Cervicofacial Lymphadenitis in Children Caused by Mycobacterium haemophilum

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Background. Nontuberculous mycobacterial (NTM) lymphadenitis in children is most often caused by Mycobacterium avium. In a prospective, multicenter trial of the optimal treatment, 23.7% of the NTM cervicofacial lymphadenitis cases in children were caused by Mycobacterium haemophilum. In this article, we describe the epidemiological and clinical features of M. haemophilum cervicofacial lymphadenitis.

Methods. The diagnosis of Mycobacterium avium or M. haemophilum infection was established by culture or polymerase chain reaction. Demographic characteristics and data regarding clinical presentation and possible environmental exposure were compared for patients infected with M. avium and those infected with M. haemophilum.

Results. Ninety-four (69.9%) of 135 infections were caused by M. avium, 32 (23.7%) by M. haemophilum, and 9 (6.4%) by other NTM species. The median age of the M. haemophilum–infected children was 72 months, compared with 41 months for the M. avium–infected children (P < .001), with an equal distribution for both sexes. Involvement of multiple lymph nodes was frequently observed among the M. haemophilum–infected patients (56% of patients). Extranodal localizations were only observed in M. haemophilum–infected patients. Children with M. haemophilum infection were more likely to have a non-Dutch background (P = .001), and in most cases, they had a history of contact with swimming water (P = .03), whereas M. avium–infected patients were more likely to have a history of playing in sandpits (P = .01). In a multivariate analysis, only older age and a non-Dutch background were predisposing risk factors for M. haemophilum infection, compared with M. avium infection.

Conclusion. Higher age, non-Dutch background, and involvement of multiple cervicofacial lymph nodes with extranodal localizations distinguished M. haemophilum infection from M. avium infection.

Nontuberculous mycobacteria (NTM) are an important cause of chronic lymphadenitis in the head and neck region in children. Many species of NTM have been linked to this condition, most commonly Mycobacterium avium–intracellulare complex, Mycobacterium scrofulaceum, Mycobacterium fortuitum, and Mycobacterium chelonae [1].

Mycobacterium haemophilum is an uncommon pathogen in humans and mainly causes infections in immunocompromised patients. Cutaneous lesions, septic arthritis with or without osteomyelitis, and disseminated infection with skin lesions and osteomyelitis are the principal manifestations [2]. Since the first case report of a cervicofacial lymphadenitis in an immunocompetent child in 1981 [3], only 7 additional cases of children with head and neck lymphadenitis have been described in the literature [2, 4–6] (table 1). In a retrospective study from Israel, M. haemophilum was identified in 12 (41%) of 29 children with culture-proven NTM submandibular lymphadenitis [7].

During the course of the CHIMED study, a randomized, prospective, multicenter, multidisciplinary trial of the optimal treatment of NTM cervicofacial lymphadenitis in children, M. haemophilum was recognized as an important pathogen. It is not known whether the clinical picture of M. haemophilum lymphadenitis is in all respects comparable with that of the more frequently described M. avium cervicofacial lymphadenitis. In the present report, we compared the epidemiological and clinical features of M. haemophilum cervicofacial lymphadenitis with those of M. avium cervicofacial lymphadenitis.
clinical features of *M. haemophilum* cervicofacial lymphadenitis with those of *M. avium* cervicofacial lymphadenitis. Additionally, the possible mode of acquisition of the *M. haemophilum* infection was explored.

**MATERIALS AND METHODS**

**Patients.** Children with chronic cervicofacial lymphadenitis were enrolled in the study between 2001 and 2004. Patients were referred by pediatricians, otolaryngologists, oral-maxillofacial surgeons, and general practitioners from all over the country to the principal investigator (J.L.). After informed consent was obtained, parents were interviewed using a standardized questionnaire. In addition to age, sex, and ethnic background, the following data were analyzed for each patient for the previous 6 months: known underlying diseases, length of lymph node swelling prior to presentation, clinical symptoms, results of laboratory tests (erythrocyte sedimentation rate, WBC count, and differentiation), contacts with pets, travel history, and history of activities such as swimming, playing in sandpits, and visiting children’s farms. The season of onset of symptoms was also analyzed. In all children, ultrasound of the cervical region was performed. The Medical Ethics Committee of the Academic Medical Center of the University of Amsterdam (Amsterdam, The Netherlands) approved the study, and parents were interviewed using a standardized questionnaire. In addition to age, sex, and ethnic background, the following data were analyzed for each patient for the previous 6 months: known underlying diseases, length of lymph node swelling prior to presentation, clinical symptoms, results of laboratory tests (erythrocyte sedimentation rate, WBC count, and differentiation), contacts with pets, travel history, and history of activities such as swimming, playing in sandpits, and visiting children’s farms. The season of onset of symptoms was also analyzed. In all children, ultrasound of the cervical region was performed. The Medical Ethics Committee of the Academic Medical Center of the University of Amsterdam (Amsterdam, The Netherlands) approved the study, and parents gave written consent before study enrolment.

