of the porcine isolates were at least 75% similar, reflecting extensive conformity. This study also demonstrated that 25% of the human isolates, a proportion much more significant than porcine isolates, represented other groupings within MAC than *M. avium,* presumably indicating sources of infections for humans, which are not shared with pigs (84). On the other hand, a Portuguese study on genetic relatedness between isolates of human and porcine origins demonstrated extensive genetic heterogeneity among the strains. It revealed no clear correlation between the respective geographical, host and biological sample sources. However, some porcine and human isolates showed close genetic relatedness (85).

Thus, it remains unclear if interactions between humans and pigs, such as consuming insufficiently cooked pork meat, play a role in the pathogenesis of mycobacterial infections in humans.

Unlike pigs, cattle is generally not prone to infection by *M. avium and M. intracellulare,* except for *M. avium* subsp. *paratuberculosis*, which is not associated with NTM cervicofacial lymphadenitis in humans *(76).* Fish species can also develop disseminated mycobacterial disease, known as piscine mycobacteriosis. *M. fortuitum* and *M. chelonae*, rare etiologic agents of NTM cervicofacial lymphadenitis, are able to cause these deadly infections reported in more than 150 fish species (86).

Finally, amoebas function as reservoirs for *M. avium*. *A. lenticulata*, which is frequently recovered from environmental water sources and also found in household water sources, also harbors intracellular *M. avium* replication (87-89).

To date, prolonged occupational soil exposure has been the only environmental risk factor linked to MAC infection. A history of animal contact has not been associated with NTM lymphadenitis (90, 91).

A relatively extremely high incidence of *M. avium* lymphadenitis was found in a region in the northern part of Sweden, with a high incidence of familial amyloidotic polyneuropathy, a genetic disease. The human population in this region has been historically isolated. Children with localized *M. avium* lymphadenitis might not have the severe impairment of interferon–γ-mediated immunity, known as Mendelian susceptibility, but their immune system might be less severely impaired, suggesting a genetic predisposition to NTM disease (92-94).

Neill et al. state that the routes by which cattle are infected by *M. bovis*, along with the host immune response and organism/s virulence, largely determine the manifestation of this

disease (95). This hypothesis may also apply to human pathophysiology. Unlike pulmonary tuberculosis, bovine tuberculosis appeared as oral and ear infections in the families of milkers (96). Moreover, outbreak studies have concluded that the clinical picture of milk epidemics differs from that of pulmonary tuberculosis. Five to eight weeks after the ingestion of tuberculous milk, a sore throat and swelling of tonsils and cervical lymph nodes were observed, among other symptoms, whereas disease following inhalation results in disease primarily related to the respiratory system (97).

Based on the predominant anatomical locations of cervicofacial lymphadenitis, children are thought to acquire the disease through the oropharynx, conjunctivae, nose, middle ear, or oral mucosa. Mouthing behaviors of children and mucosal injuries associated with tooth eruption and shedding might be important (98-100). *M. avium* can bind and invade human epithelial cells (101).

Nontuberculous mycobacteria have been cultured from tonsillar tissue, nose, and throat swabs from healthy children in early studies (102, 103). The presence of mycobacterial genetic material was also found in oral, oropharyngeal and nostril samples from healthy individuals in later studies using sequencing methods (104, 105). Another study demonstrated isolation of *M. kansasii* from lymph node tissue and tonsil and adenoid tissue samples from the same patient (106). The findings of NTM taxa in these niches prompt questions on their role as a possible portal of entry. The presence of NTM in the oral cavity and oropharynx has not yet been investigated systematically.

Diagnosis

Initial mild, non-specific symptoms often hamper an early clinical diagnosis of NTM lymphadenitis, but the disease's further clinical course is characteristic.

Many additional diagnostic aids exist. Conventional diagnostic methods include histopathologic examination, acid-fast staining procedures (Ziehl-Neelsen and auramine staining), and culturing. On the other hand, molecular detection of NTM is possible with polymerase chain reaction (PCR). Moreover, immunological diagnostic techniques include PPD skin testing, and PPD stimulated IFN-γ release assays (IGRA). Sonography can be used as an imaging technique to aid in the diagnostic process.

A definitive diagnosis requires isolation of the causative organism, and culturing has traditionally been regarded as the gold standard (107). Most mycobacteria causing NTM cervicofacial lymphadenitis grow on various commercially available media. However, *M. haemophilum* requires specific culturing conditions: a lower temperature of 28-30°C instead of 35-37°C and iron supplementation such as hemin or ferric ammonium citrate (4, 108). Growth of *M. chelonae* requires a temperature between 28-33°C. These specific growth

conditions may result in underdiagnosis of these causative agents. *M. avium* usually grows within 2-3 weeks, but *M. haemophilum* growth ranges from 23-80 days, with most isolates requiring 30 days of incubation (109).

PCR techniques are significantly faster than culturing, eliminating the long incubation period problem.

Auramine and Ziehl Neelsen stains are regularly performed as acid-fast staining techniques to identify the presence of mycobacteria, and histopathologic examinations generally reveal caseating granulomatous inflammation and necrosis, epithelioid histiocytes and occasional giant cells, as demonstrated in Figure 1e (35, 78). Unlike PCR and culturing, species identification is not possible with these techniques.

Unfortunately, clinical specimens are required to identify mycobacteria directly. Material for these procedures can be obtained in three different ways: fine needle aspiration cytology, tissue biopsy, or complete excision. These procedures are invasive, especially in children. Moreover, both a biopsy and fine needle aspiration are associated with fistula formation (110-113). These disadvantages elucidate the need for noninvasive, accurate diagnostic methods.

Skin testing using mycobacterial derivatives (tuberculin) is a minimally invasive procedure that relies on an immunologic host response. The technique of injecting these substances intradermally was first described by Charles Mantoux in 1910, and this procedure is still often referred to as the 'Mantoux test' (114). Although injected substances were originally derived from *M. tuberculosis*, the value of skin testing with a solution of tuberculin prepared from an 'avian strain' was already acknowledged in 1914 by Van Es and Schalk. Positive reactions were observed after intradermal injection into the wattle in 98% of the chickens, subsequently showing symptoms of avian tuberculosis at necropsy (115). Later, standardized NTM-PPD's were produced by the Statens Serum Institut, Denmark. The diagnostic accuracy of these tests was high, but the production of commercially available NTM-PPD was terminated. Cross-reactivity is observed in individuals infected by NTM after tuberculin skin testing with proteins derived from *M. tuberculosis*, because NTM contain proteins analogous to those of *M. tuberculosis*. Due to this cross-reactivity, tuberculin skin testing is still used to screen patients suspected of NTM lymphadenitis without BCG vaccination or a history of *M. tuberculosis* infection (116). Hill's editorial commentary stated that the reliability of skin tests, with their inherent cross-reactivity, may wane as more specific tests of immune responsiveness, comparable with IFN-γ for *M. tuberculosis* infections, become available (117).

Serodiagnosis is another relatively minimally invasive tool, but it has not yet been investigated in patients with NTM cervicofacial lymphadenitis. However, an enzyme immune-assay

measuring anti-glycopeptidolipid-core IgA antibodies is currently commercially available (118). Glycopeptidolipids are the significant and specific cell surface antigens of *M. avium complex*. In patients with *M. avium complex* pulmonary disease, the sensitivity of these tests is estimated to be 70% (119).

There remains significant need for accurate, noninvasive diagnostic tests.

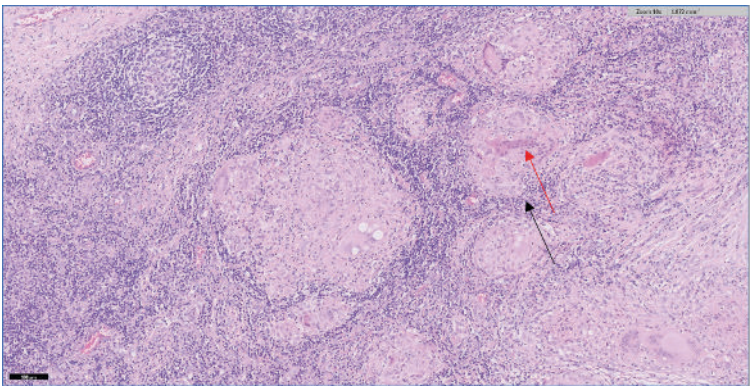

Figure 1e Hematoxylin and eosin staining of excised lymph node tissue showing visible granuloma formation (black arrow) containing areas of necrosis (red arrow)

Treatment

Several treatment modalities exist for NTM cervicofacial lymphadenitis. Surgical interventions include complete surgical excision (Figure 1e, 1f), curettage, and incision drainage. Conservative treatment options comprise antibiotic treatment and a wait-and-see strategy. Furthermore, some authors suggest the option of performing fine needle aspiration. Cure is generally defined as total skin closure without local recurrence or de novo lesions, as assessed by clinical and ultrasound evaluation (120).

The current evidence to guide the optimal treatment of NTM cervicofacial lymphadenitis is limited, as demonstrated in a systematic review of different treatment regimens. Although available literature suggests that complete excision is the best treatment option, treatment options should be carefully considered on an individual patient basis, weighing potential risks against benefits (44). To date, only one randomized controlled trial has been conducted comparing surgical excision with antibiotic treatment. This study showed that complete surgical excision leads to the quickest disease resolution. However, seven subjects in this study experienced temporary facial nerve weakness (14%), and one (2%) was affected by

permanent facial nerve palsy after surgical excision. Due to its anatomical location, the marginal branch of the facial nerve is especially at risk. Although most drug-related side effects were considered mild in intensity in this study, two patients had to cease antibiotic therapy due to jaundice or an allergic reaction (56). Despite the superior effectiveness of surgical excision, the optimal treatment strategy remains an issue of debate because of the invasive nature of the surgery, the risk of complications, and the lack of long-term outcome data (121-123).

One of the main issues in surgical management is that complete surgical removal is not always achievable due to the proximity of vital structures such as peripheral branches of the facial nerve or the accessory nerve, or extensive skin necrosis (44, 56, 120, 124-130). Curettage is believed to result in fewer facial nerve injuries, and the incision can be smaller than complete surgical excision procedures (129, 131). During a curettage procedure, curettes are used to thoroughly remove necrotic tissue after blunt tunneling to the lesion. The granulation tissue is scraped off all the walls of the cavity, including the undersurface of the skin (132). Outcomes of curettage and complete surgical excision were compared in one randomized controlled trial. Wound healing was demonstrated to take significantly longer, and recurrent swelling occurred in 36% of cases after curettage in this randomized controlled trial. Complete surgical excision resulted in a definitive resolution of the disease. It was concluded that curettage could be considered an alternative if complete excision of necrotized lymph nodes is technically too difficult or too risky concerning the safety of the facial nerve (120). Consequently, it is essential to properly estimate the ability to perform complete surgical removal of all infected lymph node tissue preoperatively without causing unnecessary morbidity of surgery. However, clinical determinants predicting the (in)ability to perform safe and complete surgical removal of the infected tissue have not been studied yet.

NTM cervicofacial lymphadenitis is a benign disease that eventually regresses without treatment (53, 133). One randomized controlled trial, also performed in The Netherlands, compared a wait-and-see strategy with antibiotic treatment. It was demonstrated that antibiotic treatment does not lead to quicker healing time. Considering costs and side effects, an antibiotic regimen does not seem beneficial (134). Incision and drainage and fine needle aspiration as treatment regimens generally result in disease recurrences and are therefore considered suboptimal (44, 135).

Either with or without treatment, NTM cervicofacial lymphadenitis may leave scars in obvious areas, which may affect self-consciousness and anxiety (136). A clinical example is represented by Figure 1h. Only one previous study has systemically evaluated the short-term esthetic outcome of different treatments. After a one-year follow-up period, the esthetic outcome of surgical excision was demonstrated to be superior to the outcome with antibiotic treatment (137). However, the opinions of the patients could not be obtained due

to their young age after one year of follow-up. Besides, little is known about the long-term development of the scars acquired when treated at a young age for this disease.

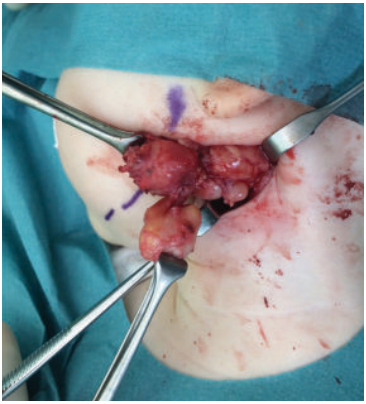

Figure 1f Clinical example of surgical excision of infected lymph nodes (same patient as image 1b)

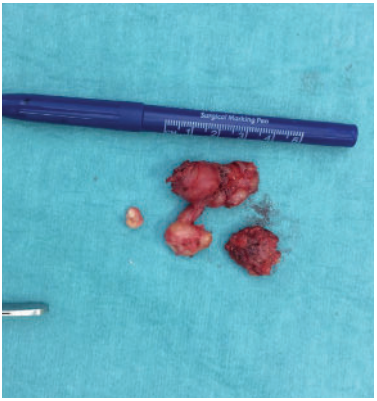

Figure 1g Clinical example of surgically removed lymph nodes (same patient as image 1b)

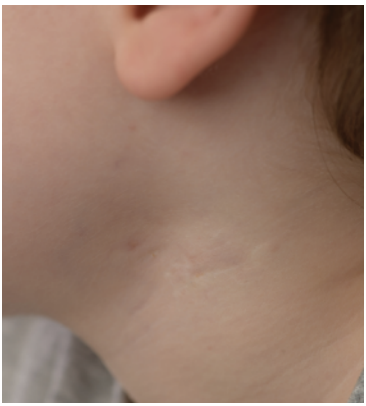

Figure 1h Clinical example of a scar 3 years after surgery (same patient as image 1b)

Aims and outline of this thesis

This thesis focuses on different aspects of NTM cervicofacial lymphadenitis. It addresses minimally invasive diagnostic possibilities, potential portals of entry, surgical treatment aspects, and long-term treatment outcomes. The aim is to contribute to an early diagnosis, a better understanding of the pathophysiology, and prediction of successful outcomes.

The thesis is divided into three parts. The first part focuses on diagnostics and possible portals of entry, and the second part on surgical challenges and long-term treatment outcomes. The last part contains a general discussion, future perspectives, summaries and appendices.

Chapter 1 is the general introduction of this thesis.

Chapter 2 describes a systematic review of all available diagnostic studies on NTM cervicofacial lymphadenitis to provide an overview of the diagnostic possibilities and insight into promising new diagnostic techniques.

Chapters 3 and 4 describe a cross-sectional clinical study. Chapter 3 investigates the diagnostic accuracy of the Capilia MAC antibody enzyme-linked immunosorbent assay® in children with a clinical suspicion of NTM cervicofacial lymphadenitis compared with controls. Chapter 4 focuses on the presence of NTM in the oral cavity and oropharynx, which could be possible portals of entry. Moreover, the potential role of oral and oropharyngeal swabs as a minimally invasive diagnostic alternative is investigated. '

Chapter 5 describes a retrospective case-control study to determine clinical determinants that may predict the (in)ability to safely and completely remove the infected tissue in patients with NTM cervicofacial lymphadenitis.

Chapter 6 describes a case series to investigate long-term recurrence rates of surgically treated NTM cervicofacial lymphadenitis patients with a follow-up of at least 10 years.

Chapter 7 describes a cohort study to evaluate the long-term esthetic outcome of different treatment modalities for NTM cervicofacial lymphadenitis.

Chapter 8 provides a general discussion Finally, the appendices include English and Dutch summaries, a list of contributing authors, chapter information, a PhD portfolio, a list of publications, acknowledgments, and a section about the author of this thesis.

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Part I

Diagnostics and possible portals of entry

Chapter 2

Diagnosing nontuberculous mycobacterial cervicofacial lymphadenitis in children: A systematic review

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Abstract

Introduction

Widespread controversy exists regarding the correct diagnosis of nontuberculous mycobacterial (NTM) cervicofacial lymphadenitis. This study intends to gather the available evidence regarding this diagnosis.

Materials & Methods

A review protocol was developed based on the Preferred Reporting Items for Systematic Reviews and Meta-Analysis (PRISMA)-statement (www.prisma-statement.org). A comprehensive search was performed using the bibliographic databases PubMed, Embase.com, and Wiley/Cochrane Library. 10 Articles fulfilled the inclusion criteria and were included in the review. The articles' risk of bias was assessed using the revised Quality Assessment of Diagnostic Accuracy (QUADAS-2) tool.

Results

This systematic review shows that diagnostic studies of high methodological quality are scarce. The diagnostic accuracy of polymerase chain reaction, culture, skin testing, auramine staining, Ziehl-Neelsen staining, and immunodiagnostic assays was studied. Culture sensitivity proved to be 41,8%, while polymerase chain reaction (PCR) has a sensitivity of 71,6%. Both methods showed a specificity of 100%. The sensitivity of immunodiagnostic assays ranged between 87,5%-100% and specificity between 81%-100%. Overall sensitivity of skin tests containing purified protein derivative (PPD-S) was 70% (95% CI [62% - 78%]) with an overall specificity of 94% (95% CI [88% - 100%]).

Conclusion

In patients with a high clinical suspicion of NTM cervicofacial lymphadenitis, a positive PPD-S skin test is indicative of the diagnosis of NTM cervicofacial lymphadenitis. Either PCR or culture is necessary to confirm the diagnosis. Interferon-γ release assays with purified protein derivative stimulation appear to provide good sensitivity and specificity as a minimally invasive preoperative test, but the evidence is weak. More studies of high methodological quality are needed to validate the results of this systematic review.

Introduction

Nontuberculous mycobacterial (NTM) cervicofacial lymphadenitis is a disease that most frequently occurs in young immunocompetent children. With a reported incidence ranging between 0,6-4,5 per 100.000 children, it is a rare disease (1-5). The condition has a characteristic clinical course, eventually leading to cutaneous fistula formation and spontaneous drainage (6). Treatment strategies for NTM cervicofacial lymphadenitis were recently systematically reviewed (7). A clinical example can be found in Figure 2a. Surgical excision of the affected lymph nodes appeared to be superior to antibiotic therapy. However, surgical treatment of advanced stages of the disease is associated with the risk of facial nerve palsy and poor esthetic outcomes (7-10). Hence, it is crucial to establish the diagnosis at an early stage. Nevertheless, widespread controversy exists regarding the correct diagnosis of NTM cervicofacial lymphadenitis (11-16). The aim of this study was to systematically review and critically appraise the existing literature on diagnostic methods of NTM cervicofacial lymphadenitis in immunocompetent children. Moreover, this study intends to provide recommendations for a structured approach with respect to diagnostic methods for nontuberculous mycobacterial cervicofacial lymphadenitis in children. To the best of our knowledge, this is the first study to review this subject systematically.

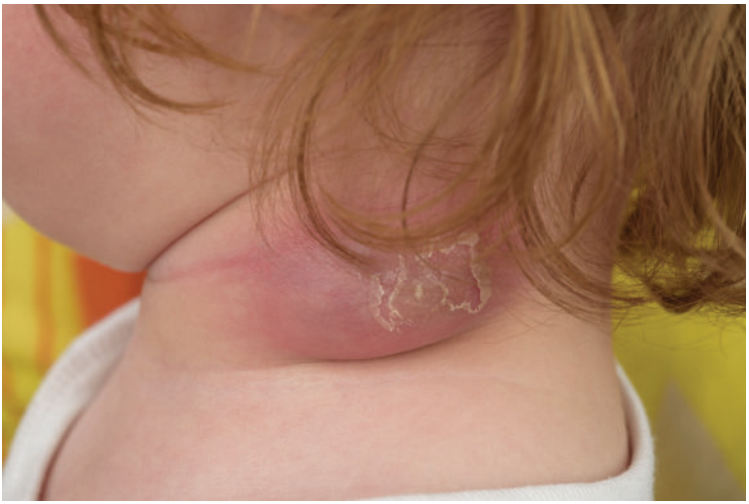

Figure 2a Clinical example

Materials and methods

Search strategy

A review protocol was developed based on the Preferred Reporting Items for Systematic Reviews and Meta-Analysis (PRISMA)-statement (www.prisma-statement.org) (17). A comprehensive search was performed in the bibliographic databases PubMed, Embase.com and Wiley/Cochrane Library. PubMed was searched from inception up to 2 March 2017, Embase.com from inception up to 16 March 2017 and Wiley/Cochrane Library from inception up to 21 March 2017. The following terms were used (including synonyms and closely related words) as index terms or free-text words: "Mycobacterium Infections, Nontuberculous", "Lymphadenitis", "Child*". The full search strategies for all databases can be found in the Appendix. The search was performed without date, language or publication status restriction. Currently, no methodological filters are available to find primary diagnostic test accuracy reports (18).

Selection Process

Duplicate articles were excluded. All titles were screened and appropriate abstracts reviewed by two reviewers (SW and LK). Discrepancies were resolved either by consensus or the use of a third reviewer (MO). The selection process was done using www.covidence.org. Two reviewers also performed full-text review of the selected articles independently (SW and MO) and discrepancies were resolved either by consensus or consulting a third reviewer (LK). Articles were selected for full review according to the following a priori eligibility criteria: (1) studies reporting on NTM cervicofacial lymphadenitis ;(2) studies reporting on patients under the age of 18 ;(3) Studies investigating diagnostic methods. Studies were excluded if: (1) the study included immuno-incompetent children or patients using immunosuppressive drugs ;(2) studies that included adults ;(3) non-clinical studies (technical notes, laboratory studies, letters, systematic reviews) or case series.

Reference lists of articles that were included for full-text review were checked for additional studies.

Methodological quality assessment

Assessing risk of bias of the articles was done using the revised Quality Assessment of Diagnostic Accuracy (QUADAS-2) tool (19). Discrepancies between the two reviewers were resolved by consensus. When inconsistent answers were given to signal questions within one domain, the following downgrading was done; if signaling questions were both answered with 'unclear' and 'yes', the risk of bias in that domain was scored as 'unclear'. If signaling questions within one domain were both answered with 'unclear' and 'no', the risk of bias in that domain was scored as high.

Results

After removing duplicate studies, 569 articles were independently screened by title and abstract by two reviewers. Of the 569 articles, 58 articles were eligible for full-text review. Full text review yielded 10 articles that met the inclusion criteria, including 2 articles that were found through reference list searching. The corresponding Prisma Flow Diagram can be found in Figure 2b. The results are displayed in Table 2a.

