INTERNATIONAL REPORT

Epidemiology and Clinical Significance of Nontuberculous Mycobacteria in Patients Negative for Human Immunodeficiency Virus in Switzerland

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Over the last decades, the rate of isolation of tubercle bacilli has declined in the developed countries, while the incidence of infection with nontuberculous mycobacteria (NTM) has increased. In a retrospective study, we analyzed all cases of patients negative for human immunodeficiency virus (HIV) and from whom NTM were isolated in the Zurich area of Switzerland from 1983 to 1988. During the 6-year study period, 513 patients infected with NTM were identified, 34 of whom had clinically significant disease. The presentation of mycobacteriosis was found to be lung disease in 23 cases, soft-tissue disease in 10 cases, and disseminated disease in one case. The highest attack rate of pulmonary mycobacteriosis was 0.49% and was found in the group of patients 41–50 years old. During the 6-year period, the incidence of tuberculosis declined from 16.2 to 13.2 per 100,000 population, while the incidence of mycobacteriosis increased from 0.4 to 0.9 per 100,000 population. Clinically nonsignificant NTM isolates were found more frequently in patients with chronic lung diseases ($P < .01$) and especially in patients with a history of tuberculosis ($P < .001$).

The genus *Mycobacterium* comprises more than 50 different species [1]. The two most prevalent species *Mycobacterium tuberculosis* and *Mycobacterium leprae* are responsible for diseases that have threatened humankind in the past and present. In Western Europe, disease caused by tubercle bacilli has been a diagnostic and therapeutic challenge for generations of physicians and microbiologists and is regaining new importance during the ongoing AIDS epidemic. The diagnosis of leprosy, which is now rare in Western Europe, is made on the basis of clinical and histopathologic findings, since *M. leprae* cannot be cultivated in the routine laboratory. In the present study, we focus our attention on the other species of this genus, the so-called nontuberculous mycobacteria (NTM).

Terminology of NTM

A variety of epithets have been proposed for designating NTM [2, 3]. *Anonymous, unclassified, unknown, tuberculoid, environmental, opportunistic, nyrocin,* and *MOTT* (mycobacteria other than tubercle bacilli) have been suggested. Each of these terms is subject to criticism. The designation *atypical* is still one of the most common, but it is actually a misnomer because these organisms are typical for the genus *Mycobacterium* when analyzed microbiologically. In agreement with the editor of the *American Review of Respiratory Disease* [4] and the official statement of the American Thoracic Society [5], we shall resort to the more neutral term of *nontuberculous mycobacteria.* For disease due to NTM, we propose to adopt the term *mycobacteriosis* as used by Runyon [6] and reemphasized by the editor of the *American Review of Respiratory Disease* [4]. This designation would permit a clear differentiation from tuberculosis, the term commonly used for disease caused by the *M. tuberculosis* complex (TBC; *M. tuberculosis, Mycobacterium bovis,* and *Mycobacterium africanum*).

Characteristics of NTM

Fundamental differences exist between TBC and NTM. NTM are ubiquitous in nature, as has been demonstrated in environmental studies [7–10]. Therefore, it has been possible to postulate mode and source of infection. Person-to-person transmission of NTM disease generally does not occur [11]. TBC is, when isolated, always associated with disease. In contrast, the interpretation of a positive NTM culture is complicated by the following facts: first, the pathogenic potential of the different species varies greatly; second, colonization of an individual without the development of infection or even invasive disease is possible; and third, a coincidental isolation of a NTM species can happen because of its ubiquitous presence.

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Historical Perspective

The first reports on NTM date back to 1885 [12]. During the first half of the century, no serious interest was paid to this group of mycobacteria, although occasional isolates from clinical specimens were reported [13]. Until recently, disease caused by TBC has been prevalent [14] to the degree that only marginal attention was paid to disease due to NTM [15].

In 1954 Timpe and Runyon [16] were the first to correlate the known facts about the relationship between human disease and NTM and provided the first working classification of these organisms. In 1959 Runyon [17] established an updated classification into four groups, which is valid to this day and is known as the so-called Runyon classification of NTM. His classification is based exclusively on phenotypic characteristics of the various species, such as growth rate and colonial pigmentation. Within a Runyon group, however, the various species differ in their clinical relevance. Clinical interpretation of a positive NTM culture remains unresolved, thus calling for reproducible criteria on interpretation of the clinical relevance of cultured NTM. In 1967 Yamamoto et al. [18] established criteria for distinguishing disease from colonization and thus provided a diagnostic aid, which still is valid today. In 1979 Wolinsky [13] reviewed major aspects of NTM. He provided a summary of the clinical manifestations, the microbiological properties, and the historical perspective of NTM.

Over the last decades, the rate of isolation of TBC has declined in the western hemisphere, while the incidence of NTM infection has increased [19, 20]. The increase in the incidence of AIDS has renewed interest in mycobacteria. Patients with AIDS are at increased risk of developing a wide spectrum of diseases due to NTM as well as to TBC [21–24].