**Methods.** In children with a persistent cervicofacial lymphadenitis, a fine-needle aspiration biopsy of the affected lymph node was performed to establish the diagnosis NTM. Mycobacterial infection was diagnosed as described elsewhere [8, 9]. In summary, after fine-needle aspiration of the affected lymph node, clinical materials were decontaminated with a NaCl-NaOH decontamination protocol. Auramine staining was performed on the decontaminated material to detect acid-fast rods. Mycobacterial cultures were performed at 35°C in liquid mycobacteria growth indicator tube medium and on solid Lowenstein-Jensen medium. *M. haemophilum*-specific cultures were performed at 30°C on Lowenstein-Jensen medium with added iron citrate and in mycobacteria growth indicator tube medium with X-factor strip added. Mycobacterial species were identified by using the Inno-Lipa assay (InnoGenetics) and, more recently, the Inno-Lipa V2 assay (InnoGenetics). In addition, real-time PCR was performed for detection of the genus *Mycobacterium* and the species *M. haemophilum* and *M. avium* [8, 9]. *M. haemophilum* infection was diagnosed if results of *M. haemophilum*-specific cultures or *M. haemophilum*-specific PCR were positive.

All patient materials were analyzed by the medical microbiological laboratory at the Leiden University Medical Center (Leiden, The Netherlands) [8, 9]. All specimens arrived at the laboratory within 6 h after collection, and they were processed immediately.

**Statistical analysis.** Comparisons between patients with cervicofacial lymphadenitis caused by *M. avium* and *M. haemophilum* were performed by univariate analysis with the χ² test for categorical variables and Student’s t test or, if variables were not normally distributed, the Mann-Whitney test for continuous variables. ORs and 95% CIs were derived from unadjusted logistic regression to express the strength of the associations between clinical and epidemiological features and the underlying pathogenic organism. Variables with a *P* < .1 in the univariate analysis were included in a multivariate logistic regression, using a backward-step selection with a *P* > .10 for removal. Adjusted ORs and their 95% CIs estimate the potential independent contribution to the risk of contracting infection with either *M. avium* or *M. haemophilum*. All analyses were performed with SPSS, version 12.0 (SPSS).

**RESULTS**

**Patients.** During the study period, 210 children with chronic cervicofacial lymphadenitis were referred to the principal investigator. Mycobacterial infection was diagnosed in 138 children (3 cases involving *M. tuberculosis* and 135 involving...
NTM); _Bartonella henselae_ infection was diagnosed in 22 children; streptococcal infection was diagnosed in 14; staphylococcal infection was diagnosed in 11; and toxoplasmosis was diagnosed in 1. The remaining 24 children had persistent lymph node swellings due to other causes. The 135 patients with NTM infection had a confirmed infection diagnosed either by positive culture results only (n = 13), PCR results only (n = 30), or both (n = 73). In 19 patients, the diagnosis of NTM infection was based on a positive culture result, and no PCR was performed.

Ninety-four (69.9%) of 135 infections were caused by _M. avium_, 32 (23.7%) were caused by _M. haemophilum_, 3 by _M. malmoense_, 2 by _M. kansasi_, 1 by _M. chelonei_, 1 by _M. intracellularis_, and 1 by _M. fortuitum_. For 1 patient with NTM infection, specification was not possible.