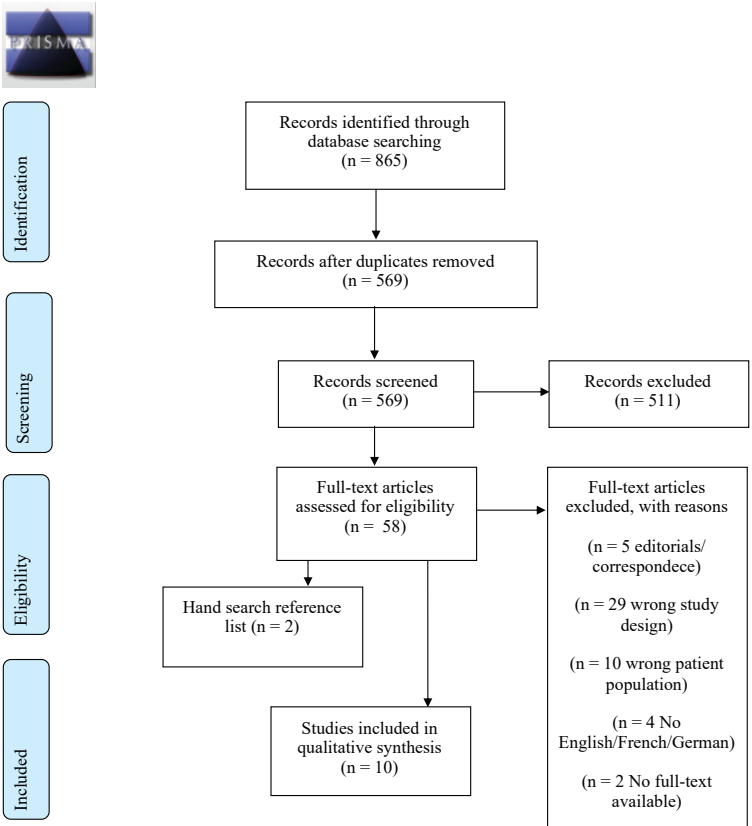

Figure 2b PRISMA 2009 Flow Diagram

Table 2a Diagnostic accuracy of included studies

Study	Data collection	Study population	Index test	Reference standard	Reference standard test result derived from	Index Test result derived from	Sensitivity	Specificity	N
April et al. (1996)	Prospective	12 otherwise healthy children with painless masses of the neck or face.	PCR	Culture	Excisional biopsy	Excisional biopsy	Indeterminable[a]	Indeterminable[a]	12
Bruijnesteijn van Coppenraet et al. (2004)	Prospective	67 children with suspected mycobacterial skin tests based on a positive skin test. Specificity was assessed with DNA from 11 mycobacterial species and 36 other human pathogens.	PCR Culture Auramine Staining	Skin tests	Palpable induration and visible erythema	Fine needle aspiration	71,6% 41,8% 46,3%	100% 100% 100%	67
Kommaredi et al. (1983)	Prospective	22 patients with NTM lymphadenitis on the basis of clinicopathologic examination or previous microbiologic study. 16 children with solitary lesions and 6 adults with disseminated lesions. In all of these patients, biopsy specimens were available.	Auramine staining ZN staining	Culture	Excisional bi-opsy	Excisional biopsy	85% 60%	85% 71%	22
Arnold et al. (1970)	Prospective	118 children having a positive tuberculin skin test, of whom 12 children had a lymph node infection..	PPD-S	Culture	Excisional biopsy	Palpable induration and visible erythema	78%	33%	12
Lindeboom et al. (2006)	Prospective	174 children with chronic lymphadenitis, with negative serological results for other infectious causes than NTM.	PPD-S *M. avium* PPD	Culture, PCR	Fine needle aspiration	Palpable induration and visible erythema	70% 93%	98% 97%	174
Huebner et al. (1992)	Prospective	Children < 18 years of age who had had at least one enlarged lymph gland in the head or neck for more than 2 weeks during the previous 5 years.	Tuberculin skin tests	Culture, histology	Excisional bi-opsy	Palpable induration and visible erythema	Indeterminable[a]	Indeterminable[a]	123

Table 2a (Continued)

Study	Data collection	Study population	Index test	Reference standard	Reference standard test result derived from	Index Test result derived from	Sensitivity	Specificity	N
Marks et al. (1977)	Prospective	12 children with proven NTM lymphadenitis and 8 children with proven lymphadenitis caused by *M. tuberculosis.*	Composite antigen	Culture	Unknown	Palpable induration and visible erythema	100%	17%	20
Detjen et al. (2007)	Prospective	73 children, 28 with confirmed *M. tuberculosis* lymphadenitis, 23 with confirmed NTM lymphadenitis and 22 with other respiratory tract infections.	IFN-g as-say	Bacterio-logical confirmation	Clinical sam-ples	Blood sampling	0%	Irrele-vant[b]	73
Davidson et al. (1993)	Prospective	*M. avium* All children presenting with a probable NTM cervicofacial lymphadenitis diagnosis between 1987 and 1988.	*M. avium* PPD stimulated IFN-g assay	Culture, histology	Excisional bi-opsy	Blood sampling	87,5%	100%	38
Kontturi et al. (2016)	Retrospective	10 children with microbiologically confirmed disease and 21 healthy asymptomatic children. Both groups were non-BCG vaccinated.	PPD-S-stimulated IFN-g assay	Culture	Excisional biopsy, fine needle aspiration, draining fistula	Blood sampling	100%	81%	31

[a]Sensitivity and specificity values could not be calculated as there was a significant number of cases with suspicion of NTM lymphadenitis, but no microbiological confirmation. [b]In the group with confirmed NTM lymphadenitis, the test was solely negative.
N indicates number of included subjects; PPD, purified protein derivative; IFN, interferon; ZN, Ziehl-Neelsen; *M*, *Mycobacterium*.

Diagnostic test accuracy

Polymerase chain reaction and culture

The diagnostic test accuracy of polymerase chain reaction was studied in two included articles (20, 21). In a study including 67 children, PCR was superior to culture in diagnosing NTM cervicofacial lymphadenitis with sensitivities of 71,6% and 41,8%, respectively. Both methods showed a specificity of 100%. The microbiological tests were done on fine needle aspiration specimens (21). In the report of April et al. (1996), PCR was positive in all 7 bacteriologically proven cases and negative in five cases of suspected mycobacterial lymphadenitis. In two control cases, PCR was positive once. No sensitivity and specificity values could be calculated based on these results (20).

Skin tests

Four studies included in this systematic review focused on skin testing (22-25). Skin testing is performed by injecting purified protein derivative (PPD), a non-infectious extract from bacteria, under the top layer of the skin. As a result of a type IV hypersensitivity, the skin reaction can be measured 24-72 hours later. PPD-S is a protein extract from *M. tuberculosis* (26). Overall sensitivity of PPD-S skin tests was 70% (95% CI [62% - 78%]) with an overall specificity being 94% (95% CI [88% - 100%]). (22, 23). *M. avium* skin tests were found to have excellent diagnostic value with a sensitivity of 93% and a specificity of 98% in 174 children with negative serological results for other infectious causes than NTM and thus a high clinical suspicion. However, *M. avium* skin tests are not commercially available anymore (22).

With a cut-off value of 5 mm, the diagnostic test accuracy of a skin test with composite antigen prepared from strains of miscellaneous NTM was investigated. The sensitivity was 100%, but the specificity was merely 17% due to a control group mainly consisting of patients with tuberculous infection (24).

Huebner et al. (1992) showed that children with lymphadenopathy and positive NTM cultures are 12 times more likely to produce ≥ 3 mm. or more induration to a PPD-B (protein extract from *M. intracellulare*) skin test than children with lymphadenopathy and negative cultures (27). No sensitivity or specificity measurements were performed in this study due to the fact that culture confirmation was possible in only 44% of the children. It is stated that there were most likely children with negative culture results who had disease resulting from mycobacteria as well as lymphadenopathy with another causative agent (25).

Acid-fast staining techniques

Auramine staining sensitivity ranged between 46%-85%, whereas the specificity ranged between 60%-100% (21, 28). Ziehl-Neelsen staining was found to have a sensitivity of 60%

and specificity of 85%. In this study, the sensitivity and specificity were calculated, including all 22 children. However, only 15 children had a neck lesion, whereas the other subjects suffered from NTM lymphadenitis at different sites (28).

Immunodiagnostic assays

In three included articles the diagnostic accuracy of several interferon-γ release assay (IFN-γ assay) was studied (29-31). Sensitivity of PPD-stimulated IFN-γ was found to be 81% with a specificity of 100% (31).

In a retrospective study, an IFN-γ assay stimulated with *M. avium* PPD and PPD-S proved to have 100% sensitivity and 81% specificity (29).

A study was carried out on the regular IFN-γ assay to determine whether it was useful in discriminating tuberculous from NTM lymphadenitis. The test proved to have a sensitivity of 93% and a specificity of 100% for children with tuberculous lymphadenitis, and the IFN-γ assay was negative in all cases of bacteriologically proven NTM cervicofacial lymphadenitis (30).

QUADAS-2

As for the risk of bias assessment by the QUADAS-2 tool, the study of Arnold et al. (1970) was the only study with a low risk of bias in all domains (23). Most studies used a threshold that was not pre-specified. Moreover, when case-control designs were used, it was often unclear if the index test was interpreted without knowledge of the reference standard and conversely. Confirming the diagnosis of NTM cervicofacial lymphadenitis is difficult due to suboptimal sensitivities of the possible tests. Therefore, this domain was scored as having a low risk of bias in the case of a good description of the method used. The complete assessment is shown in Table 2b.

Table 2b QUADAS score

Study	Risk of bias			
	Patient selection	Index test	Reference standard	Flow and timing
April et al. (1996)	☹[a]	☹	☹	☺[b]
Arnold et al. (1970)	☺	☺	☺	☺
Bruijnesteijn van Coppenraet et al. (2004)	☹	☹	☺	☺
Davidson et al. (1993)	☺	☹	?[c]	☺
Detjen et al. (2007)	☺	?[c]	☺	☺
Huebner (1992)	☺	☺	☺	☹
Kommaredi et al. (1983)	☹	☺	☺	☺
Kontturi et al. (2016)	☹	☹	☹	☹
Lindeboom et al. (2006)	☺	☹	☺	☺
Marks et al. (1977)	☹	☹	☹	☹

[a]☹High Risk, [b]☺Low Risk, [c]? Unclear Risk

Discussion

Specimen collection

In cases of NTM cervicofacial lymphadenitis, definitive diagnosis is dependent on the isolation of the mycobacterial specimen, which can either be achieved by PCR or microbiological culture. Material for these procedures can be obtained in three different ways; fine needle aspiration cytology, biopsy or complete excision. PCR on material obtained by fine needle aspiration yields significantly more positive results. However, these findings are insignificant for culture and acid-fast staining (21). Tissue biopsy and fine needle aspiration are associated with fistula formation and have lower cure rates than wait-and-see therapy (7, 11, 32). Specimen quality is also of importance with regard to the performance of a diagnostic test. A standardized approach of microbiological specimen collection and handling is essential for obtaining valid diagnostic test accuracy results. As shown in Table 2b, some studies included in this review did not accurately report how the index test was performed, possibly resulting in biased diagnostic test accuracy values.

Skin testing

This systematic review shows that many different skin tests are investigated to diagnose NTM cervicofacial lymphadenitis in children. Several articles that did not meet the inclusion criteria studied dual skin testing. Experimentally induced animal infections form the basis for the evidence that homologous antigens result in a stronger reaction than the heterologous variant (15, 16). Based on culture-proven mycobacterial disease, dual skin testing with PPD-B and PPD-T seems valuable in successfully discriminating tuberculous disease from nontuberculous disease in adolescents and children (33-35). However, specificity of dual skin testing seems to be lacking (13, 36).

Unfortunately, most PPD skin tests, including *M.avium* skin tests, are no longer commercially available. This has led the PPD-S skin test to remain the only skin test currently available in daily practice. Lindeboom et al. (2006) have proved that in children with no prior TBC exposure and no BCG vaccination who live in a country with low TBC incidence, the PPD-S test can reach a specificity of 98%. Moreover, with these baseline characteristics, the PPD-S skin test had a positive predictive value of 98% (22). However, these findings come from a specific population and thus lack external validity. The different types of antigens for skin testing have not been standardized for NTM cervicofacial lymphadenitis, which results in clinical heterogeneity in the available literature.

Immunodiagnostic assays

The results of this systematic review show that IFN-γ assay with PPD stimulation seems promising in diagnosing NTM cervicofacial lymphadenitis preoperatively (29-31). An

advantage of immunodiagnostic tests is the minimally invasive nature, since there is no fine needle aspiration or excisional biopsy needed. It is also shown that a regular IFN-γ assay has great discriminatory value regarding tuberculous and nontuberculous lymphadenitis. Whereas Detjen et al. (2007) studied the discriminatory value of the IFN-γ assay in a prospective manner, Hermansen et al. (2014) found comparable results in a retrospective study (37). The IFN-γ assay may be appropriate if is a degree of uncertainty whether the neck lesion is caused by NTM or *M. tuberculosis*. However, prospective research with sufficient enrolled children should be conducted to confirm these findings.

QUADAS-2

A possible explanation for the lack of proper quality diagnostic reports of NTM cervicofacial lymphadenitis is that definitive diagnosis is difficult due to suboptimal culturing and PCR sensitivity.

Chaparas (1994) acknowledges this problem. In this editorial, he stated that standardization of skin tests is essential for diagnosing NTM disease. However, standardization is complicated due to a lack of definitive diagnosis (38). Moreover, in the study of Huebner et al. (1992), it is stated that no sensitivity and specificity measurements could be performed due to the inability to diagnose mycobacterial disease and indeterminate cultures. This would introduce misclassification bias (25).

Moreover, because NTM cervicofacial lymphadenitis in children is a rare disease, enrolling a sufficient number of subjects might be problematic.

Diagnostic algorithm

Staufner et al. (2005) suggested an algorithm for the diagnosis of mycobacterial cervical lymphadenitis. In this algorithm, it is advised to always perform an IFN-γ assay test in case of a positive tuberculin skin test and clinical suspicion for mycobacterial lymphadenitis (39). However, the reviewers only recommend the use of IFN-γ assay tests in cases of tuberculous suspicion (22). Moreover, in contrast to what is stated in the algorithm of Staufner, Bruijnesteijn van Coppenraet et al. (2004) proved that a negative fine needle aspiration does not necessarily exclude the possibility of mycobacterial lymphadenitis (40).

General discussion

This study's strength is the fact that the available literature was gathered systematically according to the PRISMA guidelines. However, the results of this systematic review must be interpreted with caution, as there was a wide variance in the setup and baseline characteristics of the available literature on diagnosing NTM cervicofacial lymphadenitis in children. The

extensive clinical heterogeneity of the results limited the ability to perform a meta-analysis or calculate overall diagnostic accuracy values. Moreover, the quality of the included studies was assessed based on the information contained in the reports. It is important to recognize that a study with a biased design that is well reported could be judged to be of high quality and contrariwise. To validate the results of this systematic review, more studies of high methodological quality are needed.

Implications for practice

This systematic review shows that reports of high methodological quality on the diagnostic accuracy of these methods are scarce and that the sensitivity remains suboptimal. It clarifies that a stepwise approach is recommended in diagnosing NTM cervicofacial lymphadenitis, since no single diagnostic method can provide both optimal sensitivity and specificity.

In clinical practice, it is essential to exclude other causes for cervicofacial lymphadenitis and thereby indicating a high suspicion of an NTM infection at first. Subsequently, a PPD-S skin test can be used. A positive PPD-S skin test is indicative of NTM lymphadenitis in cases with a high clinical suspicion and if the possibility of *M. tuberculosis* infection is ruled out. Definitive diagnosis is dependent on isolation of the mycobacterial specimen, which can either be achieved by PCR or microbiological culture. A fine needle aspiration is indicated to establish a definitive diagnosis preoperatively. However, it is important to realize that PCR and culture do not provide 100% sensitivity, partly depending on the sample quality.

Implications for future research

IFN-γ assays with purified protein derivative appear to be promising in diagnosing NTM cervicofacial lymphadenitis as an immunodiagnostic test. Prospective studies should be conducted with a sufficient number of enrolled children and definitive diagnosis to validate this technique's potential and use it in daily practice. Moreover, the ability of IFN-γ assay to discriminate tuberculous from nontuberculous lymphadenitis should be studied prospectively based on definitive diagnosis and a sufficient number of enrolled children.

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Appendix

Supplementary Data

PubMed Session Results (02 Mar 2017)

#	Query	Results
#4	#1 AND #2 AND #3	384
#3	child*[tw] OR schoolchild*[tw] OR infan*[tw] OR adolescen*[tw] OR pediatri*[tw] OR paediatr*[tw] OR neonat*[tw] OR boy[tw] OR boys[tw] OR boyhood[tw] OR girl[tw] OR girls[tw] OR girlhood[tw] OR youth[tw] OR youths[tw] OR baby[tw] OR babies[tw] OR toddler*[tw] OR teen[tw] OR teens[tw] OR teenager*[tw] OR newborn*[tw] OR postneonat*[tw] OR postnat*[tw] OR perinat*[tw] OR puberty[tw] OR preschool*[tw] OR suckling*[tw] OR picu[tw] OR nicu[tw]	3,837,196
#2	"Lymphadenitis"[Mesh] OR lymphadenit*[tiab] OR adenitis[tiab] OR adenitides[tiab] OR lymphoadenitis[tiab]	11,483
#1	"Mycobacterium Infections, Nontuberculous"[Mesh] OR "Nontuberculous Mycobacteria"[Mesh] OR nontuberculous mycobacter*[tiab] OR non-tuberculous mycobacter*[tiab] OR mycobacterium flavescens[tiab] OR mycobacterium gilvum[tiab] OR mycobacterium terrae[tiab] OR mycobacterium obuense[tiab] OR mycobacterium szulgai[tiab] OR mycobacterium duvalii[tiab] OR mycobacterium gordonae[tiab] OR atypical mycobacter*[tiab] OR Mycobacterium avium Complex[tiab] OR Mycobacterium chelonae[tiab] OR Mycobacterium fortuitum[tiab] OR Mycobacterium kansasii[tiab] OR Mycobacterium marinum[tiab] OR Mycobacterium scrofulaceum[tiab] OR Mycobacterium smegmatis[tiab] OR Mycobacterium ulcerans[tiab] OR Mycobacterium xenopi[tiab]	19,125

Embase.com Session Results (16 Mar 2017)

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#2	'lymphadenitis'/exp OR lymphadenit*:ab,ti OR adenitis:ab,ti OR adenitides:ab,ti OR lymphoadenitis:ab,ti	23,851
#1	'atypical mycobacteriosis'/exp OR 'atypical Mycobacterium'/exp OR 'nontuberculous mycobacter*':ab,ti OR 'non-tuberculous mycobacter*':ab,ti OR 'mycobacterium flavescens':ab,ti OR 'mycobacterium gilvum':ab,ti OR 'mycobacterium terrae':ab,ti OR 'mycobacterium obuense':ab,ti OR 'mycobacterium szulgai':ab,ti OR 'mycobacterium duvalii':ab,ti OR 'mycobacterium gordonae':ab,ti OR 'atypical mycobacter*':ab,ti OR 'atypic mycobacter*':ab,ti OR 'Mycobacterium avium Complex':ab,ti OR 'Mycobacterium chelonae':ab,ti OR 'Mycobacterium fortuitum':ab,ti OR 'Mycobacterium kansasii':ab,ti OR 'Mycobacterium marinum':ab,ti OR 'Mycobacterium scrofulaceum':ab,ti OR 'Mycobacterium smegmatis':ab,ti OR 'Mycobacterium ulcerans':ab,ti OR 'Mycobacterium xenopi':ab,ti	20,186

Wiley / Cochrane Library Session Results (21 Mar 2017)

#	Query	Results
#4	#1 AND #2 AND #3	11
#3	(child* or schoolchild* or infan* or adolescen* or pediatri* or paediatr* or neonat* or boy or boys or boyhood or girl or girls or girlhood or youth or youths or baby or babies or toddler* or teen or teens or teenager* or newborn* or postneonat* or postnat* or perinat* or puberty or preschool* or suckling* or picu or nicu):ab,ti,kw	205,719
#2	(lymphadenit* or adenitis or adenitides or lymphoadenitis):ab,ti,kw	168
#1	("nontuberculous mycobacter*" or "non-tuberculous mycobacter*" or "mycobacterium flavescens" or "mycobacterium gilvum" or "mycobacterium terrae" or "mycobacterium obuense" or "mycobacterium szulgai" or "mycobacterium duvalii" or "mycobacterium gordonae" or "atypical mycobacter*" or "atypic mycobacter*" or "Mycobacterium avium Complex" or "Mycobacterium chelonae" or "Mycobacterium fortuitum" or "Mycobacterium kansasii" or "Mycobacterium marinum" or "Mycobacterium scrofulaceum" or "Mycobacterium smegmatis" or "Mycobacterium ulcerans" or "Mycobacterium xenopi"):ab,ti,kw	241

Searches:

Embase:

(('atypical mycobacteriosis'/exp OR 'atypical mycobacterium'/exp OR 'nontuberculous mycobacter*':ab,ti OR 'non-tuberculous mycobacter*':ab,ti OR 'mycobacterium flavescens':ab,ti OR 'mycobacterium gilvum':ab,ti OR 'mycobacterium terrae':ab,ti OR 'mycobacterium obuense':ab,ti OR 'mycobacterium szulgai':ab,ti OR 'mycobacterium duvalii':ab,ti OR 'mycobacterium gordonae':ab,ti OR 'atypical mycobacter*':ab,ti OR 'atypic mycobacter*':ab,ti OR 'mycobacterium avium complex':ab,ti OR 'mycobacterium chelonae':ab,ti OR 'mycobacterium fortuitum':ab,ti OR 'mycobacterium kansasii':ab,ti OR 'mycobacterium marinum':ab,ti OR 'mycobacterium scrofulaceum':ab,ti OR 'mycobacterium smegmatis':ab,ti OR 'mycobacterium ulcerans':ab,ti OR 'mycobacterium xenopi':ab,ti) AND ('lymphadenitis'/exp OR lymphadenit*:ab,ti OR adenitis:ab,ti OR adenitides:ab,ti OR lymphoadenitis:ab,ti)) AND ('child'/exp OR 'adolescent'/exp OR 'pediatrics'/exp OR child*:ab,ti OR schoolchild*:ab,ti OR infan*:ab,ti OR adolescen*:ab,ti OR pediatri*:ab,ti OR paediatr*:ab,ti OR neonat*:ab,ti OR boy:ab,ti OR boys:ab,ti OR boyhood:ab,ti OR girl:ab,ti OR girls:ab,ti OR girlhood:ab,ti OR youth:ab,ti OR youths:ab,ti OR baby:ab,ti OR babies:ab,ti OR toddler*:ab,ti OR teen:ab,ti OR teens:ab,ti OR teenager*:ab,ti OR newborn*:ab,ti OR postneonat*:ab,ti OR postnat*:ab,ti OR perinat*:ab,ti OR puberty:ab,ti OR preschool*:ab,ti OR suckling*:ab,ti OR picu:ab,ti OR nicu:ab,ti)

Pubmed:

"Mycobacterium Infections, Nontuberculous"[Mesh] OR "Nontuberculous Mycobacteria"[Mesh] OR nontuberculous mycobacter*[tiab] OR non-tuberculous mycobacter*[tiab] OR mycobacterium flavescens[tiab] OR mycobacterium gilvum[tiab] OR mycobacterium terrae[tiab] OR mycobacterium obuense[tiab] OR mycobacterium szulgai[tiab] OR mycobacterium duvalii[tiab] OR mycobacterium gordonae[tiab] OR atypical mycobacter*[tiab] OR Mycobacterium avium Complex[tiab] OR Mycobacterium chelonae[tiab] OR Mycobacterium fortuitum[tiab] OR Mycobacterium kansasii[tiab] OR Mycobacterium marinum[tiab] OR Mycobacterium scrofulaceum[tiab] OR Mycobacterium smegmatis[tiab] OR Mycobacterium ulcerans[tiab] OR Mycobacterium xenopi[tiab]) AND ("Lymphadenitis"[Mesh] OR lymphadenit*[tiab] OR adenitis[tiab] OR adenitides[tiab] OR lymphoadenitis[tiab]) AND (child*[tw] OR schoolchild*[tw] OR infan*[tw] OR adolescen*[tw] OR pediatri*[tw] OR paediatr*[tw] OR neonat*[tw] OR boy[tw] OR boys[tw] OR boyhood[tw] OR girl[tw] OR girls[tw] OR girlhood[tw] OR youth[tw] OR youths[tw] OR baby[tw] OR babies[tw] OR toddler*[tw] OR teen[tw] OR teens[tw] OR teenager*[tw] OR newborn*[tw] OR postneonat*[tw] OR postnat*[tw] OR perinat*[tw] OR puberty[tw] OR preschool*[tw] OR suckling*[tw] OR picu[tw] OR nicu[tw])

Cochrane:
("nontuberculous mycobacter*" or "non-tuberculous mycobacter*" or "mycobacterium flavescens" or "mycobacterium gilvum" or "mycobacterium terrae" or "mycobacterium obuense" or "mycobacterium szulgai" or "mycobacterium duvalii" or "mycobacterium gordonae" or "atypical mycobacter*" or "atypic mycobacter*" or "Mycobacterium avium Complex" or "Mycobacterium chelonae" or "Mycobacterium fortuitum" or "Mycobacterium kansasii" or "Mycobacterium marinum" or "Mycobacterium scrofulaceum" or "Mycobacterium smegmatis" or "Mycobacterium ulcerans" or "Mycobacterium xenopi"):ab,ti,kw AND (lymphadenit* or adenitis or adenitides or lymphoadenitis):ab,ti,kw and (child* or schoolchild* or infan* or adolescen* or pediatri* or paediatr* or neonat* or boy or boys or boyhood or girl or girls or girlhood or youth or youths or baby or babies or toddler* or teen or teens or teenager* or newborn* or postneonat* or postnat* or perinat* or puberty or preschool* or suckling* or picu or nicu):ab,ti,kw

Chapter 3

Evaluation of anti-glycopeptidolipid-core immunoglobulin A antibody detection for the diagnosis of nontuberculous mycobacterial cervicofacial lymphadenitis

Willemse SH, Oomens MAEM, Karssemakers LHE, Lindeboom JA, Schreuder WH, Ho JPTF, Van der Kuip M, Vlaming KE, Kaptein TM, De Lange J

This chapter is based on the following publication: Willemse SH, Oomens MAEM, Karssemakers LHE, Lindeboom JA, Schreuder WH, Ho JPTF, Van der Kuip M, Vlaming KE, Kaptein TM, De Lange J. Evaluation of Anti-Glycopeptidolipid-Core Immunoglobulin A Antibody Detection for the Diagnosis of Nontuberculous Mycobacterial Cervicofacial Lymphadenitis. Pediatr Infect Dis J. 2024;43(11):e416–8. doi: 10.1097/INF.0000000000004462.