In Woods and Washington's later review [25], a description of the widened spectrum of clinical manifestations due to infections with various species in patients who have AIDS was published. Case reports of infections due to species considered to be apathogenic and of less common sites of infection were discussed. A clinical classification based on two major groups was developed: namely, NTM potentially pathogenic and rarely pathogenic for humans.

In 1989 another approach to the same problem was put forth by Davidson [26], in which the mycobacterial species were correlated with their relative clinical significance. Correspondingly, a number was assigned to each NTM species, ranging from 0 to 10; the higher the number, the greater the clinical relevance. Furthermore, diagnostic criteria, comparable to those set up by Yamamoto et al. [18], were established for supporting the diagnosis of disease due to NTM.

Aims of This Study

The main goal of this study was to assess the incidence of NTM infection and the clinical significance of NTM isolates in patients negative for human immunodeficiency virus (HIV) in Switzerland, on the basis of data collected from patients in and around Zurich, its most populous city. We also analyzed the incidence of tuberculosis in the same area during the same period and the demographic differences between patients with TBC disease and those with disease due to NTM. Third, special attention was given to preexisting clinical conditions of patients from whom NTM had been isolated as well as to the interpretation of positive NTM cultures by the clinician.

Materials and Methods

Patients

This study is a retrospective analysis of all HIV-negative patients from whom NTM were isolated in the Department of Medical Microbiology of the University of Zurich, from 1 January 1983 to 31 December 1988. Only specimens that were processed in our mycobacteriology laboratory were included, not cultures sent to us from other laboratories.

To guarantee a complete and thorough registration of positive NTM cultures, we used various methods of data retrieval. We systematically checked the laboratory slips for NTM isolates from the years 1983 and 1984. For the period between 1985–1988, we were able to extract all specimens with TBC and NTM isolates with the aid of a computer-assisted data bank. Aside from this data bank, we had used a record card system in which all isolated mycobacteria were manually recorded. This system was a valuable control of all data analyzed for the entire period of our study. We traced the submitting institutions (private practices, outpatient clinics, and hospitals) from their orders.

Clinical and Demographic Data: Medical Conditions

To obtain clinical information about our patients, we sent a questionnaire for clarifying the interpretation of NTM isolates (table 1). If the questionnaire was not returned or was incomplete, we personally contacted the responsible clinician. In some cases, one of us (M.D.) reviewed the patients' records. In certain cases we consulted the physician for follow-up of the clinical course. When confronted with discrepancies between the clinicians' and our interpretation of the clinical relevance of a culture positive for NTM, we abided by the criteria of Yamamoto et al. [18]. Definitions of data used in descriptive statistics are as follows: (1) when the same species was isolated repeatedly from the same patient, we counted it only once in the first 12 months of its detection; (2) when the following events occurred, more than one NTM isolate was considered or counted for the same patient —(a) on detection of more than one isolate belonging to different species in the same 12-month period, (b) on isolation of different species in consecutive years, and (c) on re-

Table 1. Patient questionnaire for significance of nontuberculous mycobacteria (NTM).

Was one of the following medical conditions present at the time the specimen was collected?	
Chronic pulmonary disease	Yes ☐ No ☐
Chronic obstructive pulmonary disease (COPD)	☐
Bronchiectasis	☐
History of tuberculosis	☐
Others	☐
Malignancy	Yes ☐ No ☐
If yes, what kind of therapy:	
Radiotherapy	☐
Chemotherapy	☐
No therapy	☐
Serology for human immunodeficiency virus (HIV)	Positive ☐
	Negative ☐
	Not performed ☐
AIDS	Yes ☐ No ☐
Tuberculin skin test	Positive ☐
	Negative ☐
	Not performed ☐
Diabetes mellitus	Yes ☐ No ☐
Alcohol abuse	Yes ☐ No ☐
Steroid therapy/immunosuppressive therapy	Yes ☐ No ☐
Congenital immunodeficiency	Yes ☐ No ☐
Connective tissue disease	Yes ☐ No ☐
Was diagnosis made before receiving the mycobacteriologic result?	
What was the final clinical diagnosis?	
Was the isolation of NTM considered clinically significant?	
Was chemotherapy initiated because of this result?	

peated detection of the same NTM species after an interval of >2 years; and (3) when the interval between the isolation of TBC and NTM was >6 months, we considered the patient to have a history of tuberculosis.

Specimens

We analyzed the number of specimens received per patient and calculated the percentage of cultures that yielded NTM. These data were extracted from a list of ~72,000 specimens (~12,000 per year) sent to our laboratory for examination of acid-fast bacilli (AFB). Our laboratory performs this service at no extra cost.