**Demographics and clinical characteristics.** Table 2 shows the demographic and clinical characteristics of children with NTM lymphadenitis caused by either _M. avium_ or _M. haemophilum_. All children were healthy without underlying disease. The age of patients with NTM infection ranged from 9 months to 138 months, with a median age of 46 months and an even overall distribution of sex. The median age of the _M. avium_-infected children was 41 months (range, 14–167 months), whereas the median age of the _M. haemophilum_-infected patients was 72 months (range, 9–183 months; P < .001). The parents of 119 (88%) of the 135 children were of Dutch origin. Six (6%) of the _M. avium_-infected patients were from another ethnic background, compared with 9 (28%) of the _M. haemophilum_-infected patients (P = .01). With the exception of 2 patients, all non-Dutch children with NTM lymphadenitis were born in The Netherlands. Most of the _M. haemophilum_-infected patients lived in Amsterdam and surrounding areas, whereas patients with _M. avium_-associated infections were equally distributed over the country.

The mean duration (_± SD_) of lymph node swelling until initial presentation at our clinic was 12.4 ± 7.7 weeks among patients with _M. avium_ lymphadenitis versus 11.4 ± 8.2 weeks among patients with _M. haemophilum_ lymphadenitis (P = .56). Besides the local abnormalities related to NTM infection, children lacked systemic symptoms.

**Laboratory results showed a moderate increase in the erythrocyte sedimentation rate in 13 of the 94 _M. avium_-infected patients versus 3 of the 32 _M. haemophilum_-infected patients. The mean erythrocyte sedimentation rate (_± SD_) in these patients was 33 ± 13 mm/h for _M. avium_-infected patients versus 30 ± 8 for _M. haemophilum_-infected patients (P = .71). In 4 children with _M. avium_ infection, temporary lymphocytosis was noted at initial presentation, with the percentage of lymphocytes ranging from 51% to 81%.

**Affected lymph nodes.** In all children, ultrasound was performed on the cervicofacial region. The right side of the head and neck region was involved in 58 patients (46%), whereas the left side was involved in 61 patients (48%) (P = .93). Two children had bilateral involvement, and 5 children had a medially located lymph node (submental and occipital location). The submandibular lymph nodes were most commonly affected; these nodes were affected in 96 patients (76%), and 12 patients (9.5%) had involvement of the preauricular lymph

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**Table 2. Demographic characteristics and clinical symptoms of children with Mycobacterium avium or Mycobacterium haemophilum cervicofacial lymphadenitis.**

<table>
<thead>
<tr>
<th>Variable</th>
<th><em>M. avium</em>-infected patients (n = 94)</th>
<th><em>M. haemophilum</em>-infected patients (n = 32)</th>
<th>P&lt;sup&gt;a&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, median months (range)</td>
<td>40.5 (14–167)</td>
<td>71.5 (9–183)</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>No. male/female (% male/female)</td>
<td>47/47 (50/50)</td>
<td>19/13 (59/41)</td>
<td>.36</td>
</tr>
<tr>
<td>Dutch background</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>88 (94)</td>
<td>23 (72)</td>
<td>.001</td>
</tr>
<tr>
<td>No</td>
<td>6 (6)</td>
<td>9 (28)</td>
<td></td>
</tr>
<tr>
<td>Duration of lymph node swelling, mean weeks ± SD</td>
<td>12.4 ± 7.7</td>
<td>11.4 ± 8.2</td>
<td>.56</td>
</tr>
<tr>
<td>Abnormal laboratory finding(s)&lt;sup&gt;b&lt;/sup&gt;</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>13 (14)</td>
<td>3 (9)</td>
<td>.71</td>
</tr>
<tr>
<td>No</td>
<td>81 (86)</td>
<td>29 (91)</td>
<td></td>
</tr>
<tr>
<td>Involvement of multiple locations</td>
<td></td>
<td></td>
<td>&lt;.001</td>
</tr>
<tr>
<td>Yes</td>
<td>3 (3)</td>
<td>18 (56)</td>
<td></td>
</tr>
<tr>
<td>No</td>
<td>91 (97)</td>
<td>14 (44)</td>
<td></td>
</tr>
</tbody>
</table>

<sup>a</sup> By the χ<sup>2</sup> test, Student’s t test, or the Mann-Whitney test, where applicable.

<sup>b</sup> Erythrocyte sedimentation rate or WBC count.

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**NOTE.** Data are no. (%) of patients, unless otherwise indicated.
nodes. In 4 patients, submental lymph nodes were affected. A combination of involved lymph stations was observed in the remainder of patients. The submandibular lymph nodes were affected in 70 M. avium–infected patients (74%) and 26 M. haemophilum–infected patients (81%) (P = .86).