Abstract

Introduction

Non-specific symptoms hamper early recognition of nontuberculous mycobacterial (NTM) cervicofacial lymphadenitis. A delay in diagnosis complicates optimal treatment, since late surgical intervention is associated with a higher surgical morbidity. The current study aims to investigate the diagnostic performance of detecting MAC-specific anti-glycopeptidolipid-core immunoglobulin A antibodies in serodiagnosis of NTM cervicofacial lymphadenitis.

Materials & Methods

A multicenter cross-sectional diagnostic study was carried out. Consecutively presenting children (0-15 years of age) who underwent surgery under general anesthesia because of a strong clinical suspicion of NTM cervicofacial lymphadenitis were eligible for enrollment. A control group comprised children (0-15 years of age) undergoing surgery under general anesthesia for reasons other than lymphadenopathy. Serum IgA antibodies against the glycopeptidolipid-core antigen were measured.

Results

In total, 45 subjects were enrolled in this study, 32 of which underwent surgery because of a strong clinical suspicion of NTM cervicofacial lymphadenitis. Of these 32 patients, 22 NTM cases were microbiologically confirmed. Seventeen of the identified mycobacteria belonged to MAC. In 5 cases histopathologic examination revealed (necrotizing) granulomatous infection suggestive of mycobacterial disease, but microbiological confirmation was not achieved. The area under the curve for bacteriologically proven NTM lymphadenitis and controls was 0.545 (N = 40, 95% CI 0.37 – 0.73), and for MAC lymphadenitis and controls 0.528 (N = 39, 95% CI 0.33 – 073) respectively.

Conclusion

The tested enzyme immunoassay has inadequate diagnostic performance in the studied population of patients with NTM cervicofacial lymphadenitis and seems to be of no additional value in detecting cases of NTM cervicofacial lymphadenitis.

Introduction

Violaceous skin discoloration, skin breakdown, and purulent discharge represent the characteristic clinical course of nontuberculous mycobacterial (NTM) cervicofacial lymphadenitis in children. However, these symptoms are indicative of a relatively late disease stage (1). Early disease symptoms such as lymph node enlargement and mild skin erythema are less specific and often interpreted as benign reactive lymphadenopathy. Therefore, early recognition is hampered. A delay in diagnosis complicates optimal treatment, since late surgical intervention is associated with a higher surgical morbidity. Treatment is preferably performed at early disease stages before fistula formation, as this may influence the esthetic outcome (2). Therefore, it is important to consider NTM as a potential causative agent of persistent lymphadenopathy in children aged 0-5 years. Obtaining specimens from lymph nodes is required for microbiological tests and confirming the diagnosis. This procedure is relatively invasive, especially for young children. Serodiagnosis is less invasive and routinely used for other causes of (sub)chronic lymphadenopathy, such as Epstein-Barr virus or *Toxoplasma gondii* infection. An enzyme immunoassay measuring serum immunoglobulin A (IgA) antibodies that bind *Mycobacterium avium* complex (MAC) specific anti-glycopeptidolipid-core antigens is currently commercially available (3). MAC is a serological complex of serovars of *M. avium* and *M. intracellulare* (4). These account for approximately 75% of NTM cervicofacial lymphadenitis cases in the Netherlands and are identified as causative agents in most cases globally (5, 6). Glycopeptidolipids (GPLs) are found in the cell envelopes of MAC organisms. In patients with MAC pulmonary disease, the sensitivity of this immunoassay is estimated to be 70% (7). However, the diagnostic accuracy has not yet been investigated in patients suspected of NTM cervicofacial lymphadenitis. This study aimed to investigate the diagnostic performance of the detection of MAC-specific anti-GPL-core IgA antibodies in serodiagnosis of NTM cervicofacial lymphadenitis.

Materials & methods

Subjects

A multicenter cross-sectional study was carried out in the Amsterdam University Medical Center (UMC) and Radboud UMC. Consecutively presenting children (0-15 years of age) who underwent surgery under general anesthesia because of a strong clinical suspicion of NTM cervicofacial lymphadenitis were eligible for enrollment. Clinical suspicion was based on cervicofacial lymphadenopathy persisting for a period of at least 3 weeks, negative serologic tests for other causes of (sub)chronic lymphadenopathy (Epstein-Barr virus, cytomegalovirus, Bartonella species, and *Toxoplasma gondii*) and suspicious sonographic features (hypoechoic lymph nodes, often with central necrosis, nodal matting and adjacent soft tissue edema) (8). The possibility of tuberculosis was ruled out based on the absence of a travel history to high-burden countries for tuberculosis, an interferon-gamma release assay

test in case of unclear history, or a negative polymerase chain reaction (PCR) on clinical specimens.

A control group comprised children (0-15 years of age) undergoing surgery under general anesthesia for reasons other than lymphadenopathy. Children using immunosuppressive drugs or having known immunodeficiencies were not eligible for enrollment.

To obtain an effect estimate of the diagnostic test accuracy, one enzyme immunoassay kit including 96 wells was used, which yielded test results of 45 patient serum samples due to duplicate testing and manufacturer's reference samples. To provide additional diagnostic value in clinical practice, a minimum sensitivity of 70% and 90% specificity was required in accordance with the diagnostic performance in patients with MAC pulmonary disease.

Specimen collection and analysis

Blood samples were taken during general anesthesia and prior to the start of the surgical procedure. Subsequently, serum was isolated and stored at -80 degrees Celsius. Excised lymph node tissue was sent for evaluation to the Department of Medical Microbiology and Pathology, and pus samples were sent to the Department of Medical Microbiology.

Microbiological laboratory procedures

Microbiological analysis of excised lymph node and pus samples of patients suspected of NTM cervicofacial lymphadenitis included a *M. tuberculosis* PCR, mycobacterial genus PCR, *M. avium* complex PCR, mycobacterial culture, auramine/Ziehl Neelsen staining, *B. henselae* PCR and a conventional microbial culture. All clinical samples were received and split for microscopy, culture and molecular diagnostics at the microbiology laboratory.

According to a routine laboratory procedure, modified auramine O (SDL Modified auramine O Stain set, Fischer Scientific) staining was performed and viewed with fluorescent microscopes by trained personnel. Samples were cultured in liquid (Mycobacterial Growth Indicator Tube [MGIT], BD BACTEC™ MGIT™ 960, USA) and on solid media (Coletsos, Artelt-ENCLIT, Germany), and incubated 6 weeks and 8 weeks respectively. Positive cultures were stained by Modified auramine O to confirm acid fastness, and identified by molecular identification through 16S-23S at the Amsterdam UMC, or by Whole Genome Sequencing by the National Reference Laboratory in Radboud UMC. The National Reference Laboratory also did phenotypic susceptibility testing.

DNA extraction was performed using the MagNA Pure 96 platform, using the small volume viral DNA/RNA kit, according to the manufacturer's specifications (Roche Diagnostics, Almere, the Netherlands). Our in-house NTM PCR assay developed specifically for

biopsies, lower respiratory tract material, and punctate, was then applied to the DNA eluate of the obtained clinical specimens collected. PCR was performed on the LC480-II platform (Roche Diagnostics, Almere, the Netherlands). Results were assessed in the Roche FLOW software (Roche Diagnostics, Almere, the Netherlands). In order to pass QC, the internal, positive, and negative control needed to be within the set limits for this assay. According to our in-house algorithm, a *M. avium* complex PCR was performed on each NTM PCR positive sample. Results were reported as positive or negative in the laboratory information system and subsequently reported in the patient information system after authorization by the clinical microbiologist.

Serum analysis

Serum was thawed upon testing. Serum IgA antibodies against the GPL-core antigen were measured using an enzyme immunoassay kit (Tauns Laboratoy Inc., Shizuoka, Japan) according to the manufacturer's instructions. Serum was diluted 41 times. Optical densities were measured at 450nM using BioTek synergy HT reader. Results were calculated based on obtained optical densities from standard samples provided by the manufacturer and calculated using the formula x=(y-b)/a. Results are given as U/mL, and negative values were kept as a product of the formula, and the cut-off value of 0.7 U/mL, as advised by the manufacturer, was used as the threshold value to differentiate between positive and negative (9, 10).

Study groups

Subjects were divided into three groups:

- Proven NTM lymphadenitis based on microbiological confirmation by PCR, culture, or acid-fast staining techniques.
- Probable NTM lymphadenitis lacking microbiological confirmation. The probable diagnosis was based on cervicofacial lymphadenopathy for a period of at least 3 weeks, suspicious sonographic features (hypoechoic lymph nodes, often with central necrosis, nodal matting, and adjacent soft tissue edema), and serologic exclusion of other infectious causes of (sub)chronic lymphadenopathy and corresponding histopathologic results (micro-abscesses, necrotizing granulomas, giant cells) (8, 11).
- Controls without any history of suspicion or clinical signs of a past or current NTM cervicofacial lymphadenitis

Patients were classified as controls if postoperative microbiological and histopathologic examination revealed a confirmed cause of lymphadenopathy other than NTM cervicofacial lymphadenitis.

A subgroup analysis evaluating MAC lymphadenitis was performed based on these three groups:

- Proven MAC lymphadenitis based on microbiological confirmation by PCR and/or culture
- Possible MAC lymphadenitis. This group consisted of patients with a microbiological confirmation of NTM lymphadenitis but lacking microbial species isolation.
- Controls without NTM cervicofacial lymphadenitis caused by MAC

Statistics

Data were collected using Castor EDC, and statistical analyses were performed with IBM SPSS Statistics version 28 (IBM Corp., Armonk, NY, USA) and GraphPad Prism version 9 (GraphPad Software, San Diego, CA, USA) for Windows. Descriptive statistics are provided for all study groups.

Ethical approval

The study was formally approved by the Medical Ethics Review Committee of the Amsterdam UMC, location AMC (reference number 2018_315), and the boards of directors of the Amsterdam UMC and Radboud UMC. Written informed consent was required from all parents/guardians and, in subjects 12-15 years of age, from the subjects themselves as well. The study was performed in compliance with the World Medical Association Declaration of Helsinki.

Results

In total, 45 subjects were enrolled in this study between October 2019 and November 2023, 32 of whom underwent surgery because of a strong clinical suspicion of NTM cervicofacial lymphadenitis. Of these 32 patients, 22 NTM cases were microbiologically confirmed postoperatively with either culture, PCR, or acid-fast staining techniques. The identifying species are demonstrated in Table 3a. In 5 cases, histopathologic examination revealed (necrotizing) granulomatous infection suggestive of mycobacterial disease, but microbiological confirmation with PCR, culture, or staining techniques was not achieved. None of the parents/guardians reported a recent travel history to high-burden countries for tuberculosis.

The other 5 patients turned out to have different diagnoses (*B. henselae* lymphadenitis, group A *Streptococcus* lymphadenitis, methicillin-resistant *S. aureus* lymphadenitis, ruptured epidermoid cyst, suppurative lymphadenitis without identified species) than previously suspected and were classified as controls. An additional 13 patients without any history

of clinical suspicion or signs of a past or current NTM cervicofacial lymphadenitis were included in the control group. The diagnoses and surgical procedures of subjects in the control group are demonstrated in the appendix. Table 3a summarizes the characteristics of the included subjects. Four of the included subjects were born in countries other than the Netherlands (India, Italy, Syria, and in the United States).

Figure 3a demonstrates the individual serum IgA antibody levels against the GPL-core antigen. Tables 3b and 3c summarize the diagnostic test accuracy values for the different (sub)groups. For bacteriologically proven NTM lymphadenitis and controls, the sensitivity was 36% and specificity 83% (N = 40, 95% confidence interval (CI) 17% - 35% and 59% - 96%). For MAC lymphadenitis and controls, the sensitivity was 35% and specificity 86% (N = 39, 95% CI 14% - 62% and 65% - 97%).

As illustrated in Figures 3b and 3c, the area under the curve for bacteriologically proven NTM lymphadenitis and controls was 0.55 (N = 40, 95% (CI) 0.37 – 0.73) and for MAC lymphadenitis and controls 0.53 (N = 39, 95% CI 0.33 – 073) respectively.

Table 3a Subject characteristics

	Microbiologically confirmed NTM lymphadenitis (N=22)	**Probable NTM lymphadenitis (N=5)**	**Controls (N=18)**
Sex, n (%)			
Male	10 (45)	3 (40)	9 (50)
Female	12 (55)	2 (60)	9 (50)
Mean age at time of sampling in years ± SD (range)	2.9 ± 1.5 (1.5-6.2)	3.7 ± 3.4 (1.1-9.7)	4.3 ± 4.0 (1.0-16.0)
Mean time from first symptoms to date of sampling in weeks ± SD (range)	15 ± 23 (3-114)	35 ± 31 (4-73)	
Causative species, n (%)			
Mycobacterium avium complex	17 (77)		
M. avium	16 (73)		
M. florentinum	1 (5)		
M. haemophilum	1 (5)		
M. chimaera	1 (5)		
M. kansassi	1 (5)		
M. malmoense	1 (5)		
Unspecified	1 (5)		

N indicates number of included subjects; SD, standard deviation; *M, Mycobacterium*

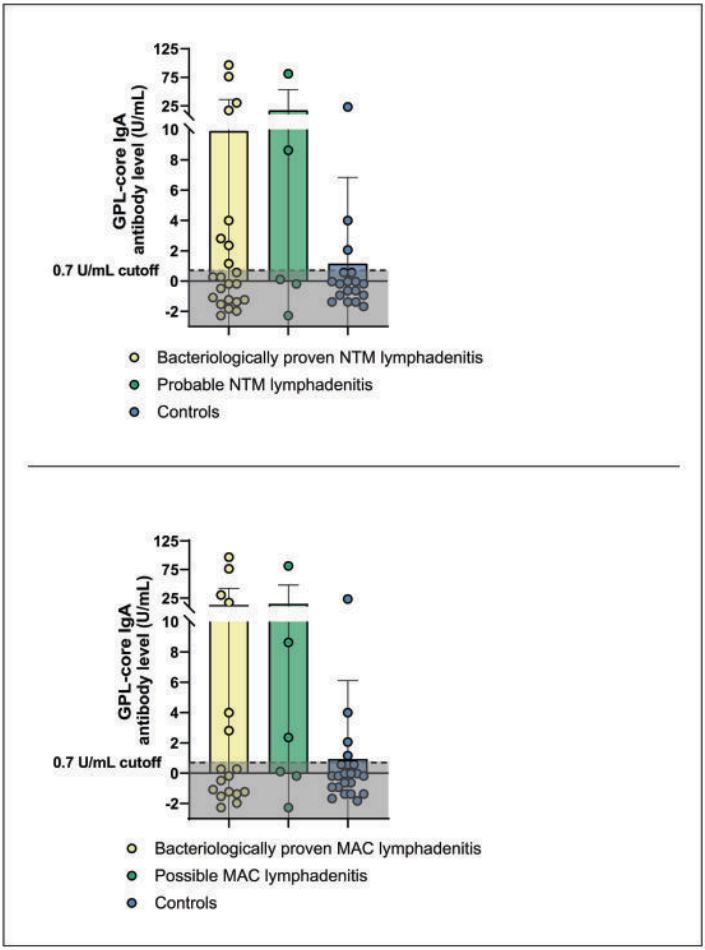

Figure 3a Serum IgA antibodies against the GPL-core antigen of patients with bacteriologically proven NTM lymphadenitis, probable NTM lymphadenitis and controls (upper graph). Serum IgA antibodies against the GPL-core antigen of patients with bacteriologically proven MAC lymphadenitis, possible MAC lymphadenitis and controls (lower graph). The horizontal broken line indicates the cut-off value (0.7 U/mL). Results were calculated based on obtained optical densities from standard samples provided by the manufacturer and calculated using the formula x=(y-b)/a. Negative values were kept as a product of the formula, and the cut-off value of 0.7 U/mL.

Table 3b Test results of study groups

	Microbiologically confirmed NTM lymphadenitis	Probable NTM lymphadenitis	Controls	
Positive[a] anti-GPL IgA titer	8	2	3	13
Negative[a] anti-GPL IgA titer	14	3	15	32
	22	5	18	45

[a]Based on a cut-off value of 0.7 U/mL
NTM indicates nontuberculous mycobacterial; GPL, glycopeptidolipid; IgA, immunoglobulin A.

Table 3c Test results of subgroups

	Microbiologically confirmed MAC lymphadenitis	Possible MAC lymphadenitis	Controls	
Positive[a] anti-GPL IgA titer	6	3	3	12
Negative[a] anti-GPL IgA titer	11	3	19	33
	17	6	22	45

[a]Based on a cut-off value of 0.7 U/mL
MAC indicates *Mycobacterium avium* complex; GPL, glycopeptidolipid; IgA, immunoglobulin A.

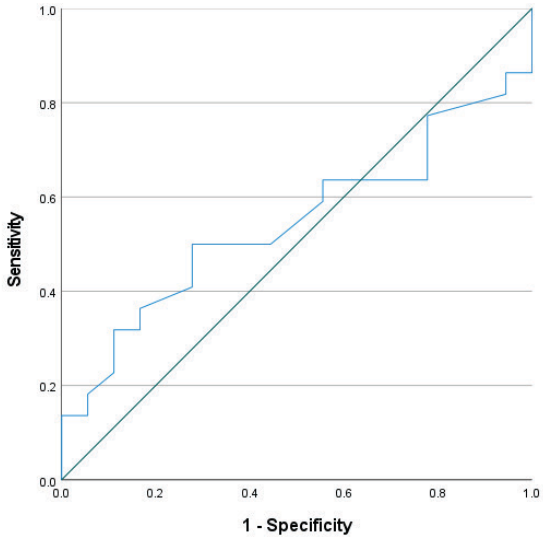

Figure 3b Receiver operating characteristic curve for bacteriologically proven NTM lymphadenitis and controls (AUC = 0.55)

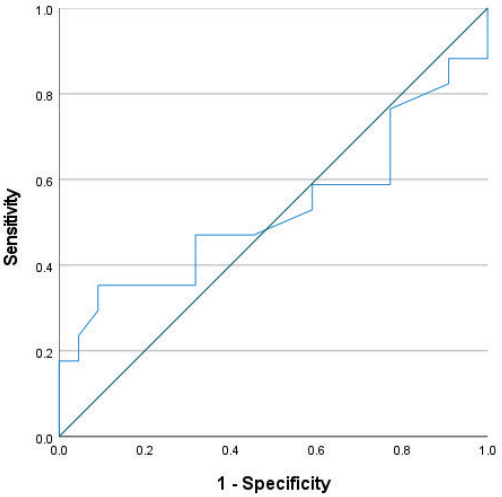

Figure 3c Receiver operating characteristic curve for bacteriologically proven MAC lymphadenitis and controls (AUC = 0.53)

Discussion

The current study evaluated the diagnostic accuracy of an enzyme immunoassay kit measuring MAC-specific anti-GPL-core IgA antibodies in children with and without NTM cervicofacial lymphadenitis. Although some microbiologically proven patients had a positive titer, the test lacked adequate sensitivity and specificity. Subgroup analyses revealed no additional diagnostic value of the MAC-specific anti-GPL-core IgA antibodies assay for subjects with NTM lymphadenitis caused by MAC.

As far as the authors are aware, this is the first study to systematically investigate this immunoassay in subjects with NTM cervicofacial lymphadenitis. The values of the areas under the curve found in this study (0.55 for NTM lymphadenitis and 0.53 for MAC lymphadenitis) are lower than the area under the curve reported in a systematic review from 2016 by Shibata et al. (0.87), which focused on the diagnostic test accuracy for MAC pulmonary disease. This discrepancy indicates a comparatively poorer diagnostic performance of the tested assay in patients with MAC cervicofacial lymphadenitis (7). Patients with NTM cervicofacial lymphadenitis are, in contrast to MAC pulmonary disease, not immunocompromised, and are generally younger. Pulmonary infections include larger infected tissue surfaces, and the immunogenic pathway of MAC cervicofacial lymphadenitis

might be different from that of MAC pulmonary disease. Children are known to have a more immature cellular immunity compared to adults, and IFN- γ release of NTM cervicofacial lymphadenitis patients has been demonstrated to be impaired after mitogenic or antigenic stimulation during their infection (12, 13). It could be hypothesized that patients with NTM cervicofacial lymphadenitis exhibit increased salivary IgA levels instead of increased serum IgA levels due to the location of the infection in the head and neck area and the absence of general disease symptoms (14). Moreover, none of the included studies in the systematic review of Shibata et al. was carried out in a European center, reflecting heterogeneity in the included study subjects compared with those in the he current study.

An interesting finding of the current study was the positive test result in 3 negative controls. This could be due to false positive test detection of IgA or due to an immunogenic response reflecting latent M. avium infection.

A strength of the current study is the completeness of data. No historical data were used, and all subjects received similar reference diagnostics. However, a limitation stems from the relatively small sample of included subjects, resulting in wide 95% confidence intervals around the reported areas under the curve. On the other hand, considering the low incidence of the disease, the number of subjects included is substantial, and the study provides enough information to determine the clinical utility of the test.

Antigen-based skin testing is an alternative minimally invasive diagnostic method to support the clinical suspicion of NTM cervicofacial lymphadenitis. However, skin tests containing NTM antigens are not commercially available anymore despite their high diagnostic accuracy, (15, 16). Due to sensitization to protein purified derivative (PPD) from *M. tuberculosis*, skin tests containing *M. tuberculosis*-derived antigen mixture may be used as a surrogate test in a population of patients with a clinical suspicion of NTM lymphadenitis. After ruling out a history of BCG vaccination and the possibility of tuberculosis, the sensitivity of this test is 70% (16).

Immunodiagnostic assays with PPD stimulation are another minimally invasive diagnostic tool and seem promising. These modified interferon-gamma release assays were described as early as 1990 and have also been evaluated in later studies (17-19). The sensitivity and specificity are estimated to be 100% and 81%, respectively. However, these assays are not commercially available and have yet to be validated in prospective studies. More recently, an NTM-specific interferon-γ release assay stimulated by glycopeptidolipids, was investigated. The sensitivity and specificity estimates of this immunodiagnostic assay were 92% and 100%, respectively, suggesting this test could be used as a tool in the diagnosis of NTM cervicofacial lymphadenitis. These results should be validated in larger and more heterogeneous samples (20).

Conclusion

The tested enzyme immunoassay measuring MAC-specific anti-GPL-core IgA antibodies has inadequate diagnostic performance in the studied population of patients with NTM cervicofacial lymphadenitis in contrast to those with MAC pulmonary disease. It seems to be of no additional value in detecting cases of NTM cervicofacial lymphadenitis. To optimize outcomes in these patients with NTM cervicofacial lymphadenitis, further research on (relatively) noninvasive methods for early detection is needed.

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Appendix

Table 3d Supplementary content. Diagnosis and procedure of controls without previous suspicion of NTM lymphadenitis (N=13)

Diagnosis	Procedure
Congenital pulmonary airway malformation	Thoracoscopic pulmonary resection
Median neck cyst	Excision of median neck cyst
Femoral fracture	Removal of hardware after osteosynthesis
Inguinal hernia	Laparoscopic inguinal hernia repair
Inguinal hernia	Laparoscopic inguinal hernia repair
Inguinal hernia	Laparoscopic inguinal hernia repair
Inguinal hernia	Laparoscopic inguinal hernia repair
Severe constipation	Proctoscopy with full thickness rectal biopsy
Stenosing tenosynovitis of the abductor pollicis longus muscle	A1 pulley division
Preauricular appendage	Excision of preauricular appendage
Inguinal hernia	Open inguinal hernia repair
Anal pain	Proctoscopy
Severe constipation	Full-thickness rectal biopsy

Chapter 4

Can oral swabs be used to diagnose nontuberculous mycobacterial cervicofacial lymphadenitis?

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Abstract

Introduction

We investigated the presence of mycobacteria in the oral cavity and oropharynx of children with nontuberculous mycobacterial (NTM) cervicofacial lymphadenitis compared to negative controls. Our aim was to evaluate whether oral cavity and oropharyngeal swab sampling could serve as a minimally invasive alternative to diagnostic sampling of excised lymph node tissue or punctate.

Materials & Methods

Children who underwent surgery under general anesthesia due to a strong clinical suspicion of NTM cervicofacial lymphadenitis were enrolled in this multicenter cross-sectional study. The control group comprised children undergoing surgery under general anesthesia for reasons other than lymphadenopathy. The obtained oropharyngeal, dorsal tongue and gingival swabs were subjected to mycobacterial polymerase chain reaction (PCR) analysis.

Results

A total of 67 subjects were enrolled in this study, 31 of whom underwent surgery due to strong clinical suspicion of NTM cervicofacial lymphadenitis. Positive genus and *Mycobacterium avium* specific PCR results were found in one patient from the control group, and in none of the patients with (suspected) NTM cervicofacial lymphadenitis.

Conclusion

Our in-house routine diagnostic PCR assay on oral and oropharyngeal swabs was not suitable as a minimally invasive alternative to diagnostic sampling of excised lymph node tissue or punctate. It remains unclear whether the oral cavity and oropharynx might be a portal of entry for mycobacteria in NTM cervicofacial lymphadenitis.

Introduction

Nontuberculous mycobacteria (NTM) are ubiquitous organisms that are typically present in freshwater reservoirs and soils (1). They can cause a wide variety of infections, mainly in immunocompromised individuals. Remarkably, NTM can also cause chronic cervicofacial lymphadenitis in immunocompetent children. This is a rare infection, affecting only 0.6-4.5 per 100,000 children annually, most commonly in the head and neck region (2-6).

The underlying pathophysiological mechanisms remain unclear to this day (2, 3, 7). The literature postulates that the oral cavity is a likely portal of entry due to the predominant anatomic locations of affected lymph nodes (8). Additionally, significant factors might be children's mouthing behaviors and mucosal injuries associated with tooth eruption and shedding (9-11). However, no systematic investigation has explored the presence of NTM in the oral cavity and oropharynx of patients with NTM cervicofacial lymphadenitis.

The diagnosis of NTM cervicofacial lymphadenitis can be challenging. The characteristic clinical presentation becomes apparent at an advanced disease stage with skin changes. It is necessary to obtain specimens from lymph nodes for microbiologic diagnostic confirmation. This procedure is regarded as invasive, especially for young children. Surgical excision leads to the quickest resolution of the disease, especially in cases without skin involvement. Early detection at a stage without specific symptoms, such as violaceous skin discoloration, is crucial. This facilitates early surgical intervention and optimizes the long-term esthetic outcome (12, 13). Considering this context, the implementation of a minimally invasive diagnostic tool prior to the necessity of an invasive surgical treatment procedure may offer significant benefits for this pediatric population.

Therefore, we investigated the presence of mycobacteria in the oral cavity and oropharynx of children with NTM cervicofacial lymphadenitis in comparison to negative controls. Our aim was to evaluate whether oropharyngeal swab sampling could serve as a minimally invasive alternative to diagnostic sampling of excised lymph node tissue or punctate.

Materials & Methods

Subjects

We conducted a multicenter cross-sectional study at the Amsterdam University Medical Center (UMC) and Radboud UMC Nijmegen. Enrolled patients included consecutively presenting children (0-15 years of age) who underwent surgery under general anesthesia due to a strong suspicion of NTM cervicofacial lymphadenitis. The preliminary diagnosis was based on cervicofacial lymphadenopathy persisting for at least 3 weeks, negative serologic tests for other potential causes of (sub)chronical lymphadenopathy (Epstein-Barr virus, cytomegalovirus, *Bartonella* species, and *Toxoplasma gondii*) and distinctive sonographic features (i.e. hypoechoic lymph nodes, often with central necrosis, nodal matting and adjacent soft tissue edema)(14). The possibility of tuberculosis was excluded based on the absence of risk factors for tuberculosis, a negative interferon-gamma

release assay (IGRA) test in cases of unclear history, or a *Mycobacterium tuberculosis complex* negative polymerase chain reaction (PCR) analysis on excised lymph node tissue.

Our control group comprised children, 0-15 years of age, undergoing surgery under general anesthesia for reasons other than lymphadenopathy. Children using immunosuppressive therapy or with known immunodeficiencies were not eligible for enrollment. The use of systemic antibiotics during the three months prior to study participation was documented for all subjects.

Specimen collection

We collected excised lymph node tissue samples from children with suspected NTM lymphadenitis and sent them to the Department of Medical Microbiology & Infection Prevention (MMI), and Pathology for evaluation. We also collected pus samples, which were sent to the MMI for evaluation.

We collected oropharyngeal, dorsal tongue, and gingival swabs from all included patients during general anesthesia. Samples were obtained using FlOQswabs® (FLOQswab, Copan Diagnostics, Murrieta, California, USA, Flocked Swabs 520CS01) and put in a dry tube following a standard operating procedure developed for the current study. NTM have been successfully recovered from FlOQswabs® elsewhere, and these swabs provide a high yield of DNA recovery (15, 16). All swabs were collected from the side of the affected lymph node and stored at -80 degrees Celsius.

Laboratory procedures

All clinical lymph node tissue and pus samples were sent to the MMI, where they were split for microscopy (Ziehl Neelsen staining by the Department of Pathology, Auramine staining by the MMI), culture (conventional and mycobacterial), and molecular diagnostics (PCR analysis for *M. tuberculosis* complex, *Mycobacterium* genus and *Bartonella henselae*).

According to routine laboratory protocol, trained personnel performed modified Auramine O (SDL Modified Auramine O Stain set, Fischer Scientific) staining with visualization using fluorescent microscopes. Samples were cultured in liquid (Mycobacterial Growth Indicator Tube [MGIT], BD BACTEC™ MGIT™ 960, USA) and on solid media (Coletsos, Artelt-ENCLIT, Germany), and incubated for 8 weeks. Positive cultures were stained with Modified Auramine O to confirm acid-fastness and were identified by molecular identification through 16S-23S sequencing at the Amsterdam UMC, or by Whole Genome Sequencing performed at the National Reference Laboratory in Radboud UMC Nijmegen. Phenotypic susceptibility testing was also performed by the National Reference Laboratory in Radboud UMC Nijmegen.

Upon testing, Floqswabs® were thawed, 1 ml phosphate buffered saline was added, and the suspension was vortexed. An aliquot of 300 µl was enzymatically lysed using proteinase K (Qiagen, Venlo, the Netherlands), after which was added an equal amount of MagNA Pure external lysis buffer (Roche Diagnostics, Almere, the Netherlands) and an in-house developed

internal control. DNA extraction was performed on the MagNA Pure 96 platform, using the small volume viral DNA/RNA kit, following the manufacturer's specifications (Roche Diagnostics, Almere, the Netherlands). The DNA eluate of the oropharyngeal swabs was analyzed using our in-house NTM PCR assay, specifically developed for biopsies, lower respiratory tract material, and punctate. PCR was performed on the LC480-II platform (Roche Diagnostics, Almere, the Netherlands), and the results were assessed using the Roche FLOW software (Roche Diagnostics, Almere, the Netherlands). Quality control testing ensured that the internal, positive, and negative controls were within the set limits for this assay. In accordance with the in-house algorithm, *Mycobacterium avium* complex PCR was performed on each NTM positive sample. This PCR was also performed to analyze excised lymph nodes and pus samples. Results were reported as positive or negative in the laboratory information system and subsequently reported in the patient information system following authorization by the clinical microbiologist.

None of the oral swabs were cultured, because they were stored at -80°C, and mycobacterial culture from frozen patient samples has not been validated in Amsterdam UMC.

Study groups

Subjects were divided into three groups:

- Proven NTM lymphadenitis: proven by microbiological confirmation with PCR, culture, or acid-fast staining techniques.
- Probable NTM lymphadenitis: based on the aforementioned preliminary diagnosis and corresponding histopathologic results (micro-abscesses, necrotizing granulomas, and giant cells), without microbiological confirmation (14, 17).
- Controls: Without any history of suspicion or clinical signs of a past or current NTM cervicofacial lymphadenitis

Patients were also classified as controls in cases where postoperative microbiological and histopathologic examination confirmed an infectious neck mass other than NTM cervicofacial lymphadenitis.

Statistics

Data were collected using Castor EDC. Statistical analyses were performed with IBM SPSS Statistics for Windows version 28 (IBM Corp., Armonk, NY, USA).

Ethical approval

The study was formally approved by the Medical Ethics Review Committee of the Amsterdam UMC, location AMC (reference number 2018_315), and the boards of directors of the Amsterdam UMC and Radboud UMC Nijmegen. Written informed consent was required from all parents/guardians, and from the subjects themselves if they were 12-15 years of age. The study was performed in compliance with the World Medical Association Declaration of Helsinki.

Results

Subjects

A total of 67 subjects were enrolled between October 2019 and February 2024, 31 of whom underwent surgery due to a strong suspicion of NTM cervicofacial lymphadenitis. Among these 31 patients, 20 cases were microbiologically confirmed as NTM infection postoperatively by culture, PCR, or acid-fast staining techniques. Six cases were classified as probable NTM lymphadenitis because histopathologic examination revealed (necrotizing) granulomatous infection suggestive of mycobacterial disease, but no microbiological confirmation was achieved with PCR, culture, or acid-fast staining technique. The remaining 5 patients with an infectious neck mass were found to have different diagnoses than previously suspected, including *B. henselae* lymphadenitis, group A *Streptococcus* lymphadenitis, methicillin-resistant *Staphylococcus aureus* lymphadenitis, ruptured epidermoid cyst, and suppurative lymphadenitis without identified species, and were classified as controls. The control group also included 36 individuals without any history of clinical suspicion or signs of past or current NTM cervicofacial lymphadenitis. Table 4a summarizes the characteristics of the included subjects. It was documented for all subjects that none had used tuberculostatic drugs during the three months prior to study participation. In the group with NTM lymphadenitis and probable NTM lymphadenitis, 15 out of 26 subjects had used systemic antibiotics during this period (2 amoxicillin, 12 amoxicillin-clavulanic acid, and 1 amoxicillin + ceftriaxone). In the control group, 15 out of 41 subjects had used systemic antibiotics during the same period, including amoxicillin (1 subject), amoxicillin-clavulanic acid (6 subjects), amoxicillin–clavulanic acid + gentamycin (2 subjects), cefazolin (2 subjects), flucloxacillin (1 subject), clindamycin (1 subject), metronidazole (1 subject), and nitrofurantoin (1 subject). The supplementary content presents the diagnoses and surgical procedures of subjects in the control group and can be found in the Appendix.

Table 4a Subject characteristics

	Microbiologically confirmed NTM lymphadenitis (N=20)	**Probable NTM lymphadenitis (N=6)**	**Controls (N=41)**
Sex, n (%)			
Male	8 (40)	4 (67)	25 (61)
Female	12 (60)	2 (33)	16 (39)
Mean age at time of sampling in years ± SD (range)	3.0 ± 1.5 (1.5-6.2)	3.3 ± 3.3 (1.1-9.7)	3.8 ± 4.2 (0.2-16.0)
Mean time from first symptoms to date of sampling in weeks ± SD (range)	16 ± 24 (4-114)	29 ± 31 (2-73)	
Causative species, n (%)			
M. avium	14 (70)		
M. florentinum	1 (5)		
M. haemophilum	1 (5)		
M. chimaera	1 (5)		
M. kansasii	1 (5)		
M. malmoense	1 (5)		
Unspecified	1 (5)		

N indicates number of included subjects; SD, standard deviation; *M, Mycobacterium*.

Microbiological swab analysis

In none of the patients with bacteriologically proven NTM lymphadenitis or probable NTM lymphadenitis (lacking bacteriologic confirmation) and in one patient from the control group, positive mycobacterial genus and *M. avium*-specific PCR swab results were obtained. Only the gingival swab was positive in the patient with positive PCR results, not the tongue or oropharyngeal swab. This patient had undergone a strabismus correction for decompensated esotropia and was 5 years of age at the time of sampling. The parents reported a negative history of lymph node enlargement in their child. For another subject in the control group, the gingival swab yielded an indeterminate PCR result.

Discussion

In this study, we explored the presence of NTM in the oral cavity and oropharynx of patients with NTM cervicofacial lymphadenitis compared to controls by performing a routine diagnostic NTM PCR analysis of oral and oropharyngeal swabs. Our results indicate that NTM can colonize the oral cavity, as evidenced by the detection of a positive PCR result in a gingival swab of one control patient.

The abundant PCR-negative results from swabs in the current study may have several different explanations, including the absence of NTM, a low mycobacterial load (below PCR detection level), and suboptimal sampling of specimens by means of (thawed) swabs instead of fresh biopsies and punctate (18). Overall, based on the results of the current study, it is impossible to determine whether NTM are present during disease manifestation. Considering the possibility of false-negative results, a higher colonization prevalence cannot be ruled out. From a pathophysiological point of view, a disruption of the microbial balance, facilitating mycobacterial overgrowth, might be part of the pathophysiology. Little is currently known about the mechanisms leading to asymptomatic NTM colonization, onset of disease, disease progression, or persistence in human body sites (19).

Given the absence of tuberculostatic drug use during the three months prior to study participation and the resistance of mycobacteria against the antibiotics used by some of the included subjects, the study results are unlikely to be influenced by systemic antibiotics (20).

In earlier studies, NTM have been cultured from tonsillar tissue and nose and throat swabs obtained from healthy, predominantly school-aged children (21, 22). In later studies using sequencing methods, mycobacterial DNA has been detected in oral, oropharyngeal and nostril samples obtained from healthy adult individuals. Samples in these studies were obtained by dental plaque curettage and swabs (BBL CultureSwab; Becton, Dickinson, and Co)(23, 24). The subjects included in these studies were generally older than those included in the current study, which might indicate that colonization occurs later in childhood.

The findings of NTM taxa in these niches by these previous studies and the current study, prompt questions regarding their role as a possible portal of entry. This question cannot yet be answered based on the currently available evidence. We only focused on the presence of

NTM at the time of disease, but the presence of NTM in these niches before disease onset also remains subject to further investigation

The current study found no correlation between NTM cervicofacial lymphadenitis and positive PCR results of swabs from the oral cavity or oropharynx. This finding indicates that our in-house routine diagnostic PCR assay on these swabs cannot serve as a minimally invasive alternative to the analysis of excised lymph node tissue or punctate. A recently published study evaluated an NTM-specific interferon-γ release assay, stimulated by glycopeptidolipids as a minimally invasive diagnostic alternative. The immunodiagnostic assay's estimated sensitivity and specificity were 92% and 100%, respectively (25). Their findings suggest that, in contrast to PCR analysis of oral and oropharyngeal swabs, the tested immunodiagnostic assay could be used to diagnose NTM cervicofacial lymphadenitis, after further validation.

A strength of the current study is the completeness of data. No historical data were used, and all subjects received similar reference diagnostics. On the other hand, a limitation stems from the non-validated (experimental) sampling technique by means of oral and oropharyngeal swabs instead of tissue samples. The currently utilized PCR is applied in routine clinical mycobacterial diagnostics, and has not been validated on cells from swabs in contrast to biopsies, lower respiratory tract material, and punctate. This complicates our interpretation of the abundant negative PCR results from the current study. However, in light of ethical considerations, it was not possible to take extra biopsies from the oral cavity or oropharynx. Another limitation is the relatively small sample of included subjects. A larger study would have yielded a more accurate estimate of the prevalence of colonization. However, considering the low incidence of NTM cervicofacial lymphadenitis, the number of subjects included is substantial.

In our center, the treatment protocol is to perform surgery in cases of high clinical suspicion, except when there is a high risk of injury to the accessory nerve or marginal branch of the facial nerve, or extensive skin involvement. Without a reliable noninvasive test, the diagnosis is generally confirmed after surgical excision. This situation underscores the ongoing need for non-invasive diagnostic sampling, particularly in the pediatric population, which is predominantly burdened by NTM cervicofacial lymphadenitis.

Future research should be conducted to determine whether sequencing methods can detect NTM in oral and oropharyngeal swabs from patients with NTM cervicofacial lymphadenitis and to prospectively validate promising immunodiagnostic assays.

Conclusion

In conclusion, we found no correlation between NTM cervicofacial lymphadenitis and positive NTM PCR results from oral and oropharyngeal swabs. This renders our in-house routine diagnostic PCR assay on these swabs unsuitable as a minimally invasive alternative to analysis of excised lymph node tissue or punctate. Secondly, it remains unclear whether the oral cavity and oropharynx might be a portal of entry in subsequently occurring cervicofacial lymphadenitis.

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Appendix

Table 4b Diagnosis and procedure of controls without previous suspicion of NTM lymphadenitis (N=36)

Diagnosis	Procedure	N
Anal pain	Proctoscopy	1
Congenital pulmonary airway malformation	Thoracoscopic pulmonary resection	1
Coronal hypospadias	Hypospadias repair with foreskin reconstruction	1
Cryptorchidism	Laparoscopic orchidopexy	2
Decompensated esotropia	Strabismus correction	1
Femoral fracture	Removal of hardware after osteosynthesis	1
Hereditary spherocytosis with splenomegaly	Splenectomy	1
Gastroschisis	Primary closure of scar hernia	1
Hirschsprung's disease	Anal dilatation and injection with corticosteroids	1
Hirschsprung's disease	Sigmoidoscopy	1
Infantile esotropia	Strabismus correction	1
Inguinal hernia	Laparoscopic inguinal hernia repair	4
Inguinal hernia	Open inguinal hernia repair	11
Lateral neck fistula	Excision of lateral neck fistula	1
Median neck cyst	Excision of median neck cyst	2
Occipital dermoid cyst	Excision of occipital dermoid cyst	1
Perianal fistula	Fistulotomy	1
Preauricular appendage	Excision of preauricular appendage	1
Severe constipation	Proctoscopy with rectal biopsy	2
Stenosing tenosynovitis of the abductor pollicis longus muscle	A1 pulley division	1

N indicates number of included subjects

Part II

Surgical challenges and long-term treatment outcomes

Chapter 5

Cervicofacial non-tuberculous mycobacterial lymphadenitis: clinical determinants of incomplete surgical removal

Willemse SH, Karssemakers LHE, Oomens MAEM, Schreuder WH, Lindeboom JA, Van Wijk AJ, De Lange J

This chapter is based on the following publication: Willemse SH, Karssemakers LHE, Oomens MAEM, Schreuder WH, Lindeboom JA, Van Wijk AJ, De Lange J. Cervicofacial non-tuberculous mycobacterial lymphadenitis: clinical determinants of incomplete surgical removal. Int J Oral Maxillofac Surg. 2020;49(11):1392-1396. doi: 10.1016/j.ijom.2020.03.019.

Abstract

Introduction

In patients with nontuberculous mycobacterial cervicofacial lymphadenitis, incomplete surgical removal of infected lymph nodes leads to delayed healing time and higher recurrence rate, with eventually spontaneous drainage through the skin. However, complete surgical removal is not always achievable due to the extent of the infected tissue and proximity of vulnerable structures such as the facial or accessory nerve. The aim of this study was to identify the clinical determinants of the (in)ability to perform complete surgical removal.

Materials & Methods

We analyzed electronic health records of patients aged 0-15 years with a bacteriologically proven nontuberculous mycobacterial cervicofacial lymphadenitis who underwent surgical treatment and preoperative sonographic imaging. The study design was a case-control study.

Results

A total of 103 patients met the inclusion criteria. Most of the infections were unilateral submandibular and caused by *Mycobacterium avium*. Multiple logistic regression analysis revealed that higher age (odds ratio 1.24, 95% confidence interval 1.04 – 1.47) and fistulization (odds ratio 3.15, 95% confidence interval 1.13-8.75) were significantly associated with a limited ability to surgically remove all infected tissue. However, a larger sonographic lymph node size was not significantly associated.

Conclusion

These findings could aid clinicians in informing the patient's parent(s) or guardian(s) preoperatively and in properly estimating the intraoperative and postoperative course.

Introduction

Nontuberculous mycobacteria (NTM) are among the possible causes of head and neck infectious lymphadenopathy in children. With an incidence rate of 0.6-4.5 per 100,000 children, NTM cervicofacial lymphadenitis is a rare infection (1-5). It usually occurs in children aged 1-5 years. An early definitive diagnosis is often hampered by initial mild symptoms, but the further clinical course of the disease is characteristic. At first, there is a painless mass adherent to the skin. Subsequently, fluctuation appears, followed by discoloration of the skin and eventually cutaneous fistulization (6-8). Definite confirmation of the clinical diagnosis requires a positive result of polymerase chain reaction or microbiological culture (9-12).

Cure is generally defined as total skin closure without local recurrence or de novo lesions, as assessed by clinical and ultrasound evaluation (13). Complete surgical removal of the infected lymph node tissue results in definitive resolution of disease (14, 15). In contrast, incomplete surgical removal leads to delayed healing time and higher recurrence rate with eventually spontaneous drainage through the skin (14, 16). However, the main issue in surgical management is that complete surgical removal is not always achievable due to the proximity to vital structures such as peripheral branches of the facial nerve or extensive skin necrosis (13, 14, 16-23). Consequently, it is important to properly estimate the ability to perform complete surgical removal of all infected lymph node tissue preoperatively without causing unnecessary morbidity from surgery.