In the M. haemophilum–infected patients, multiple lymph nodes were affected in 18 (56%) of 32 patients, compared with 3 (3%) of 94 M. avium–infected patients (P < .001) (figure 1). In 4 patients infected with M. haemophilum, there was also involvement of the medial canthus of the eye (figure 2), with an infection of the nasolacrimal duct in 4 patients. In addition, in 2 M. haemophilum–infected patients, a lesion was observed at the outer aspect of the ear lobe, and in 1 M. haemophilum–infected patient, a lesion on the cheek was also observed. No extra-nodal lesions were observed among the M. avium–infected patients.

No association was found between age and involvement of multiple lymph node locations. In the M. haemophilum lymphadenitis group, 7 patients with infection of multiple lymph nodes were <46 months old (i.e., less than the median age of all patients), and 11 patients were >46 months old (P = .71).

Most patients with NTM cervicofacial lymphadenitis (83%) presented during the stage of skin discoloration with lymph node fluctuation. Only 17% of patients had clinical features of a cervicofacial swelling or a nodal mass without abscess formation or skin-color changes. M. avium–infected and M. haemophilum–infected patients did not differ in this regard.

Environmental risk assessment for contracting either M. avium or M. haemophilum infection. Possible environmental risk factors were first analyzed by univariate analysis (table 3). Patients with NTM infection experienced the first clinical symptoms of a lymph node swelling during autumn (29% of patients), followed by winter (25%), spring (25%), and summer (21%). There was no statistical significant difference in season of onset between the M. avium–infected and the M. haemophilum–infected patient groups.

Only 5 patients traveled abroad before the onset of the symptoms, and although there was a significant difference between M. haemophilum–infected and M. avium–infected patients, the
number of patients was too small to draw any conclusions. There was no difference between *M. haemophilum*–infected and *M. avium*–infected children with respect to contact with pets such as cats, birds, or guinea pigs. Patients with *M. avium* infection were more likely to have played in sandpits (*P* .01), whereas patients with *M. haemophilum* infection were more likely to have a history of contact with swimming water (*P* .03). There was no significant difference in the number of visits to children’s farms (*P* .18).

In the multivariate analysis, age, non-Dutch background, history of playing in sandpits, and history of contact with swimming water were entered. Given the low number of children who had traveled abroad, this variable was not entered (table 4). Older age (OR, 1.02; *P* .03) and a non-Dutch background (OR, 5.25; *P* .008) were the only characteristics significantly associated with *M. haemophilum* infection.

**DISCUSSION**

To our knowledge, this report is the first prospective study to describe the epidemiological and clinical features of *M. haemophilum* cervicofacial lymphadenitis in children and to compare them with features of the more frequently described *M. avium* infection. *M. avium*–infected and *M. haemophilum*–infected patients did not differ with respect to sex, duration of lymph node swelling prior to presentation at our clinic, or clinical symptoms, but *M. haemophilum*–infected patients were older than patients with *M. avium* lymphadenitis (median age,
M. haemophilum infections were more common in Amsterdam and surrounding areas, whereas M. avium infection was distributed more equally over the country. There was also a higher incidence of M. haemophilum infection among non-Dutch children. It cannot be excluded that the higher incidence might be explained by the fact that the pediatric population of Amsterdam largely consists of children from non-Dutch descent. Exposure to sandpits and swimming water were significant in the univariate analysis, but not in the multivariate analysis, so this difference is probably related to the difference in age. In patients with M. haemophilum infection, multiple lymph nodes were affected significantly more often, and extranodal localizations were only observed in these patients.

Recently, a survey study in The Netherlands estimated the annual incidence of NTM infection in children to be 0.77 cases per 100,000 children [10]. In the youngest age group (<5 years), the incidence was even higher (2.3 cases per 100,000 children). Several studies [11–13] have reported NTM cervicofacial lymphadenitis to be most frequently observed in persons aged 1–5 years, which corresponds with the median age of the M. avium–infected group in this study. The higher median age of the M. haemophilum–infected patients in our study is a new finding. The incidence of M. haemophilum cervicofacial lymphadenitis in children in The Netherlands is unknown, but infections due to M. haemophilum could be underdiagnosed, because the specific culture methods required for its recovery are not routinely used [9]. Moreover, combining a Mycobacterium genus-specific, real-time PCR with amplicon sequencing and a M. haemophilum–specific PCR resulted in the recognition of M. haemophilum in another 12 patients (who represented 38% of all diagnosed M. haemophilum infections).