The aim of this study was to identify clinical determinants that may predict the (in)ability to perform safe and complete surgical removal of the infected tissue. We hypothesized that fistulization and a larger sonographic size of the infected lymph nodes would be associated with incomplete surgical removal.

Materials and Methods

Study design

A case-control study was performed to investigate determinant outcome relationships. Patients treated with incomplete surgical removal were classified as cases, and patients treated with complete surgical removal were classified as controls. Incomplete surgical removal was either based on an explicit statement in the operative report that there was some extent of infected tissue left in situ or on the statement that excision was not possible and curettage was performed. The study design is graphically presented in Figure 5a. This study is reported according to the STROBE statement (24).

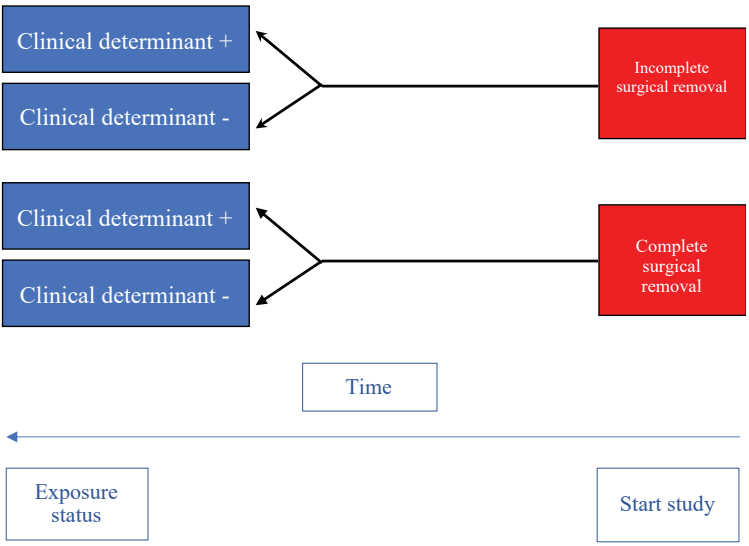

Figure 5a Study design flow chart

Data collection

Historical data were used to conduct the study. Health records were screened systematically for patients referred to the Department of Oral and Maxillofacial Surgery of the Amsterdam University Medical Center (AUMC) with bacteriologically proven or clinically suspected NTM cervicofacial lymphadenitis from July 2001 to October 2018. The inclusion criteria for the subjects were (1) age 0-15 years, (2) bacteriologically proven NTM cervicofacial lymphadenitis, (3) surgical treatment, and (4) availability of sonographic imaging information. The exclusion criteria were unclear or incomplete information in the health records, immuno-incompetence, usage of immunosuppressive drugs, and randomization between surgical removal or curettage due to participation in a study-protocol (13). Data regarding sonographic lymph node size, treatment method, location of the infection, fistulization, bacteriological characteristics, treatment method, sex, and time from first symptoms until presentation were collected. Fistulization was defined as an infection that has reached the skin surface or subcutaneous tissue, either sonographically or clinically. Relevant information was found in health records, operative reports, referral letters, microbiology lab reports, and ultrasound reports.

All patients were treated with the intention to completely remove all infected tissue, as this is considered to be the primary treatment strategy (14).

Statistical analysis

A univariate logistic regression analysis was performed to identify possible associations between the determinants (largest infected lymph node size, age, fistulization, location of the infection) and the surgical outcome (completer or incomplete surgical removal). Every determinant with a *P*-value < 0.2 was analyzed by multiple logistic regression analysis. The level of significance (α) was set at 0.05, and two tailed tests were used. All statistical analyses were performed using IBM SPSS Statistics for Macintosh, version 25 (IBM Corp., Armonk NY, USA).

Ethical approval

The Medical Ethics Review Committee of the AUMC was formally asked if the Medical Research Involving Human Subjects Act was applicable to the present study (reference number W17_387 # 17.453). They decided that the act did not apply to the current study. Therefore, official approval by the committee was not required. Nor was patient consent needed.

Results

The study population comprised 103 patients (49.5% male and 50.5% female). A flow chart of the patient selection process is shown in Figure 5b. The mean age at presentation was 3.93 years, and the mean sonographic size of the largest infected lymph node was 2.21 cm. Most infections were unilateral, localized in the submandibular triangle (level 1B), and caused by *M. avium*. *M. haemophilum* was the second most common causative species (Table 5a). The results of the univariate logistic regression analysis are presented in Table 5b. Fistulization was present in 42.7% of all cases. Surgical removal was incomplete in 23.3% of all included subjects. Higher age was significantly associated with incomplete surgical removal (odds ratio (OR) 1.18, 95% confidence interval (CI) 1.00 – 1.38; *P*=0,043). However, submandibular involvement, multiple locations of infection, and mean sonographic size were not. Fistulization was included in the multiple logistic regression as the P-value was <0.2 (*P* = 0.081). As shown in Table 5c, multiple logistic regression analysis revealed a statistically significant association between incomplete surgical removal and both higher age (OR 1.24, 95% CI 1.04 – 1.47; *P*=0.081) and fistulization (OR 3.15, 95% CI 1.13-8.75; *P*=0.028).

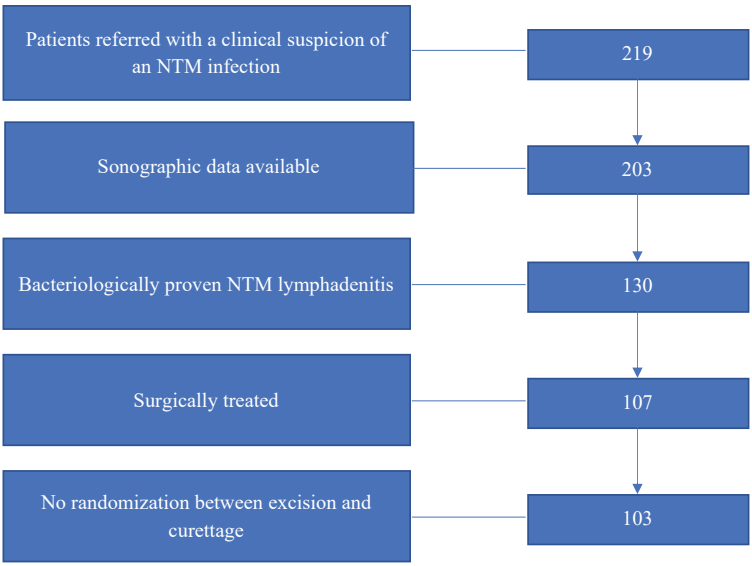

Figure 5b Flow chart of patient selection

Table 5a Subject characteristics

Variables	All (n = 103)
Sex	
Male	51 (49.5%)
Female	52 (50.5%)
Mean age at presentation in years ± SD (range)	3.93 ± 2.70 (0.55 – 13.20)
Mean sonographic size in cm ± SD (range)	2.21 ± 0.88 (0.40 – 5.30)
Mean time from first symptoms to presentation in days ± SD (range)	85 ± 59 (11 - 305) n = 94[a]
Treatment	
Complete surgical removal	79 (77%)
Incomplete surgical removal	24 (23%)
Location	
Submental involvement (Level IA)	3 (2.9%)
Submandibular involvement (Level IB)	83 (80.6%)
Preauricular involvement (Parotid)	25 (24.3%)
Multiple locations	17 (16.5%)
Left side	58 (56.3%)
Right side	43 (41.7%)
Both sides	2 (1.9%)

Table 5a (Continued)

Variables	All (*n* = 103)
Causative species	
Mycobacterium avium	74 (71.8%)
M. haemophilum	21 (20.4%)
M. malmoense	3 (2.9%)
M. kansassi	3 (2.9%)
M. intracellulare	1 (1.0%)
M. bovis	1 (1.0%)

[a]Data available for 94 of the 103 patients
SD indicates standard deviation; N, number of included subjects.

Table 5b Results of the univariate logistic regression analysis

Determinant	Incomplete surgical removal (*n* = 24)	Complete surgical removal (*n* = 79)	OR (95% CI)	*P*-value
Mean age (years) (95% CI)	4.95 (3.52-6.39)	3.63 (3.10-4.17)	1.18 (1.00-1.38)	0.043[a]
Fistulization			2.29 (0.90-5.80)	0.081
Yes	14 (58.3%)	30 (38.0%)		
No	10 (41.7%)	49 (62.0%)		
Submandibular involvement			0.52 (0.14-1.96)	0.334
Yes	21 (87.5%)	62 (78.5%)		
No	3 (12.5%)	17 (21.5%)		
Multiple locations of infection			0.68 (0.21-2.17)	0.516
Yes	5 (20.8%)	12 (15.2%)		
No	19 (79.2%)	67 (84.8%)		
Mean sonographic size of the largest affected lymph node (cm) (95% CI)	2.25 (1.80-2.70)	2.20 (2.02-2.38)	1.07 (0.64-1.79)	0.806

[a]Statistically significant
N indicates number of included subjects; OR, odds ratio; CI, confidence interval.

Table 5c Results of the multiple logistic regression analysis

Variable	Odds ratio (95% CI)	P-value
Age	1.2 (1.04 – 1.47)	0.016[a]
Fistulization	3.1 (1.13 – 8.75)	0.028[a]

[a]Statistically significant
OR indicates odds ratio; CI, confidence interval

Discussion

Proximity of the facial nerve, extensive skin necrosis, and surgical skill and experience have been reported to be factors that may affect complete surgical removal (13, 17, 18, 22). Transient facial nerve paresis occurs in 10% of surgically treated children with NTM cervicofacial lymphadenitis, whereas 2% develop permanent paresis. The additional value of intraoperative monitoring of facial nerve integrity monitoring on nerve preservation is still under debate (16, 25-27). The level of evidence of available studies is low due to the absence of randomized studies, case heterogenicity, and the lack of standardized monitoring. It is important to recognize that the facial nerve in surgically treated children is more prone to iatrogenic damage because its anatomical location is more superficially in children than it is in adults (28). Taking into consideration the fact that NTM cervicofacial lymphadenitis is a benign disease, one should always consider whether the benefits of complete surgical removal outweigh the risk of potential life-long facial nerve paresis.

This is novel in critically and systemically evaluating the association between potential clinical determinants and incomplete surgical removal in a sample with bacteriologically confirmed NTM cervicofacial lymphadenitis. Given the low incidence of NTM cervicofacial lymphadenitis, the outcome data for the substantial number of subjects included in this study provide valuable information to answer this pivotal question in clinical decision-making.

Analysis of the data in the present study demonstrates that incomplete surgical removal occurs relatively often in older patients and in patients with fistulization.

Complete surgical removal results in optimal esthetic outcomes. Haimi-Cohen et al. (2016) reported that 44% of the patients were disturbed by the appearance of the scar after a follow-up period of at least 2 years following management with observation only (29). In addition, Lindeboom et al. (2009) demonstrated an improved cosmetic outcome for successful surgical treatment compared to successful antibiotic treatment, while delayed surgery after antibiotic treatment failure had a comparable esthetic outcome to successful antibiotic treatment (30). This emphasizes that prediction of complete surgical removal plays a substantial role in this population with regard to the treatment planning and stresses the importance of an individualized assessment of potential long-term morbidities associated with either treatment approach.

Fistulization is a sign of advanced disease and occurs after fluctuation of the infected tissue (6). It leads to unclear distinction of surgical planes, resulting in a loss of anatomical orientation. Moreover, fistulization means that there is a certain extent of necrotic skin involvement, which may complicate wound closure. Thus, fistulization may favor a more conservative surgical approach, which is in line with the results of the present study.

An explanation for the association between higher age and inability to perform complete surgical removal may be that early symptoms, such as mild swelling, are possibly recognized more easily by parents of younger children. However, no supporting evidence for this assumption was found in the literature.

Due to the use of historical data in this study, the assessment of clinical features could not be standardized, resulting in information bias. Furthermore, no objective criteria could be used to define incomplete surgical removal. This outcome depended on information found in operative reports, since no histologic examination was possible to confirm there was complete surgical removal of infectious tissue. Moreover, no absolute nor relative risks of incomplete surgical removal could be calculated due to the case-control design of the present study.

This study demonstrated that higher age and fistulization were associated with a limited ability to excise all infected tissue in patients with NTM cervicofacial lymphadenitis. In contrast, a larger sonographic lymph node size was not associated with incomplete surgical removal of the infected lymph node tissue. These findings could be helpful in terms of shared decision-making. The data will aid clinicians in adequately informing the parent(s) or guardian(s) of the patients preoperatively and in guiding clinical decision-making based on an estimation of the intraoperative and postoperative course.

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Chapter 6

Long-Term Outcome of Surgical Treatments for Nontuberculous Mycobacterial Cervicofacial Lymphadenitis in Children

Willemse SH, Schreuder WH, Apperloo RC, Lindeboom JA

This chapter is based on the following publication: Willemse SH, Schreuder WH, Apperloo RC, Lindeboom JA. Long-Term Outcome of Surgical Treatments for Nontuberculous Mycobacterial Cervicofacial Lymphadenitis in Children. J Oral Maxillofac Surg. 2022;80(3):537-544. doi: 10.1016/j.joms.2021.09.029.

Abstract

Introduction

Information on long-term treatment outcome for nontuberculous mycobacterial (NTM) cervicofacial lymphadenitis in children is scarce. The purpose of this study is to evaluate long-term outcome for surgical treatment, which is the mainstay treatment modality.

Materials & Methods

This case series describes recurrence rates of surgically treated NTM cervicofacial lymphadenitis patients with a follow-up of at least ten years. The current study data were partially collected from a randomized, prospective, multicenter, multidisciplinary trial (CHIMED study), which was conducted between 2000–2006 to determine the optimal treatment for NTM cervicofacial lymphadenitis in children. After the CHIMED trial inclusion ended, our institute continued to serve as a referral center. This enabled us to enlarge the surgical CHIMED cohort by adding patients who were treated during 2007–2010 in our center and collect the rest of the current study data.

Results

427 children with chronic cervicofacial lymphadenopathy were analyzed. Among these, 290 had microbiologically confirmed cervicofacial mycobacterial infections (n=3 *M. tuberculosis*, n=1 *M. bovis*, n=286 NTM). Of these 286 children with NTM cervicofacial lymphadenitis, 189 were treated surgically (median age: 41 months, range: 9–144, 46.0% males). The affected lymph nodes were excised in 151 children (79.9%), and curettage was performed in 38 children (20.1%). One patient (0.7%) experienced a reactivation/recurrence two years after surgical excision and required another surgical excision. Three children (7.9%) experienced infection reactivation/recurrences after curettage, confirmed by redness or a draining fistula, within the first year after healing. Two of these three patients were treated with additional surgical excisions.

Conclusion

The long-term outcome of surgical excision for NTM cervicofacial lymphadenitis is favorable with a low recurrence rate. Curettage or a conservative wait-and-see approach can be considered an alternative in advanced and surgically challenging cases. However, healing will take longer, and late recurrences are possible.

Introduction

When a lymphadenopathy persists for more than 3 weeks and does not resolve with adequate antibiotics in a child, a nontuberculous mycobacterial (NTM) infection should be suspected (1). NTM account for most mycobacterial head and neck infections, and it is an important cause of chronic cervicofacial lymphadenitis in otherwise healthy young children (2-4). NTM cervicofacial lymphadenitis typically occurs in children between the ages of 1 and 5 years (median age: 3 years), and it rarely occurs in children over 12 years old (3-5).

The annual incidence rate in the Netherlands is estimated to be 0.77 NTM infections per 100,000 children and the age-specific infection rate is highest in the youngest age group, at 2.3 cases per 100,000 children (3). The oropharyngeal mucosa has been described as a potential portal of entry. Thumb-sucking might be a potential risk factor in young children, and infections can follow tooth eruptions (5, 6).

The disease manifests as a chronic, unilateral lymphadenopathy, with enlarged solitary or clustered masses predominantly in the submandibular or preauricular region evolving into suppurating lymph nodes and violaceous skin discoloration (4, 7, 8). Eventually, fistulae and spontaneous drainage occur, which can lead to disfiguration of the surrounding skin (9). Moreover, without intervention, breakdown of the vulnerable skin can, without intervention, result into an open wound (Figure 6a) giving way for a prolonged clinical course of purulent discharge and intensive wound treatment.

The most effective management strategy for NTM cervicofacial lymphadenitis is surgical excision of the affected lymph nodes. This leads to a quick resolution of the disease in over 96% of patients and provides a better esthetic outcome compared to antibiotic therapy (4, 10, 11). Surgical excision should be performed as soon as possible to reduce the risk of excessive scar formation and functional sequelae due to surgical intervention, which occurs more frequently in advanced disease stages (12). Published studies on long-term treatment outcome, however, are scarce. The purpose of this study is to evaluate long-term treatment outcome in a large group of surgically treated NTM cervicofacial lymphadenitis patients. The specific aim of the study is to report recurrence rates with a follow-up of at least 10 years.

Materials and Methods

Study design/sample

To address the research purpose, the investigators designed and implemented a case series of surgically treated NTM cervicofacial lymphadenitis patients.

The study population is composed of surgically treated patients with a diagnosis of NTM cervicofacial lymphadenitis after standard clinical and ultrasound examination with a bacterial culture or polymerase chain reaction (PCR). Treatment was performed either by surgical excision or curettage.

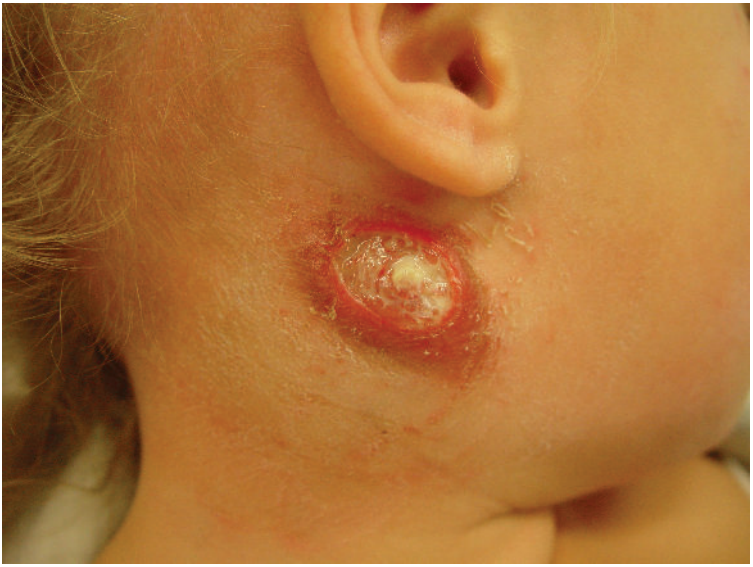

Figure 6a Image of an open wound that resulted from a nontuberculous mycobacterial infection

Patients were excluded as study subjects when they were immunocompromised or used immunosuppressive drugs.

The current study data were partially collected from the CHIMED study (Surgery versus Medication). This was a randomized, prospective, multicenter, multidisciplinary trial conducted from 2000–2006 to determine the optimal treatment for NTM cervicofacial lymphadenitis in children (4). Patients (aged 0–15 years) with suspected NTM lymphadenitis were referred by pediatricians, otolaryngologists, oral and maxillofacial surgeons, and general practitioners from various locations in the Netherlands. Inclusion criteria were enlarged cervicofacial lymphadenitis that persisted for more than 3 weeks and negative serologic test results for other common infectious causes of chronic lymphadenitis (eg, cytomegalovirus, Epstein–Barr virus, adenovirus, Bartonella species, and toxoplasmosis). After the CHIMED trial inclusion ended, our institute (Amsterdam University Medical Center, location AMC) continued to serve as a referral center. This enabled us to enlarge the surgical CHIMED cohort by adding patients who were treated during 2007–2010 in our center and collect the rest of the current study data.

Mycobacterial infections were diagnosed as described previously (13, 14). Briefly, fine-needle aspiration specimens were taken from affected lymph nodes to diagnose NTM infections. Clinical specimens were decontaminated with a *N*-acetyl-L-cysteine-sodium hydroxide

decontamination protocol. Specimens were stained with auramine to detect acid-fast rods. When auramine-positive rods were detected, Ziehl–Neelsen staining was performed to verify the presence of acid-fast rods. Mycobacterial cultures were incubated at 35°C in tubes with liquid mycobacteria growth indicator medium and in plates on solid Löwenstein–Jensen medium. *Mycobacterium haemophilum*–specific cultures were incubated at 30°C in plates on Löwenstein–Jensen medium, with added iron citrate, and in tubes with mycobacteria growth indicator medium, with an X-factor strip added. Mycobacterial species were identified with the INNO-LIPA assay (InnoGenetics, Gent, Belgium) and the INNO-LIPA V2 assay (InnoGenetics, Gent, Belgium). In addition, real-time PCR was performed to detect the genus (*Mycobacterium*) and the species (*M. haemophilum* and *M. avium*).

Ethical approval

The Medical Ethics Committee of the Academic Medical Center of the University of Amsterdam (Amsterdam, The Netherlands) approved the study, and parents provided informed, written consent before study enrollment. The Helsinki Declaration guidelines were followed.

Variables

The primary outcome of the current study is recurrence after surgical treatment. This was defined as the reappearance of NTM lymphadenitis after initial disease resolution. Disease resolution after successful treatment was defined as total skin closure without local recurrence or de novo lesions after 6 months, assessed with clinical and ultrasound evaluations (4, 15, 16). Secondary outcomes were adverse effects, caused by the surgical procedure (e.g., facial nerve weakness), and postoperative infections.

Data collection methods

Standard follow-up was performed annually for the first 5 years, by telephone, email, or a visit to the clinic. Thereafter, parents consulted the clinic when they suspected a potential recurrence. At the 10-year follow-up, patients were once more telephoned, emailed, or asked to visit the clinic to evaluate the long-term outcome. Any lymphadenopathy or suspected recurrence during follow-up was assessed by clinical observation, ultrasound examination and bacterial culture or PCR on indication.

Data analyses

Since the current study is descriptive in nature, no statistical comparative analysis was performed between groups. Descriptive statistics were used to characterize the patients using dedicated software (IBM SPSS Statistics for Windows, Version 21.0, Armonk, NY, USA). Continuous variables are presented as the mean ± standard deviation.

Results

Patients

In total 430 children with suspected NTM infections were recruited. Two children had an axillary NTM infection, and one was diagnosed with an inguinal NTM lymphadenitis infection. Thus, we included 427 patients with chronic cervicofacial lymphadenopathy. Among these, 290 had microbiologically confirmed cervicofacial mycobacterial infections (*n*=3 *M. tuberculosis*, *n*=1 *M. bovis*, and *n*=286 NTM), 53 had *Bartonella henselae* infections, 14 had streptococcal infections, 11 had staphylococcal infections, and 3 had toxoplasmosis (17). Another 18 children had persistent lymph node swelling from other causes (*n*=8 malignancies and *n*=10 congenital cysts). In 38 children, no causative agent was identified, but among these, 22 had clinical presentations consistent with an NTM infection, although no NTM species could be cultured or confirmed with PCR. Among 286 children with confirmed NTM cervicofacial infections, we studied 189 patients who were treated surgically (median age: 41 months, range: 9–144, 46.0% males). Among these 189 children, 100 patients participated (*n*=75 treated with surgical excision and *n*=25 with curettage) in two randomized controlled clinical trials (4, 16). All children were in good general health, without any underlying disease or immunosuppressive medication.

Altogether, the affected lymph nodes were excised in 151 children (79.9%), and curettage was performed in 38 children (20.1%). In the surgical excision group, 20 children were previously treated with a non-surgical method that was unsuccessful (18 with clarithromycin and rifabutin and 2 with a wait-and-see policy).

The baseline patient characteristics are shown in Table 6. We observed unilateral right and left submandibular lymph node involvement in 63 (33.3%) and 84 (44.4%) patients, respectively. Infections occurred in right preauricular nodes in 4 patients (2.1%), left preauricular nodes in 3 patients (1.6%), and both submandibular and preauricular nodes in 24 patients (12.7%). We observed submental infections in 5 patients (2.6%). Most infections were caused by *M. avium* (153 patients; 81.0%) and *M. haemophilum* (29 patients; 15.3%).

Short-term outcomes

Among the 151 patients in the surgical excision group, 149 (98.7%) had a successful outcome after surgery without evidence of recurrence. During the first 6 months, 1 recurrence occurred in the submandibular region and 1 new lesion developed in the submental region. These 2 recurrences were successfully treated with a 3-month course of clarithromycin combined with rifabutin as part of the CHIMED protocol. After the surgical excision, the mean interval until complete skin closure was 4.74 ± 0.73 weeks. In 6 (4.0%) patients, wounds became infected with *Staphylococcus aureus* within 2 weeks, despite perioperative antibiotic prophylaxis with flucloxacillin. These infections were treated with drainage and oral administration of amoxicillin/clavulanic acid (125/31.5 mg, three times

per day for seven days). We observed postoperative peripheral neuropathy of the marginal mandibular branch of the facial nerve in 13 patients (8.6%); of these, 1 patient (0.6%) experienced persistent grade 1 facial nerve weakness according to the House Brackmann scale; in the other 12 patients, nerve function returned to normal within 12 weeks.

Table 6 Baseline clinical and microbiological characteristics of patients surgically treated for nontuberculous mycobacterial cervicofacial lymphadenitis

Characteristic	Excision group (n = 151)	Curettage group (n = 38)
Male, n	72	15
Female, n	79	23
Median age, months (range)	42 (14–144)	40 (9-85)
Location of NTM lymphadenitis		
Right submandibular, n (%)	46 (30.4)	17 (44.7)
Left submandibular, n (%)	69 (45.6)	15 (39.5)
Right preauricular, n (%)	2 (1.3)	2 (5.3)
Left preauricular, n (%)	3 (2.0)	0 (0)
Submental, n (%)	5 (3.3)	0 (0)
Right retro-auricular	1 (0.7)	0 (0)
Occipital	1 (0.7)	0 (0)
Right supraclavicular	2 (1.3)	0 (0)
Left supraclavicular	2 (1.3)	0 (0)
Multiple locations, both preauricular and submandibular (%)	20 (13.2)	4 (10.5)
***Mycobacterium* species**		
***M. avium*, n (%)**	121 (80.1)	32 (84.2)
***M. haemophilum*, n (%)**	25 (16.6)	4 (10.5)
***M. kansasii*, n (%)**	2 (1.3)	0 (0)
***M. fortuitum*, n (%)**	1 (0.7)	0 (0)
***M. malmoense*, n (%)**	3 (2)	1 (2.6)
Positive cultures, n (%)	105 (69.5)	28 (73.7)
Susceptibility to clarithromycin, %	90	89
Susceptibility to rifabutin, %	90	89

N indicates number of included subjects; *M, Mycobacterium*

Among the 38 patients in the surgical curettage group, 25 were treated successfully. No postoperative facial nerve weakness or infections were observed. After successful curettage, the mean healing time was 8.78 ± 2.9 weeks, which was significantly longer than the mean wound healing time in the surgical excision group (P <.05). In 13 patients (34.2%), swelling recurred within 6 weeks after surgery and subsequent drainage occurred spontaneously. Of these patients, 7 were successfully treated with surgical excision. The other 6 patients did not consent to surgery. Thus, a conservative wait-and-see policy was adopted. In those patients, the chronic draining fistula subsided in time, and the disease resolved within 4-6 months.

Long-term outcomes

In 1 patient (0.7%), a reactivation/recurrence was observed 2 years after a surgical excision. The recurrence was also treated with surgical excision. In 3 patients treated with curettage (7.9%), reactivation/recurrences of the infection occurred within the first year after healing. In these 3 patients, the recurrence appeared as a redness in the area or a draining fistula. Two of these patients were treated with surgical excision, but the parents of the third patient refused an additional surgical intervention. Thus, a conservative wait-and-see policy was adopted. In this latter patient, fistulation and drainage persisted, and after 8 months, the lesion was considered cured. Interestingly, these recurrences were not associated with positive cultures or positive PCR results for NTM species.

Figure 6b shows the patient of Figure 6a after surgical excision of the NTM lymphadenitis at 6 months and an image of the same patient 16 years later. Figure 6c shows another patient before and 16 years after surgical excision.

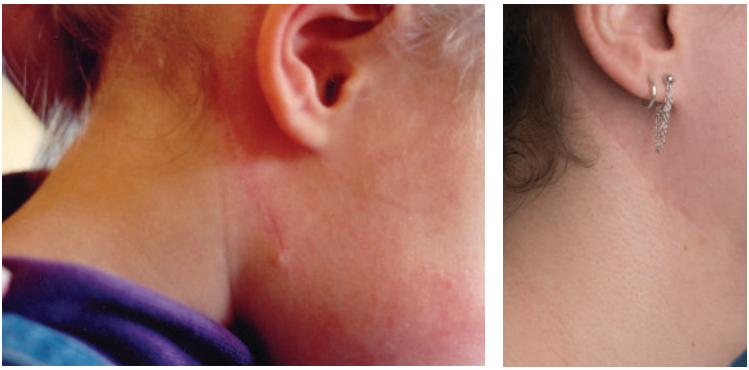

Figure 6b Image of the patient of Figure 6a after surgical excision of the NTM lymphadenitis at 6 months (left) and the image of the same patient 16 years later (right).

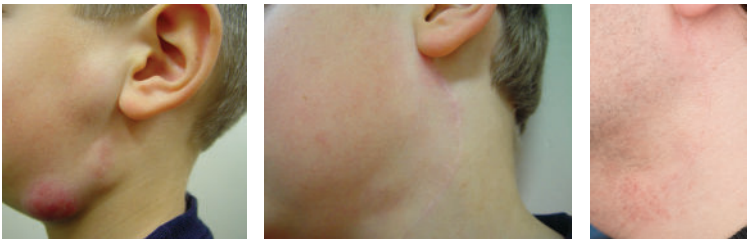

Figure 6c Image of a patient with a preauricular and submandibular NTM infection treated with surgical excision. The patient before surgery (left), the same patient at one year (middle) and 16 years after surgery (right).

Discussion

The purpose of this study was to evaluate long-term surgical treatment outcome in a large group of surgically treated NTM cervicofacial lymphadenitis patients. We found that surgical excisions were curative in 98.7% of cases, and we observed only 1 case of a late recurrence. In the children treated with curettage, 13 (34.2%) experienced swelling with subsequent drainage within 3 weeks after surgery, and 3 reactivations/recurrences occurred (7.9%) within the first year after healing.

The current study also reports facial nerve injury and postoperative wound infection rates as complications of surgery. An injury to the marginal branches of the facial nerve can cause facial nerve dysfunction, when the lesion is located in the submandibular triangle. This injury is the most severe complication of surgery for NTM cervicofacial lymphadenitis. It occurs when the infected lymph nodes are in close proximity to the facial nerve branches (1, 4, 9, 12, 18-22). In the present study, marginal mandibular nerve weakness was observed in 13 patients (8.6%) after surgical excision. Of these, 12 patients regained normal nerve function within 12 weeks, but one patient (0.6%) experienced persistent grade 1 facial nerve weakness. Intraoperative continuous nerve integrity monitoring can reduce the risk of permanent nerve injury and avoid direct damage to the nerve. This monitoring is performed with a nerve stimulator, which is applied during surgery to identify the facial nerve branches. However, in many cases, the infected lymph nodes adhere to the facial nerve without the possibility to separate the nodes from the nerve branches (16, 21). In those cases, it is better to leave part of the node capsule in place. In the present study, 1 infected lymph node was attached to the carotid artery, and it was not removed during surgery to avoid possible severe hemorrhage. This remnant could explain the late recurrence we observed in one patient at 2 years after the surgical excision.

Wound infection was another complication of surgery, occurring in 6 (4.0%) surgically treated patients. The risk of wound infection can be reduced with prophylactic administration of amoxicillin clavulanic acid, because postoperative wound infections are typically caused by gram-positive staphylococcus or streptococcus species (4, 16). In the CHIMED study, which compared surgical excision to antibiotic therapy for NTM cervicofacial lymphadenitis, we observed postoperative wound infections caused by *Staphylococcus aureus* in 6 (12.0%) patients within 2 weeks of treatment, despite perioperative flucloxacillin prophylaxis (4). Subsequently, we changed the prophylaxis protocol by adding oral amoxicillin/clavulanic acid (125/31.5 mg in three doses for five days), and no subsequent postoperative wound infections were observed.

In a previous prospective study of 105 patients treated for NTM infections over a 32-year period, Wolinsky reported that 5 children (4.7%) experienced recurrences from 3½ months to 7 years after the original lymphadenitis was cured (5). Additionally, Reuss et al. reported a

recurrence rate of 6.6% (n=4/61 children), and infections recurred from 6 months to 5 years after the initial disease course had completed (23). Recently, we investigated NTM infection recurrences in children treated with a nonsurgical approach (antibiotics or wait-and-see) (24). In 10 patients (10.0%), the infections recurred 1 to 4 years after the lymphadenitis had completely resolved.

Surgical excisions aim to remove all enlarged lymph nodes, which implies that apparently normal lymph nodes must also be excised because 70% of apparently normal nodes contain microscopic infection foci (25). In a retrospective study by Loizos et al., 22 patients with confirmed NTM infections were treated with excisions (26). However, in 12 cases, recurrences were observed. Five of these occurred in the first 2 months after surgery and required a second operation. Recurrences occurring in such a short time after surgery are mainly due to an incomplete excision or residual infected lymph nodes, similar to normal findings after a surgical curettage. Torretta et al. described 10 patients who underwent a modified neck dissection technique and thereby achieved complete surgical excision of diseased lymph nodes. In advanced cases, with skin involvement and a discharging fistula, the skin was removed en bloc with the lymph nodes (12). We also used a similar approach in our previous clinical trial, where we compared surgical excisions to antibiotic treatments with clarithromycin and rifabutin. Because the en bloc approach leaves a relatively long scar, we modified the approach by not removing the affected skin. Instead, we performed a remote access approach, with the primary incision located in a skin crease of healthy skin 1-2 cm away from the vulnerable affected skin. A red scar appeared in the skin, which returned to a normal color, but only after several months. The scars left after this approach were significantly smaller and less obvious than the scars left after an en bloc incision.

Other studies reported postoperative marginal mandibular nerve weakness rates of 7.2 to 24.6%, all related to surgery involving infected submandibular lymph nodes (9, 18).

An early diagnosis of NTM cervicofacial lymphadenitis is important for a better outcome, but a high index of suspicion is needed. In early stages, before lymph node fluctuations and involvement of the skin and subcutaneous tissue, the procedure is easier, with less risk of morbidity and a better esthetic outcome (4, 10, 27-29). Although surgical excision is the current gold standard for treating NTM cervicofacial lymphadenitis, complete excisions are not always possible in extensive lesions (9). Surgical curettage is an alternative to surgical excision, particularly when lesions are in close proximity to the facial nerve branches or when skin involvement is extensive (22). However, healing may take considerable time, and scarring is often prominent. Briefly, curettage is performed by making a small incision, then using a small scoop to remove soft, necrotic tissue inside the affected lymph nodes (30). After removing necrotic tissue from the lymph node, there is less tissue to drain, which may shorten the draining period. Curettage leaves the affected lymph nodes in place, and these

nodes may retain the potential for disease reactivation. Other studies reported recurrence rates of 30-50% in treated children, and subsequent surgical excision (18, 22, 25). In addition, a staged approach might be considered, where curettage is performed initially, and surgical excision is performed in a second-stage procedure.

Unfortunately, most NTM lymphadenitis patients are diagnosed in the lymph node fluctuation stage, which is associated with skin discoloration. In our previous study, only 17% of patients were diagnosed at an early stage, before skin discoloration or lymph node fluctuations occurred (4, 7). Advanced stages of NTM cervicofacial lymphadenitis are associated with abscess formation, skin discoloration, and adherence to surrounding structures, which makes surgery challenging. In advanced, surgically challenging NTM infections, non-surgical treatment with a wait-and-see approach can be considered (15, 24, 30-32). However, healing will take months to years, and late recurrences are possible. A randomized study found no difference in the time to disease resolution between clarithromycin and rifabutin antibiotics (median, 36 weeks) and a wait-and-see policy (median, 40 weeks) for treating NTM cervicofacial lymphadenitis (15). In addition, antibiotics were associated with several side effects, including fever, fatigue, and abdominal pain. Thus, the wait-and-see approach is a reasonable alternative, particularly in advanced cases with a high risk of facial nerve damage (15, 28, 30-32). An observational study reported disease resolution within 6–9 months in 71% of patients with the wait-and-see approach (31). Those patients experienced spontaneous regression and healing. Most of those patients had, however, minimal disease with single lymph node involvement and limited skin involvement.

The main strength of the current study is the large sample size with detailed and complete descriptive information of microbiologically confirmed NTM cervicofacial lymphadenitis patients and a long follow-up period. The lack of standardized long-term follow-up information about the esthetic outcome is a limitation.

Conclusion

The long-term outcome of surgical excision for NTM cervicofacial lymphadenitis is favorable with a low recurrence rate. Surgery is easier to perform in early cases that lack abscess formation and skin involvement. Moreover, damage to the nerve can be avoided with the use of nerve stimulation. In addition, an incision in a skin crease leads to a better esthetic outcome. For extensive, advanced cases of submandibular NTM cervicofacial lymphadenitis that are at high risk of facial nerve damage, curettage or a conservative wait-and-see approach could be considered. However, healing will take longer, and late reactivations/recurrences are possible. Future studies are needed to evaluate the long-term follow-up esthetic outcome of different treatment modalities for NTM cervicofacial lymphadenitis.

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Chapter 7

Long-term esthetic outcome of different treatment modalities for nontuberculous mycobacterial cervicofacial lymphadenitis

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Abstract

Introduction

Nontuberculous mycobacterial (NTM) cervicofacial lymphadenitis is a rare infection which almost exclusively occurs in children, most commonly children 0-5 years old. It can leave scars in highly visible areas. The present study aimed to evaluate the long-term esthetic outcome of different treatment modalities for NTM cervicofacial lymphadenitis.

Materials & Methods

This retrospective cohort study included 92 participants with a history of bacteriologically proven NTM cervicofacial lymphadenitis. All patients were diagnosed at least 10 years prior and were aged >12 years upon enrollment. Based on standardized photographs, the scars were assessed by subjects with the Patient Scar Assessment Scale, and by five independent observers with the revised and weighted Observer Scar Assessment Scale.

Results

The mean age at initial presentation was 3,9 years, and the mean follow-up time was 15.24 years. Initial treatments included surgical treatment (n = 53), antibiotic treatment (n = 29), and watchful waiting (n = 10). Subsequent surgery was performed in two patients, due to a recurrence after initial surgical treatment, and in 10 patients initially treated with antibiotic treatment or watchful waiting. Esthetic outcomes were statistically significantly better with initial surgery, compared to initial non-surgical treatment, based on patient scores of scar thickness, and based on observer scores of scar thickness, surface appearance, general appearance and the revised and weighted sum score of all assessment items.

Conclusion

The long-term esthetic outcome of surgical treatment was superior to non-surgical treatment. These findings could facilitate the process of shared decision-making.

Introduction

Nontuberculous mycobacterial (NTM) cervicofacial lymphadenitis is a rare infection which almost exclusively occurs in children, most commonly children 0-5 years old (1, 2). Despite the benign nature of the disease, it can be locally destructive and lead to cutaneous sinus tract formation (3). Several treatment modalities exist for NTM cervicofacial lymphadenitis. Complete surgical excision generally leads to the quickest resolution, but it is associated with a 2% risk of permanent facial nerve palsy (4). Alternatively, non-surgical, less invasive options include antibiotic treatment and a watchful waiting strategy (5, 6). Despite the superior effectiveness of surgical excision, the optimal treatment strategy remains an issue of debate, because of the invasive nature of the surgery and the risk of complications (7-9). NTM cervicofacial lymphadenitis leaves scars in highly visible areas, which affect self-consciousness and anxiety (10). Only one previous study has systemically evaluated the short-term esthetic outcome of different treatments. After a one-year follow-up period, the esthetic outcome of surgical excision was demonstrated to be superior to the outcome with antibiotic treatment (11). Patient opinions could not be obtained due to their young age after one-year follow-up. Besides, little is known about the long-term development of the scars acquired when treated at a young age for this disease. The present study aimed to evaluate the long-term esthetic outcome of surgical and non-surgical treatment modalities for NTM cervicofacial lymphadenitis.

Materials & Methods

Subjects

A retrospective cohort study was carried out. An existing database of all consecutively presenting NTM cervicofacial lymphadenitis patients at our department between 2001-2010 was screened for eligible subjects. Inclusion criteria were: (*i*) a history of bacteriologically proven NTM cervicofacial lymphadenitis, established either by polymerase chain reaction or culture; (*ii*) time of diagnosis at least 10 years prior to enrollment; and (*iii*) current age >12 years. Exclusion criteria were laser therapy or surgery to improve the scar after healing.

Study groups

Some eligible subjects had participated in the CHIMED study, a multicenter randomized controlled clinical trial, with a surgical excision arm and an antibiotic treatment arm. The antibiotic regimen consisted of a 12-week course of clarithromycin and rifabutin (4). Patients who did not participate in the CHIMED study were surgically treated, unless the parents/guardians preferred conservative treatment. All subjects were treated by a single, experienced oral and maxillofacial surgeon (JAL) at the Department of Oral and Maxillofacial Surgery of the Academic Medical Center (AMC), Amsterdam. In the present study, the esthetic outcome of surgical treatment, antibiotic treatment, and watchful waiting was evaluated. Antibiotic treatment and watchful waiting were combined to form a non-surgical treatment

group. The surgical group was compared to the non-surgical group in an intention-to-treat analysis**.** A subgroup analysis was performed on subjects that had undergone (*i*) successful surgical treatment, (*ii*) successful non-surgical treatment and (*iii*) delayed surgery after initial non-surgical treatment failure. Treatment was classified as antibiotic treatment when macrolide antibiotics were used. When the lymphadenitis persisted after antibiotic treatment, the option to perform subsequent surgery was discussed with the parents/guardians.

Successful treatment was defined by all of the following criteria:
- regression of the lymph node by at least 75%, compared to the lymph node size at the initial clinical presentation and ultrasound assessment
- cure of the fistula and total skin closure; and
- no local recurrence or new lesions after 6 months.

Recurrence was defined as the reappearance of NTM lymphadenitis following an initial resolution.

Data collection

All eligible subjects were contacted by phone. Upon consent to participate, an appointment was scheduled at the Amsterdam University Medical Center, location AMC. Subjects were included between 2020-2022.

During the visit, standardized photographs were taken of the scars at the medical photography department by medical photographers according to a preset protocol. A ruler was used to assess the size of the scars. When it was not possible to schedule a visit at the research location, either due to the subject's preference or COVID-19 measures, subjects were asked to take photographs of their scars at home, according to standardized instructions sent by secure e-mail. A ruler was sent to their home address.

Subjects were asked if cosmetic skin products were used and if they had undergone any cosmetic or surgical procedures to improve the esthetic outcome.

Outcome

The esthetic assessment, based on the images, was independently performed by a blinded panel of 4 oral and maxillofacial surgeons (JAL, LK, MO, WS) and a PhD student (SW) with the revised and weighted Observer Scar Assessment Scale (OSAS) based on images. The weights for different items on this scale were previously determined with a Rasch Analysis. A sum score reflected different scar characteristics and overall scar quality (12). The items on the OSAS were vascularization, pigmentation, thickness, surface appearance, size, and general appearance. All items were rated on a 1-10 scale (1 = best, 10 = worst). The weighted sum score was converted to a 0-100 scale (0 = best, 100 = worst). The images were shown to the

assessors in random order. Before the individual assessments were carried out, three random images were discussed in an online calibration among assessors to agree on definitions.

Subjects were asked to complete the patient scale of the Patient Scar Assessment Scale version 2 (PSAS). This is a reliable patient tool for self-evaluations of physical, tactile, and visual characteristics of burns and linear scars (13, 14).

Statistical analysis

Different treatment modalities were compared, based on the PSAS and OSAS scores, with the independent sample *t*-test, Mann-Whitney U test, and Kruskal-Wallis test, as appropriate. Means, standard deviations (SD), and ranges are presented for normally distributed continuous variables, and medians and interquartile range (IQR) for non-normally distributed continuous variables. The level of significance was set to 0.05, and two-tailed tests were used. The intra- and interrater reliability of the observers was tested with the intraclass correlation coefficient (ICC), based on the two-way random-effects model and absolute agreement setting. The average scores per item were analyzed for inter-rater reliability, and single scores per item to analyze the intra-rater reliability. All observers reassessed the same 22 randomly chosen images at least two weeks after the initial assessment.

All statistical analyses were performed with IBM SPSS Statistics for Windows version 26 (IBM Corp., Armonk, NY, USA).

Ethical approval

The Medical Ethics Review Committee of the Amsterdam University Medical Center, location AMC, was formally asked whether the Medical Research Involving Human Subjects Act was applicable to the present study (reference number W18_152). It was decided that the act did not apply to the present study. The study was exempt from a formal review. All subjects provided written informed consent. For subjects 12-15 years of age, written informed consent from parents/guardians was required. The study was performed in compliance with the World Medical Association Declaration of Helsinki.

Results

Record screening yielded 194 eligible subjects, of which 108 could be contacted via phone. Of these, 92 subjects participated in the study, including 21 subjects that had their photographs taken at home. The reasons not to visit the clinic were a lack of time or a travel distance that was found to be too large in 12 cases and COVID-19 measures in 9 cases. One subject underwent laser therapy 5 years after antibiotic treatment to improve the scar appearance due to dissatisfaction with the esthetic result and was therefore not included in the current study. The baseline characteristics are displayed in Table 7a.

All included subjects were immunocompetent, and none had undergone surgical procedures to improve the scar quality. In total, 18 subjects had used cosmetic skin products in the past to improve the esthetic outcome (two subjects after conservative treatment and 12 subjects after surgical treatment). Silicone gels and vitamin E creams were used, but most subjects did not remember the exact product type. Five subjects did not remember if they had used any cosmetic skin products.

At start of treatment, both the surgical and non-surgical group had similar disease stage characteristics, i.e. skin redness, fluctuation, and fistula formation.

The mean age at initial presentation was 3,9 years and the mean follow-up time was 15.24 years (range 11-19 years). A total of 47 included subjects had previously participated in the CHIMED study. Of these randomized subjects, 26 initially received surgical treatment and 21 antibiotic treatment. All other subjects in the present study were not randomized and were surgically treated, unless the parents/guardians preferred conservative treatment.

Among the 92 included subjects, 53 (58%) initially underwent surgical treatment, 29 (32%) initially underwent antibiotic treatment, and 10 (11%) initially underwent watchful waiting. A subsequent surgical procedure was carried out in 10 subjects that experienced non-surgical treatment failure. Two subjects developed a recurrence after initial surgery and underwent a second surgical intervention.

Three surgically treated subjects developed a wound infection, which was treated with amoxicillin/clavulanic acid and, in one case, with incision and drainage.

PSAS and OSAS scores for the different treatment modalities are displayed in Table 7b.

The ICC correlation coefficient for the inter-rater reliability of the different PSAS items varied between 0.85 and 0.94, which indicated good inter-rater reliability. The ICC for the mean intra-rater reliability per item varied between 0.65 and 0.85 which indicated moderate to good intra-rater reliability (15). The ICC values of the images taken at home did not differ from the professional images taken at the hospital.