In our study, children with M. avium or M. haemophilum cervicofacial lymphadenitis seldom exhibited general clinical symptoms, although some children experienced loss of appetite. The lack of clinical symptoms or abnormal laboratory results has been reported previously by several authors [14, 15]. This might be different for other manifestations of M. haemophilum infections. Armstrong et al. [5] describe a patient with a periilar M. haemophilum lymphadenitis who presented with daily fever, anorexia, coughing, and weight loss.

Some studies claim a correlation between season and NTM infections in the head and neck. In one study, the number of NTM isolates recovered during winter was greater than the number recovered during spring and summer [16], whereas in another study, the majority of children with NTM cervicofacial lymphadenitis presented for treatment during winter and spring [17]. There was no significant seasonality for the presentation of NTM infections in our study.

Most children with NTM lymphadenitis (83%) were observed during the stage of lymph node fluctuation with discoloration of the skin, which can be explained by the diagnostic delay: the average amount of time between onset of symptoms and presentation at our clinic was ~3 months. Only 17% of patients with NTM lymphadenitis were observed during an early stage, before skin changes or lymph node fluctuation.

Lymph node involvement in both M. haemophilum–infected and M. avium–infected patients was predominantly located in the submandibular region and less commonly in the preauricular region. Although there were no differences in the percentage of M. haemophilum–infected and M. avium–infected patients with involvement of the submandibular or preauricular regions, a significant difference was found in the involvement of multiple lymph node regions. Involvement of multiple lymph nodes or extranodal localizations in M. haemophilum infection was present in 56% of patients, a finding that has not been described before in other reports. An explanation for the involvement of multiple lymph nodes in the M. haemophilum–infected patients might be that M. haemophilum is more pathogenic than is M. avium. M. haemophilum infection has been associated with skin lesions, septic arthritis, sinusitis, and pneumonia [18]. Children with M. haemophilum infection were older, but no significant relation was found between age and infection of multiple lymph nodes. This makes it unlikely for infection of multiple lymph nodes to be the result of stronger immunological reactions in older children.

An important question is where children acquire the infection. M. avium are ubiquitous and have been isolated from a wide range of environmental sources, such as soil and water. The natural source of M. haemophilum, however, is not well known, but studies suggest that the species might be found in water [19]. Geographic differences in the frequency of disease are probably related to the extent of contamination of the local environment [14]. There appeared to be a clustering of cases in the referral area of our hospital, and the cause of the geographic prevalence of M. haemophilum infection in Amsterdam and surrounding areas will be the subject of additional studies.

Although no association was found in the multivariate analysis between playing in sandpits or having exposure to swimming water and an increased risk for either M. avium or M. haemophilum lymphadenitis, a possible role for these activities can not be excluded. Therefore, additional research will be

<table>
<thead>
<tr>
<th>Variable</th>
<th>OR (95% CI)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>1.02 (1.00–1.04)*</td>
<td>.03</td>
</tr>
<tr>
<td>Non-Dutch background</td>
<td>5.25 (1.53–18.01)</td>
<td>.008</td>
</tr>
<tr>
<td>Playing in a sandpit</td>
<td>0.73 (0.25–2.10)</td>
<td>.56</td>
</tr>
<tr>
<td>Swimming</td>
<td>1.02 (0.29–3.59)</td>
<td>.98</td>
</tr>
</tbody>
</table>

* For each 1-month increase in age

Table 4. Multivariate analysis for variables related to Mycobacterium avium lymphadenitis or Mycobacterium haemophilum lymphadenitis.
necessary to elucidate the potential role of environmental sources for both NTM species.

In conclusion, *M. haemophilum* was the second most commonly recognized pathogen in children with cervicofacial NTM lymphadenitis in The Netherlands. This study has shown that patients with *M. haemophilum* lymphadenitis tend to be older than are patients with the more common *M. avium* lymphadenitis. Moreover, *M. haemophilum* infections of the head and neck are associated with infection of multiple lymph nodes and involvement of extra-nodal areas like the medial canthus, cheek, or ear lobe. Additional studies are required to explain the geographical clustering of cases in the region of Amsterdam.

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