The patient scores for scar thickness and the observer scores for scar thickness, surface appearance, general appearance, and the revised and weighted sum score of all items were significantly more favorable for patients who received initial surgery compared to those who received initial non-surgical treatment. Other items on the OSAS were either equivalent between surgery and non-surgical treatments, or they favored surgery but lacked statistical significance.

The esthetic outcome with and without the use of cosmetic skin products did not differ significantly.

The subanalyses revealed that successful surgical treatment received significantly better general appearance and revised and weighted sum scores than successful non-surgical

treatment and delayed surgery after initial non-surgical treatment failure. The observer scores for scar thickness and surface appearance were also significantly in favor of successful surgical treatment compared to successful non-surgical treatment. Furthermore, the patient scores for scar irregularity were significantly better for patients who received delayed surgery compared to those who received successful non-surgical treatment. Conversely, the patient scores for scar itching were significantly worse in patients who underwent delayed surgery compared to those who received either successful surgical treatment or non-surgical treatment.

Examples of scars after different treatment modalities are displayed in Figures 7a, 7b and 7c.

Table 7a Subject baseline characteristics

Variable	Surgical treatment (n = 53)	Non-surgical treatment (n = 39)
Sex, n (%)		
Male	25 (47)	16 (41)
Female	28 (53)	23 (59)
Mean age in years ± SD (range)	19 ± 3 (14 - 29)	19 ± 3 (14 - 27)
Mean follow-up time in years ± SD (range)	15 ± 2 (11 - 18)	15 ± 2 (11 - 19)
Mean age at presentation in months ± SD (range)	51 ± 34 (16 - 161)	42 ± 26 (11 - 128)
Mean time from first symptoms to presentation in weeks ± SD (range)	16 ± 21 (2 - 150)	12 ± 11 (1 - 52)
Disease stage at start of treatment, n (%)		
Skin discoloration	41 (77)	32 (82)[a]
Fluctuation	38 (72)	30 (77)[a]
Fistula[b]	21 (40)	17 (44)[a]
Location, n (%)		
Mid jugular	2 (4)	2 (5)
Preauricular	7 (13)	11 (28)
Retro-auricular	3 (6)	3 (8)
Submandibular	47 (89)	28 (72)
Submental	3 (6)	1 (3)
Supraclavicular	1 (2)	0 (0)
Multiple locations	9 (17)	5 (13)
Unilateral	52 (98)	38 (98)
Bilateral	1 (2)	1 (3)
Causative species, n (%)		
M. avium	43 (81)	34 (87)
M. chelonae	0 (0)	1 (3)
M. haemophilum	8 (15)	4 (10)
M. intracellulare	1 (2)	0 (0)
M. kansasii	1 (2)	0 (0)

[a]Missing information for one subject.
[b]Either cutaneous or subcutaneous fistula
N indicates number of included subjects; SD, standard deviation; *M*, *Mycobacterium*.

Table 7b Patient and Observer Scar Assessment Scale intention-to-treat analysis and subanalysis

Variable	Surgical treatment (n = 53)	Conservative treatment (n = 39)	*P*-value	Successful surgical treatment (n = 51)	Successful Conservative treatment (n = 29)	Delayed surgery[a] (n = 10)	*P*-value
	Intention-to-treat analysis			Subanalysis			
Patient scores							
PSAS subitem							
Median pain (IQS)	1.0 (1.0 - 1.0)	1.0 (1.0 - 1.0)	0.87	1.0 (1.0 - 1.0)	1.0 (1.0 - 1.0)	1.0 (1.0 - 1.3)	0.61
Median itching (IQS)	1.0 (1.0 - 1,5)	1.0 (1.0 - 2.0)	0.46	1.0 (1.0 - 1.0)	1.0 (1.0 - 1.0)	2.5 (1.0 - 4.8)	0.03*
Median color (IQS)	3.0 (2.0 - 5.0)	3.0 (2.0 - 4.0)	0.90	3.0 (2.0 - 5.0)	4.0 (2.0 - 6.0)	3.0 (1.8 - 3.3)	0.36
Median stiffness (IQS)	3.0 (1.0 - 5.0)	4.0 (1.0 - 6.0)	0.23	3.0 (1.0 - 5.0)	4.0 (2.0 – 5.5)	2.5 (1.0 - 6.3)	0.32
Median thickness (IQS)	2.0 (1.0 - 4.0)	4.0 (1.0 - 6.0)	0.02*	2.0 (1.0 - 4.0)	4.0 (1.5 - 6.0)	2.0 (1.0 - 4.3)	0.03*
Median irregularity (IQS)	2.0 (1.0 - 5.5)	3.0 (2.0 - 7.0)	0.17	2.0 (1.0 - 6.0)	5.0 (2.0 -7.0)	2.0 (1.0 - 3.3)	0.03*
Median general opinion (IQS)	3.0 (2.0 - 4.0)	3.0 (2.0 - 5.0)	0.12	3.0 (2.0 - 4.0)	3.0 (2.0 - 5.0)	3.5 (2.5 - 5.0)	0.27
Median sum score (IQS)	12.0 (8.0 - 21.5)	18.0 (11.0 - 24.0)	0.12	12.0 (8.0 - 22.0)	19.0 (11.0 – 25.0)	15.0 (8.0 - 19.8)	0.17
Observer scores							
Revised and weighted OSAS subitem							
Median average vascularization (IQS)	2.8 (2.2 - 3.7)	2.8 (2.4 - 3.6)	0.63	2.8 (2.2 - 3.6)	2.8 (2.4 - 3.6)	3.1 (2.3 - 4.9)	0.71
Median average pigmentation (IQS)	3.0 (2.4 - 4.0)	3.6 (2.4 - 4.8)	0.29	3.0 (2.4 - 4.0)	3.4 (2.4 - 4.6)	4.0 (2.8 - 5.1)	0.51
Median average thickness (IQS)	2.8 (2.3 - 3.2)	3.6 (2.8 - 4.4)	0.001*	2.8 (2.2 - 3.2)	3.6 (3.0 - 4.4)	3.7 (2.3 - 4.8)	0.01*
Median average surface (IQS)	3.0 (2.2 - 3.5)	4.0 (2.8 - 4.8)	0.001*	3.0 (2.2 - 3.6)	4.0 (2.9 - 4.7)	3.9 (2.2 - 5.8)	0.004*
Mean average size ± SD	3.6 ± 1.4	3.9 ± 1.3	0.28	3.6 ± 1.4	3.9 ± 1.1	4.2 ± 1.8	0.44
Mean average general ± SD	3.6 ± 1.6	4.3 ± 1.7	0.048*	3.6 ± 1.6	4.1 ± 1.5	5.0 ± 2.2	0.04*
Median average sum score (IQS)[b]	16.4 (4.3 – 27.1)	27.1 (12.1 - 42.1)	0.01*	16.4 (4.3 - 27.1)	25.7 (13.6 - 42.1)	35.7 (10.4 - 48.0)	0.04*

[a]Delayed surgery after initial conservative treatment failure

[b]Values are presented on a 0-100 scale.

**P*-values <0.05 were considered statistically significant.

N indicates number of included subjects; IQS, interquartile scores; PSAS, patient scar assessment score; OSAS, observer scar assessment score; SD, standard deviation.

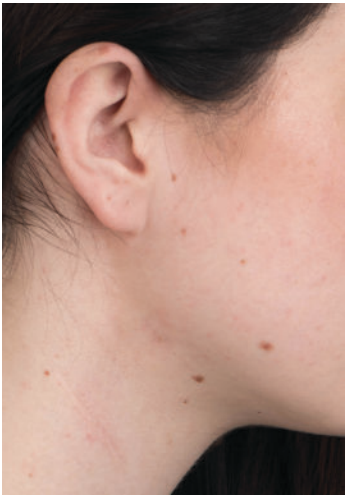

Figure 7a Example of a scar after surgical treatment. The average general score for this subject was 2.2 on the Observer Scar Assessment Scale.

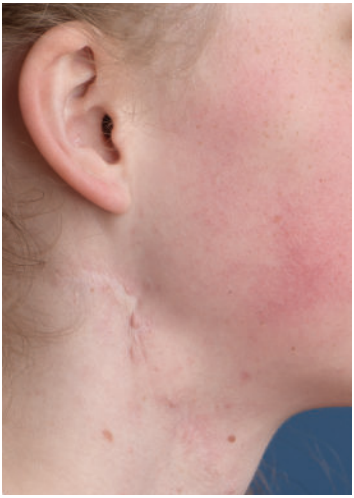

Figure 7b Example of a scar after antibiotic treatment. The average general score for this subject was 6.2 on the Observer Scar Assessment Scale.

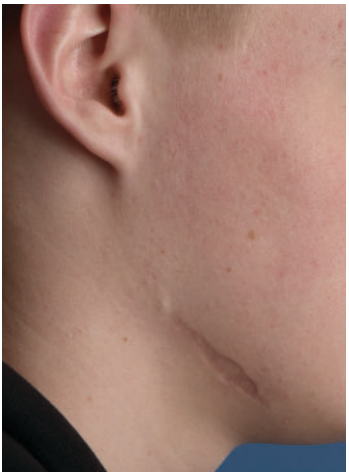

Figure 7c Example of a scar after initial antibiotic treatment failure and subsequent surgical treatment. The average general score for this subject was 6.4 on the Observer Scar Assessment Scale.

Discussion

The present study demonstrated that NTM cervicofacial lymphadenitis caused scars after a long follow-up period both after surgical and non-surgical treatment. The esthetic outcome of surgical treatment was superior to that of a non-surgical treatment strategy. There was no difference in general patient opinion between surgical and non-surgical treatment. However, the main differences in scar quality between these treatment modalities were found in scar irregularity, thickness, surface appearance and general appearance. Thus, scars were more irregular, rougher, and hypo/hypertrophic after non-surgical treatment than scars after surgery. Moreover, the esthetic outcome was generally superior after successful surgery compared to successful non-surgical treatment or delayed surgery after an initial non-surgical treatment strategy failure. Among patients that underwent delayed surgery, scars were more prone to itchiness.

In general, two different techniques are used in the surgical excisions of NTM cervicofacial lymphadenitis. One technique includes the removal of the affected thin and discolored skin, whereas the other approach is performed in a skin crease located 1-2 cm away from the affected skin (16-19). In our experience, the red color of the affected skin usually returns to normal several months postoperatively, and the latter approach results in significantly smaller scars on the condition that there is no cutaneous fistula. The majority of surgically treated subjects in the present study underwent an excision which included the affected skin. This might have negatively affected the overall surgical esthetic outcome. Moreover, a number of subjects did not respond to antibiotic treatment, and subsequently, they underwent surgical treatment. The esthetic outcome might have been worse in the non-surgical group if they had not undergone an additional surgical intervention. Consequently, these features might have led to an underestimated effect size.

Three surgically treated subjects developed a wound infection, which was treated with amoxicillin/clavulanic acid and, in one case, with incision and drainage.

To our knowledge, this study was the first to perform a systematic comparison of long-term esthetic outcomes of different treatment modalities for NTM cervicofacial lymphadenitis, which included patient opinions. Two previous studies evaluated long-term scar formation in patients with NTM cervicofacial lymphadenitis. Haimi-Cohen et al. (2016) evaluated scar formation in 21 patients with proven NTM cervicofacial lymphadenitis after observation-only management. The minimal and median follow-up times were 2 and 6.1 years, respectively. In total, 44% of the parents/guardians were disturbed by the presence of the scar. However, 94% were satisfied with the conservative management (20). Claesson et al. (2010) reported disturbing scar formation in 3/51 patients after surgical excisions and 2/12 patients after conservative treatments after 5-20 years of follow-up (21).

Considering the low incidence of NTM cervicofacial lymphadenitis and the considerable follow-up period in the present study, the number of included subjects was substantial. Although the disease stage at baseline was similar for all groups, only subjects who previously participated in the CHIMED study were randomly allocated to treatment. This might have caused a potential selection bias. The use of digital images and the revised and weighted OSAS for assessing the scars had both advantages and limitations. A strength of this approach was that it facilitated an independent assessment by five observers, who are experienced in the management of NTM cervicofacial lymphadenitis. The limitations of using digital images, instead of a physical examination, is the lack of tactile information. Therefore, it was not possible to assess the scar 'pliability', which is a standard part of the original OSAS, but was omitted from the revised and weighted OSAS. Furthermore, the intra-rater reliability of the item vascularization was moderate (average ICC 0.65). This means that this was the most difficult item to re-assess by the same observer and that the reliability of this item should be interpreted with caution. Most photographs in the current study were taken by professional photographers at the medical photography department. However, 21 subjects had photographs taken at home. These photographs were taken according to standardized instructions, and all observers agreed on the sufficiency of image quality. Moreover, the ICC values of these images did not differ from the ICC values of the professional images. Nevertheless, the overall image quality was not as good as the images taken by professionals, and this limitation might have influenced observer assessments. Furthermore, almost all included subjects in the current study have white skin, possibly limiting the external validity with regard to variation in racial profile. Finally, although the observers were blinded and the images were shown in random order, the possibility that the observers might have been able to discriminate between scars after surgery and scars after non-surgical treatment could not be ruled out.

Conclusion

In conclusion, it is reasonable to assume that children with NTM cervicofacial lymphadenitis will develop permanent scars in highly visible areas. Considering the disease stage and time to resolution, it is essential to individualize treatment planning and evaluate the risk of facial nerve palsy and the esthetic outcome. This study demonstrated that the long-term esthetic outcome obtained with surgical treatment was superior to non-surgical treatment. Moreover, surgery after initial non-surgical treatment strategy failure had a worse esthetic outcome than initial successful surgery. These findings could facilitate the process of shared decision-making in selecting the best treatments for patients with NTM cervicofacial lymphadenitis.

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Part III

General discussion, future perspectives, summaries and appendices

Chapter 8

General discussion and future perspectives

Willemse SH

General discussion

Treatment delay

Part 1 of this thesis demonstrated that diagnosing NTM cervicofacial lymphadenitis at an early stage in a noninvasive manner remains challenging (Chapters 2, 3, 4). It is problematic that the majority of children are still referred to a secondary or tertiary reference center after the appearance of skin discoloration (1-3). Only 19% of subjects included in the study in Chapter 7 were treated in the absence of skin discoloration, and 42% of subjects experienced fistulization. Several types of delay may explain this:

- Referral delay. This can be explained by various reasons. The first might be late recognition by first-line caregivers due to the rarity of the disease and the initial non-specific symptoms. Secondly, patients might not receive a prompt appointment after referral due to the absence of generalized symptoms, such as fever. Finally, patients may initially be sent to a secondary center lacking specialized (surgical) expertise, leading to an extra referral.
- Diagnostic delay due to a lack of a noninvasive test. The required invasive collection of clinical specimens may lead to delays in decision-making.
- Surgical delay due to a lack of generalized symptoms. Other patients may be prioritized on a surgical schedule because they experience (potentially) life-threatening symptoms.

Diagnostic approach

It is essential to consider NTM lymphadenitis if lymphadenopathy in young children persists for over three weeks, even if only non-specific symptoms are present. Staufner et al. (2005) suggested an algorithm for diagnosing mycobacterial cervical lymphadenitis. They advise to consistently perform an IFN-γ release assay test in case of a positive tuberculin skin test and clinical suspicion for mycobacterial lymphadenitis (4). Based on the systematic review results in Chapter 2, it seems reasonable to only perform IFN-γ release assay tests in cases of tuberculous suspicion. Moreover, the suggested negative fine needle aspiration does not necessarily exclude the possibility of mycobacterial lymphadenitis (5). The enzyme immunoassay kit measuring *M. avium* complex (MAC)-specific anti-Glycopeptidolipids (GPL)-core IgA antibodies and a routine PCR analysis on oral and oropharyngeal swabs, evaluated in Chapters 3 and 4, have insufficient diagnostic performance to be incorporated in a diagnostic algorithm. The systematic review in Chapter 2 demonstrates a lack of qualitative diagnostic reports on NTM cervicofacial lymphadenitis. Other authors acknowledge the difficulty of performing these studies, stating that a suboptimal reference standard introduces misclassification bias (6, 7). The rarity of the disease, which complicates the enrollment of a sufficient number of subjects, is another cause of the lack of qualitative reports. A great need remains for accurate, noninvasive diagnostic tests to shorten delays.

Pathophysiology

As highlighted in the introduction of this thesis, children are thought to acquire the disease through the oropharynx, conjunctivae, nose, middle ear, or oral mucosa, based on the predominant anatomical locations of cervicofacial lymphadenitis. The study conducted in Chapter 4 is the first clinical study carried out to test this hypothesis systematically. In none of the patients with bacteriologically proven NTM lymphadenitis or probable NTM lymphadenitis (lacking bacteriologic confirmation) and in one patient from the control group, positive mycobacterial genus and *M. avium*-specific PCR swab results were obtained. The abundant PCR-negative results from swabs in this study may have several different explanations, including the absence of NTM, a low mycobacterial load (below PCR detection level), and suboptimal sampling of specimens by means of (thawed) swabs instead of fresh biopsies and punctate (8). In earlier studies, NTM have been cultured from tonsillar tissue, and nose and throat swabs obtained from healthy, predominantly school-aged children (9, 10). In later studies using sequencing methods, mycobacterial genetic material has been detected in oral, oropharyngeal, and nostril samples obtained from healthy adult individuals (11, 12). The subjects included in these studies were generally older than those included in the study in Chapter 4 of this thesis, which might suggest that colonization occurs later in childhood. The findings of NTM taxa in these niches by these previous studies and the study in Chapter 4 prompt questions regarding the role of the oral cavity and oropharynx as possible portals of entry. The potential presence of NTM in these niches before disease onset also remains unclear. Obtaining fresh clinical specimens from potential portals of entry in children with NTM cervicofacial lymphadenitis may yield positive microbiological results, but this is not possible in light of ethical considerations.

Treatment and outcomes

In Part 2, this thesis demonstrated that advanced disease stages involving fistulization complicate complete surgical removal and eventually lead to poorer esthetic outcomes and higher recurrence rates (Chapters 5, 6, 7). These findings underscore the importance of early detection at a stage without specific symptoms, such as violaceous skin discoloration. The long-term esthetic outcome after surgical treatment appears superior to non-surgical treatment (Chapter 7). These findings are helpful in terms of shared decision-making. The data will aid clinicians in adequately informing the parents or guardians of the patients preoperatively and in guiding clinical decision-making based on an estimation of the intraoperative and postoperative course.

Pictures of long-term esthetic outcomes, as evaluated in Chapter 7, may help improve these tools and help patients' parents make treatment decisions that best align with their individual values and preferences.

To illustrate the importance of avoiding the aforementioned delays, two images of the introduction of this thesis are highlighted below. Both patients have NTM cervicofacial lymphadenitis. If left untreated, the neck mass of the patient in Figure 8a may progress to a draining fistula, as seen in the patient in Figure 8a. After surgical treatment, the patient in Figure 8a has a better chance of a good esthetic outcome than the patient in Figure 8b. A reliable noninvasive test would facilitate preoperative confirmation and help reduce referral, diagnostic, and surgical delay.

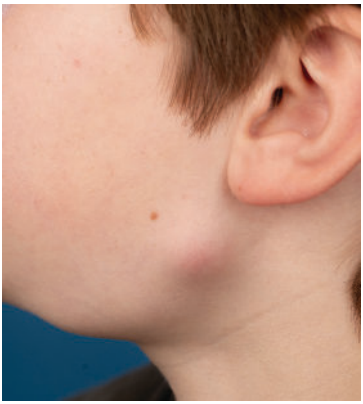

Figure 8a Clinical example of Stage I NTM cervicofacial lymphadenitis without skin discoloration

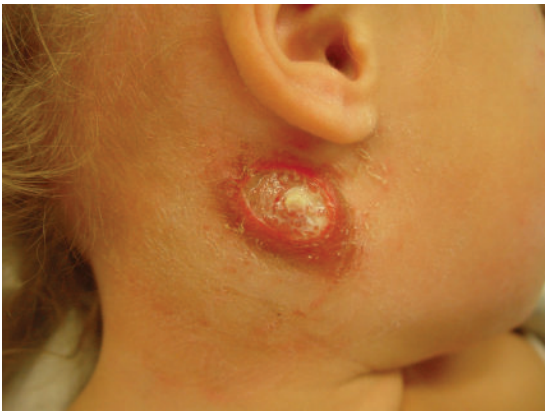

Figure 8b Clinical example of Stage IV NTM cervicofacial lymphadenitis with cutaneous fistulization

Future perspectives

Raising awareness among first-line caregivers and pediatricians through conferences and publications in field-specific journals could help shorten referral delays. Moreover, setting up regional/national centers of expertise and clear referral guidelines might reduce referral delays.

Diagnostic guidelines, based on systematic gatherings of literature as performed in Chapter 2, may reduce diagnostic delays. These should be based on a stepwise approach including clinical suspicion, tuberculin skin testing, interferon-γ (IFN-γ) release assay testing in case of tuberculous suspicion and finally isolation of the mycobacterial specimen. Future studies should focus on improving the early, noninvasive detection of NTM cervicofacial lymphadenitis. The development of new immunologic, minimally invasive diagnostic tools presents significant opportunities. While NTM-specific GPL and PPD-stimulated IFN-γ release assays seem promising, *M. avium* lysate IL-2 and IFN-γ ELISPOT assays also show diagnostic potential (1, 13, 14). After further clinical validation in more heterogeneous populations, these tests might be included in diagnostic guidelines.

Future studies investigating possible portals of entry should include relevant metadata, such as demographic factors, general health, oral health, and lifestyle factors, and use validated sequencing approaches on oral and oropharyngeal swabs instead of a routine diagnostic PCR analysis to identify mycobacteria (15).

While this thesis focused on portals of entry, other pathophysiologic aspects, such as sources of transmission and susceptibility, also warrant attention in future studies. Epidemiologic cohort studies, including questionnaires on contact with potential sources of *M. avium subsp. hominssuis* could help to identify sources of transmission. Soils, water systems, natural water and human-animal interaction may be important. Moreover, genetic cohort studies exploring newly identified polymorphisms related to the immune response against NTM could reveal increased susceptibility to NTM lymphadenitis. Finally, systematic data collection in epidemiologic cohort studies evaluating medication use, vaccination history, oral health, general health, and demographic factors may also help identify risk factors for NTM lymphadenitis.

Worse long-term esthetic outcomes after surgical delays emphasize the need for patients with NTM cervicofacial lymphadenitis to be given reasonable priority on operating schedules (Chapter 8). Surgical delay could be overcome by clear working agreements in centers with (surgical) expertise.

Although the treatment of NTM cervicofacial lymphadenitis has been studied extensively, it remains important to continue exploring less invasive alternatives to surgery. Although no evidence of effectiveness is available, future treatment studies may include intralesional

(antimicrobial) medication or other experimental treatments as alternatives or adjuncts to surgery.

Decision aids could further improve the care of patients with (suspected) cervicofacial lymphadenitis. To improve shared-decision making, such aids should incorporate various aspects of treatment:

- Disease stage information
- Risk of incomplete surgical removal
- Risks of treatment (drug side effects, general anesthesia-related risks, nerve injury, wound infection, bleeding)
- Need for hospitalization
- Number of hospital visits
- Time to resolution
- Potential esthetic outcomes
- Health-related quality of life

To develop these aids, it is essential to involve patients' parents as representatives and foster interdisciplinary collaboration between pediatricians and surgeons.

Despite their significance, quality of life outcome measures have received little attention in studies on NTM cervicofacial lymphadenitis to date. Systematic collection of these outcomes in future studies could help to identify disease aspects affecting the quality of life of patients and family members and yield information to optimize the care.

Given the rarity and complexity of NTM cervicofacial lymphadenitis, multi-center, multidisciplinary collaboration is crucial to include a sufficient number of subjects and improve the interdisciplinary research approach.

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Summary

Contemporary challenges in the diagnosis and treatment of nontuberculous mycobacterial cervicofacial lymphadenitis

Nontuberculous mycobacterial (NTM) cervicofacial lymphadenitis is a rare, infectious disease which almost exclusively manifests in immunocompetent young children. The clinical course can be indolent and eventually lead to a draining fistula in the head and neck area.

This thesis focused on different aspects of nontuberculous mycobacterial cervicofacial lymphadenitis. It addressed minimally invasive diagnostic possibilities, potential portals of entry, surgical treatment aspects, and long-term treatment outcomes. The aim was to contribute to an early diagnosis, a better understanding of the pathophysiology, and the prediction of successful outcomes.

Part 1 of the thesis, comprising Chapters 2, 3, and 4, is about diagnostics and possible portals of entry.

Many diagnostic possibilities exist to support a clinical suspicion and to establish a definitive diagnosis of NTM cervicofacial lymphadenitis. A systematic overview of the performance of available diagnostic tests is provided in **Chapter 2.** It was concluded that reports of high methodological quality on diagnostic accuracy of available diagnostic tests are scarce and that their sensitivity remains suboptimal. It was clarified that a stepwise approach is recommended in diagnosing NTM cervicofacial lymphadenitis, since there is not a single diagnostic method that can provide sufficient sensitivity and specificity. A definitive diagnosis depends on isolation of the mycobacterial specimen, which can be achieved by polymerase chain reaction (PCR) or microbiological culture. A great need remains for accurate, noninvasive diagnostic tests. An example of a minimally invasive diagnostic tool is an enzyme immune-assay measuring anti-glycopeptidolipid-core immunoglobulin A antibodies. This test was evaluated in **Chapter 3**. In a cross-sectional study, it was shown that the tested enzyme immunoassay has inadequate diagnostic performance in the studied population of patients with NTM cervicofacial lymphadenitis and seems to be of no additional value in detecting cases of NTM cervicofacial lymphadenitis (area under the curve = 0.55).

Based on the predominant anatomical locations of NTM cervicofacial lymphadenitis, children are thought to acquire the disease through the oropharynx, conjunctivae, nose, middle ear, or oral mucosa. Mouthing behaviors of children and mucosal injuries associated with tooth eruption and shedding might be important. The presence of mycobacteria in the oral cavity and oropharynx was evaluated in **Chapter 4**. In a cross-sectional study**,** no correlation was found between NTM cervicofacial lymphadenitis and positive NTM PCR results from oral and oropharyngeal swabs, deeming this method unsuitable as a minimally

invasive diagnostic alternative to analysis of excised lymph node tissue or punctate. It remains unclear whether the oral cavity and oropharynx might be a portal of entry in the subsequent development of NTM cervicofacial lymphadenitis.

The findings of Part 1 of this thesis provide clinicians with an overview of available diagnostic tests for detecting NTM cervicofacial lymphadenitis and highlight the associated diagnostic challenges.

Part 2 of the thesis, comprising Chapters 5, 6, and 7, concentrates on surgical challenges and long-term treatment outcomes.

In **Chapter 5**, clinical determinants predicting the (in)ability to perform safe and complete surgical removal of infected tissue were evaluated. A retrospective case-control study was carried out, which demonstrated that higher age and fistulization were associated with a limited ability to excise all infected tissue in patients with NTM cervicofacial lymphadenitis. In contrast, a larger sonographic lymph node size was not found to be associated with incomplete surgical removal of the infected lymph node tissue.

Chapter 6 describes a case series analyzing recurrence rates of surgically treated patients with a follow-up period of at least 10 years. In total, 290 microbiologically confirmed cases were included in this study. Among the 151 patients in the surgical excision group, 149 (98.7%) had a successful outcome after surgery without evidence of recurrence, whereas 25 (66%) out of 38 patients were treated successfully in the surgical curettage group. It was concluded that the long-term outcome of surgical excision for NTM cervicofacial lymphadenitis is favorable with a low recurrence rate.

The long-term esthetic outcome of different treatment modalities was evaluated in **Chapter 7.** A cohort study with a mean follow-up period of 15.24 years concluded that the long-term esthetic outcome obtained with surgical treatment was superior to non-surgical treatment. Moreover, surgery after initial non-surgical treatment strategy failure had a worse esthetic outcome than initial successful surgery.

The findings of Part 2 of this thesis will aid clinicians in adequately informing the parents or guardians of the patients preoperatively and in guiding clinical decision-making based on an estimation of the intraoperative and postoperative course. Considering the disease stage and time to resolution, it is essential to carefully assess the risk of facial and accessory nerve palsy and the potential esthetic outcome to individualize treatment planning.

When the findings of Part 1 and Part 2 are combined, it becomes clear that early recognition of the disease is difficult, but crucial for performing surgery at an early disease stage, enabling complete surgical removal of the infected tissue, minimizing complication rates and optimizing the esthetic outcome.

Dutch summary – Nederlandse samenvatting

Hedendaagse uitdagingen in de diagnostiek en behandeling van nontuberculeuze mycobacteriële cervicofaciale lymfadenitis

Nontuberculeuze mycobacteriële (NTM) cervicofaciale lymfadenitis is een zeldzame infectieziekte die vrijwel uitsluitend voorkomt bij immunocompetente jonge kinderen. Het klinische beloop kan traag zijn en uiteindelijk leiden tot een cutane fistel in het hoofd-hals gebied.

Dit proefschrift richt zich op verschillende aspecten van NTM cervicofaciale lymfadenitis. Minimaal invasieve diagnostische mogelijkheden, potentiële portes d'entrée, chirurgische behandelaspecten en langetermijnresultaten van de behandeling komen aan bod. Het doel was om bij te dragen aan een vroege diagnose, een beter begrip van de pathofysiologie en de voorspelling van succesvolle behandelresultaten.

Deel 1 van het proefschrift, bestaande uit Hoofdstukken 2, 3 en 4, gaat over diagnostiek en mogelijke portes d'entrée.

Er zijn veel diagnostische mogelijkheden om een klinische verdenking te ondersteunen en een definitieve diagnose te stellen voor NTM cervicofaciale lymfadenitis. Een systematisch literatuuroverzicht van de accuratesse van beschikbare diagnostische tests wordt gepresenteerd in **Hoofdstuk 2**. Er werd geconcludeerd dat er weinig studies van hoge methodologische kwaliteit zijn over de diagnostische nauwkeurigheid van beschikbare diagnostische tests en dat de sensitiviteit suboptimaal blijft. Een stapsgewijze benadering verdient de aanbeveling bij het diagnosticeren van NTM cervicofaciale lymfadenitis, aangezien er niet één diagnostische methode is die voldoende sensitiviteit en specificiteit biedt. Een definitieve diagnose is afhankelijk van de mycobacteriële isolatie middels 'polymerase chain reaction' (PCR) en/of microbiologische kweek. Er is grote behoefte aan niet-invasieve tests met goede diagnostische nauwkeurigheid. Een voorbeeld van een minimaal invasieve diagnostische test is een enzym-immunoassay die anti-glycopeptidolipid-core immunoglobuline A antilichamen meet. Deze test werd geëvalueerd in **Hoofdstuk 3**. In een cross-sectionele studie werd aangetoond dat de geteste enzym-immunoassay onvoldoende diagnostische waarde bleek te hebben in de onderzochte populatie van patiënten met NTM cervicofaciale lymfadenitis en waarschijnlijk geen aanvullende klinische waarde heeft voor de detectie van NTM cervicofaciale lymfadenitis ('area under the curve' = 0,55).

Vanwege de meest voorkomende anatomische locaties van cervicofaciale lymfadenitis wordt gedacht dat kinderen de ziekte opdoen via de orofarynx, conjunctivae, neus, middenoor of orale mucosa. Duimgedrag van kinderen en mucosale verwondingen die gepaard gaan met het wisselen van tijdelijke gebitselementen zijn mogelijk van belang. De aanwezigheid van mycobacteriën in de mondholte en orofarynx werd geëvalueerd in **Hoofdstuk 4**. In een cross-sectionele studie werd geen correlatie gevonden tussen NTM cervicofaciale lymfadenitis en positieve NTM PCR-resultaten van orale en orofaryngeale swabs, waardoor deze methode niet geschikt is als minimaal invasief diagnostisch alternatief voor de analyse van uitgenomen

lymfeklierweefsel of punctaat. Het blijft daarbij onduidelijk of de mondholte en orofarynx een toegangspoort kunnen zijn voor de later optredende cervicofaciale lymfadenitis.

De bevindingen uit Deel 1 van dit proefschrift geven de clinicus een overzicht van de beschikbare diagnostische testen voor NTM cervicofaciale lymfadenitis en geven de diagnostische uitdagingen weer.

Deel 2 van het proefschrift, bestaande uit hoofdstukken 5, 6 en 7, richt zich op chirurgische uitdagingen en lange termijn behandelresultaten van NTM cervicofaciale lymfadenitis.

In **Hoofdstuk 5** werden klinische determinanten geëvalueerd die voorspellen of veilige en volledige chirurgische verwijdering van geïnfecteerd weefsel mogelijk is. Uit de uitgevoerde case-control studie bleek dat hogere leeftijd en fistelvorming geassocieerd waren met een incomplete chirurgische verwijdering van het aangedane lymfeklierweefsel bij patiënten met NTM cervicofaciale lymfadenitis. Daarentegen werd geen associatie gevonden tussen een grotere lymfekliergrootte op echografische beeldvorming en een incomplete chirurgische verwijdering van het geïnfecteerde lymfeklierweefsel.

Hoofdstuk 6 beschrijft een case-serie waarin het recidiefpercentage werd geanalyseerd bij chirurgisch behandelde patiënten met een follow-up van minstens 10 jaar. In totaal werden 286 patiënten met microbiologisch bevestiging geïncludeerd. Van de 151 patiënten in de groep met chirurgische excisie hadden 149 (98,7%) een succesvol resultaat na de operatie zonder tekenen van recidief, terwijl 25 (66%) van de 38 patiënten in de groep met curettage succesvol werden behandeld. Er werd geconcludeerd dat de lange termijn uitkomst van chirurgische excisie voor NTM cervicofaciale lymfadenitis gunstig is met een laag recidiefpercentage.

De lange termijn esthetische uitkomst van verschillende behandelingsmodaliteiten werd geëvalueerd in **Hoofdstuk 7**. Uit een cohortstudie met een gemiddelde follow-up van 15,24 jaar werd geconcludeerd dat de lange termijn esthetische uitkomst na chirurgische behandeling beter was dan na niet-chirurgische behandeling. Bovendien had chirurgie na mislukking van een initiële niet-chirurgische behandelingsstrategie een slechtere esthetische uitkomst dan initiële succesvolle chirurgie.

De bevindingen uit Deel 2 van dit proefschrift zullen clinici helpen om ouders of wettelijk vertegenwoordigers van patiënten preoperatief goed te informeren en om de klinische besluitvorming te sturen op basis van een inschatting van het peroperatieve en postoperatieve beloop. Rekening houdend met het ziektestadium en de tijd tot genezing, is het essentieel om zorgvuldig het risico op schade van de nervus facialis en accesorius en het potentiële esthetische resultaat in te schatten om de behandelplanning te individualiseren.

Wanneer de bevindingen uit Deel 1 en Deel 2 samen worden gevoegd, kan men concluderen dat vroege herkenning van het ziektebeeld moeilijk, maar cruciaal is om in een vroeg stadium van de ziekte chirurgie te kunnen uitvoeren. Dit maakt volledige chirurgische verwijdering van het geïnfecteerde weefsel mogelijk, minimaliseert de kans op complicaties en optimaliseert het esthetische resultaat.

Appendices

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Chapter information

PhD Portfolio

List of Publications

Acknowledgments/Dankwoord

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Chapter information

Chapter 2

Published as:
Diagnosing nontuberculous mycobacterial cervicofacial lymphadenitis in children: A systematic review

Authors:
Willemse SH, Oomens MAEM, De Lange J, Karssemakers LHE.

Published in:
International Journal of Pediatric Otorhinolaryngology

Author contributions:

Study conception and design:	SW, MO, JLa, LK
Data acquisition:	SW, MO, LK
Data analysis and interpretation:	SW, MO, LK
Manuscript editing and review:	SW, MO, JLa, LK

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Chapter 3

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Evaluation of anti-glycopeptidolipid-core immunoglobulin A antibody detection for the diagnosis of nontuberculous mycobacterial cervicofacial lymphadenitis

Authors:
Willemse SH, Oomens MAEM, Karssemakers LHE, Lindeboom JA, Schreuder WH, Ho JPTF, Van der Kuip M, Vlaming KE, Kaptein TM, De Lange J.

Published in:
Pediatric Infectious Diseases Journal

Author contributions:

Study conception and design:	SW, MO, LK, JLi, JLa
Data acquisition:	SW, JH, WS, MKu, KV, TK
Data analysis and interpretation:	SW, KV
Manuscript editing and review:	SW, MO, LK, JLi, WS, JH, MKu, KV, TK, JLa

Funding sources:
None

Conflicts of interest:
None

Chapter 4

Can oral swabs be used to diagnose nontuberculous mycobacterial cervicofacial lymphadenitis?

Authors:
Willemse SH, Karssemakers LHE, Oomens MAEM, Schreuder WH, Ho JPTF, Kolader M, Van Houdt R, Van der Kuip M, Buijsers GR, De Lange J, Lindeboom JA.

Published in:
The Laryngoscope

Author contributions:

Study conception and design:	SW, MO, LK, JLi, MKo, JLa
Data acquisition:	SW, JH, WS, MKu, MKo, RH, GB
Data analysis and interpretation:	SW, MKo
Manuscript editing and review:	SW, LK, MO, WS, JH, MKo, Rh, MKu, JLa, JLi

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None

Chapter 5

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Cervicofacial nontuberculous mycobacterial lymphadenitis: Clinical determinants of incomplete surgical removal

Authors:
Willemse SH, Karssemakers LHE, Oomens MAEM, Schreuder WH, Lindeboom JA, Van Wijk AJ, De Lange J.

Published in:
International Journal of Oral & Maxillofacial Surgery

Author contributions:

Study conception and design:	SW, LK, MO, JLa
Data acquisition:	SW
Data analysis and interpretation:	SW, AW
Manuscript editing and review:	SW, LK, MO, WS, JLi, AW, JLa

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Conflicts of interest:
None

Chapter 6

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Long-term outcome of surgical treatments for nontuberculous mycobacterial cervicofacial lymphadenitis in children

Authors:
Willemse SH, Schreuder WH, Apperloo RC, Lindeboom JA

Published in:
Journal of Oral and Maxillofacial Surgery (JOMS)

Author contributions:

Study conception and design:	JLi
Data acquisition:	JLi
Data analysis and interpretation:	SW, WS, RA, JLi
Manuscript editing and review:	SW, WS, RA, JLi

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Conflicts of interest:
None

Chapter 7

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Long-term esthetic outcome of different treatment modalities for nontuberculous mycobacterial cervicofacial lymphadenitis

Authors:
Willemse SH, Lindeboom JA, Karssemakers LH, Oomens MA, Schreuder WH, De Lange J

Published in:
Journal of Pediatric Surgery

Author contributions:

Study conception and design:	SW, JLi, LK, MO, JLa
Data acquisition:	SW, JLi, LK, MO, WS
Data analysis and interpretation:	SW
Manuscript editing and review:	SW, JLi, LK, MO, WS, JLa

Funding sources:
None

Conflicts of interest:
None

PhD Portfolio

Courses	Year	Workload (ECTS)
VU Online pubmed course (basic)	2016	0,1
VU Online pubmed course (advanced)	2016	0,1
VU Endnote course	2017	0,1
UvA Talen Academic Writing	2017	1,5
ACTA postgraduate course: Statistics, Methodology and SPSS	2018	3,0
e-BROK	2018	1,5
UvA Talen Academic English	2018	3,2
ACTA postgraduate course: Epidemiology & evidence based practice in dentistry and oral health care	2018	3,0
ACTA postgraduate course: Scientific Integrity	2019	2,0
Stanford Continuing Medical Education: Statistics for Medical Professionals	2019	0,8
Castor Advanced training	2019	0,1
ACTA postgraduate course: Oral Biology	2021	4,0
Re-registration e-BROK	2022	0,5
V10: Epidemiologisch onderzoek: basisprincipes	2023	4,0
V81: Missing data	2023	2,0
V20: Principes van epidemiologische data analyse	2023	4,0
V30: Regressietechnieken	2023	4,0
WK87: Causal Inference and Propensity Score Methods	2024	2,0
WK75: Multilevel Modelling and Longitudinal Data Analysis	2024	4,0
WK80: Clinical Prediction Models and Machine Learning	2024	2,0
WV40: Clinimetrics - Assessing Measurement Properties of Health Measurement Instruments	2024	3,0
V51: Epidemiologisch onderzoek: verdieping	2024	4,0
V55: Methodologische advisering	2024	4,0
Total		52,9

Conferences	Year
KNMT lezing; diagnostiek en behandeling van hoofdhalskanker	2016
How to publish a world class paper?	2016
Kindertandheelkunde 2017	2017
ACTA Quality Practice; Hoofd-halspathologie en oncologie	2018
Congres Mens, mond, microbioom	2019
Castor Masterclass: making research fun again	2019
Castor launch event	2019
Amsterdam UMC & COVID-19, Unorthodox Teams, Accelerating Science	2020
Autumn conference Dutch Association of Oral and Maxillofacial Surgeons (online)	2020
Vitality workshop	2023
Spring conference Dutch Association of Oral and Maxillofacial Surgeons	2024
Autumn conference Dutch Association of Oral and Maxillofacial Surgeons	2024

Oral presentations	Year
Seminar organized by the Dutch Society for Veterinary Parasitology (NVP) in collaboration with the task force general microbiology (WAMM) of the Dutch Society of Medical Mirobiology (NVMM): 'Treatment of nontuberculous mycobacterial cervicofacial lymphadenitis and ongoing studies'	2022
Radboud UMC visit at department of Medical Microbiology: 'Past presence and future of NTM cervicofacial lymphadenitis research'	2022
Wageningen Bioveterinary Research visit: 'NTM cervicofacial lymphadenitis, a zoonosis?'	2022
Reunion of the department of Oral and Maxillofacial surgery, Amsterdam UMC, location AMC: 'NTM cervicofacial lymphadenitis, past, presence and the future'.	2023
Spring conference Dutch Association of Oral and Maxillofacial Surgeons: 'Esthetic outcome of NTM cervicofacial lymphadenitis'	2024
Nederlandstalige Tuberculose Diagnostiek Dagen (NTDD) 2025: 'Minimally invasive diagnostics for NTM cervicofacial lymphadenitis'	2025

Supervising	Year
Cosupervisor Master's Thesis Dentistry – Marit Bakker: Evaluating the predictive value of ultrasonic characteristics in the diagnosis of cervicofacial nontuberculous mycobacterial lymphadenitis (NTM) in children: a retrospective analysis	2017-2018
Cosupervisor Bachelor's Thesis Dentistry – Misha Tan & Karlijn Bindels: Retrospectief onderzoek naar de diagnostische waarde van de Mantouxtest en de toevoeging van de Ziehl-Neelsen kleuring bij lymfadenitis veroorzaakt door een nontuberculeuze mycobacterie	2018
Cosupervisor Bachelor's Thesis Dentistry – Fione de Reuver & Isa Bierman: Langetermijnontwikkeling van littekens en andere bijwerkingen na behandeling van NTM cervicofaciale lymfadenitis	2020
Cosupervisor Master's Thesis Dentistry – Daan Murck: Long-term follow-up esthetic outcome of treatment for nontuberculous mycobacterial cervicofacial lymphadenitis	2021-2022
Cosupervisor Master's Thesis Dentistry – Gina Buijsers: Evaluation of the presence of nontuberculous mycobacteria in the oral and oropharyngeal cavity of children	2023-2024

List of publications

In this thesis

1. Willemse SH, Oomens MAEM, De Lange J, Karssemakers LHE. Diagnosing nontuberculous mycobacterial cervicofacial lymphadenitis in children: A systematic review. Int J Pediatr Otorhinolaryngol. 2018;112(9):48-54. doi: 10.1016/j.ijporl.2018.06.034.
2. Willemse SH, Oomens MAEM, Karssemakers LHE, Lindeboom JA, Schreuder WH, Ho JPTF, Van der Kuip M, Vlaming KE, Kaptein TM, De Lange J. Evaluation of Anti-Glycopeptidolipid-Core Immunoglobulin A Antibody Detection for the Diagnosis of Nontuberculous Mycobacterial Cervicofacial Lymphadenitis. Pediatr Infect Dis J. 2024;43(11):e416–8. doi: 10.1097/INF.0000000000004462.
3. Willemse SH, Karssemakers LHE, Oomens MAEM, Schreuder WH, Lindeboom JA, Van Wijk AJ, De Lange J. Cervicofacial non-tuberculous mycobacterial lymphadenitis: clinical determinants of incomplete surgical removal. Int J Oral Maxillofac Surg. 2020;49(11):1392-1396. doi: 10.1016/j.ijom.2020.03.019
4. Willemse SH, Schreuder WH, Apperloo RC, Lindeboom JA. Long-Term Outcome of Surgical Treatments for Nontuberculous Mycobacterial Cervicofacial Lymphadenitis in Children. J Oral Maxillofac Surg. 2022;80(3):537-544. doi: 10.1016/j.joms.2021.09.029.
5. Willemse SH, Lindeboom JA, Karssemakers LH, Oomens MA, Schreuder WH, De Lange J. Long-Term Esthetic Outcome of Different Treatment Modalities for Nontuberculous Mycobacterial Cervicofacial Lymphadenitis. J Pediatr Surg. 2023;58(9):1770-1775. doi: 10.1016/j.jpedsurg.2023.01.044.
6. Willemse SH, Karssemakers LHE, Oomens MAEM, Schreuder WH, Ho JPTF, Kolader M, Van Houdt R, Van der Kuip M, Buijsers GR, De Lange J, Lindeboom JA. Can oral swabs be used to diagnose nontuberculous mycobacterial cervicofacial lymphadenitis?. Laryngoscope. 2025;135(7):2602-2607. doi: 10.1002/lary.32062.

Other publications

1. Ploumen RLM, Willemse SH, Jonkman REG, Nolte JW, Becking AG. Quality of Life After Orthognathic Surgery in Patients with Cleft: An Overview of Available Patient-Reported Outcome Measures. Cleft Palate Craniofacial J. 2023;60(4):405-412. doi: 10.1177/10556656211067120.
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About the author

Samuel Hendrik Willemse was born in Leidschendam, the Netherlands, in 1995, as the son of Theo and Louise. He grew up in Voorschoten alongside his sister, Liza.

After completing his secondary education at the Stedelijk Gymnasium Leiden in 2013, he studied dentistry at the University of Amsterdam. Upon earning his master's degree in dentistry in 2019, he began studying medicine at the University of Amsterdam while working part-time as a dentist. In 2023, he completed the master's program in medicine and worked as an oral and maxillofacial surgery resident (not in training) at the Amsterdam University Medical Center. That same year, he began a post-initial master's program in clinical epidemiology at the Free University of Amsterdam, which he completed in 2025.

In 2024, he commenced his residency program at the Amsterdam University Medical Center to become an oral and maxillofacial surgeon. As part of his training, he is currently working at Isala in Zwolle.

Sam lives happily in Utrecht with Celine and their son Mees.