NTM Disease and Tuberculosis

Apart from evaluating the epidemiology and clinical relevance of NTM, we recorded data on all patients with tuberculosis (TB). We, therefore, were able to compare these two patient cohorts.

Laboratory Procedures

Smears of most specimens sent to our laboratory were examined by the fluorochrome (auramine-rhodamine) procedure [27–29]. Exceptions were gastric aspirates and blood and urine specimens not submitted by urological clinics or from patients with a history of urogenital tuberculosis.

Laboratory techniques and methods were consistent during the study period except for the pretreatment methodology [27–29]. Until 1985 *N*-acetyl-L-cysteine (3% NaOH, 1.45% sodium citrate, 0.5% *N*-acetyl-L-cysteine) [27, 28] was used. In 1986 the modified SDS method, which provided for a mild and sufficient contamination control [30], was introduced in our laboratory.

Media and procedures used depended on the origin of the specimen. Specimens from the respiratory tract as well as other specimens considered not to be sterile (e.g., urine, feces, gastric aspirates, ejaculate, or endometrial drainage) were inoculated onto two egg-based Löwenstein-Jensen (L-J) media (produced in this department), one of which was an L-J slant and one of which was an L-J slant modified after Gruft [31]. Incubation time was 8 weeks at 36°C (4 weeks in 5%–10% CO_2 atmosphere and 4 weeks without CO_2). Growth was examined visually once a week.

One Middlebrook 7H11 plate (Difco Laboratories, Detroit) was inoculated additionally with specimens from patients with silicosis or who were positive for HIV. Incubation time was 6 weeks at 36°C with CO_2. These plates were examined with a dissecting microscope (16× magnification) once a week.

Specimens not considered to be contaminated (CSF, bone marrow, pleural and pericardial effusions, and lysis-centrifugation blood) were inoculated into two tubes with Middlebrook 7H9 broth (Difco Laboratories) and onto one Middlebrook 7H11 plate. If the smear was positive for AFB, two L-J slants (one L-J, one L-J modified after Gruft) were also inoculated. After 2 and 4 weeks, respectively, the 7H9 broths were centrifuged for 15 minutes at 3,300*g*, and the sediment was stained for microscopic examination as well as inoculated onto two L-J slants. All other specimens (e.g., pus, deep swabs, bladder aspirates, dialysate fluids, and biopsy) were cultured first on a chocolate agar plate for 24 hours at 36°C in a 5%–10% CO_2 atmosphere. If growth was detected, gram and Ziehl-Neelsen stains were done. If there was no growth after 48 hours, the procedure was the same as in the case of sterile specimens (see above). In case of non-acid-fast growth, the specimen was treated in the same manner as specimens from the respiratory tract, except that tissue was inoculated additionally onto one Middlebrook 7H11 plate.

Specimens from peripheral sites of the body (e.g., skin, superficial swabs, and pus) were first inoculated onto a choco-

late agar plate and incubated up to 48 hours at 36°C, in a 5%–10% CO_2 atmosphere. In case of non-acid-fast growth, the specimen was decontaminated, and two L-J slants each were inoculated and incubated at 30°C and 36°C. Further, two L-J slants supplemented with ferric ammonium citrate were incubated at 30°C. If there was no growth on the chocolate agar plate, one L-J slant supplemented with ferric ammonium citrate and six tubes with Middlebrook 7H9 broth were inoculated. Three of the tubes and the L-J slant were incubated at 30°C. The remaining three tubes were processed as in the case of a noncontaminated specimen (described above). When the direct smear was positive, L-J slants were inoculated first.

Identification

Mycobacteria were identified by colonial morphology, pigment production, growth rate, susceptibility pattern, and a battery of in vitro tests described in the most updated versions of laboratory manuals [27–29, 32].

Determination of Clinical Significance

The Yamamoto criteria [18] that we used in our study are based on the number of positive cultures, number of colonies in a culture, and histopathologic findings. These criteria are, therefore, applicable to a retrospective evaluation, such as the present study, in which radiographic findings were only partly available or radiography was not done at all. In addition, one extrapulmonary specimen had to be smear and culture positive or at least two specimens had to be culture positive. Dissemination was defined as a positive blood specimen or culture-positive specimens with the same organism from two different body sites.

Attack Rate

We analyzed all respiratory tract specimens obtained from 1986 to 1988 and determined the age-specific attack rate.

Exclusions

We excluded all HIV-positive patients from our study. The following cases were also excluded from our evaluation: five cases in which the *Mycobacterium scrofulaceum* isolates were submitted by a physician whose bronchoscope had been contaminated by the above-mentioned mycobacterium, one case in which the culture was contaminated, and one case in which the medium had to be decontaminated several times before isolation.

Table 2. Number of patients from whom mycobacteria were isolated, 1983–1988.

Pathogen	1983	1984	1985	1986	1987	1988
Mycobacterium tuberculosis complex	181	181	168	140	151	150
Nontuberculous mycobacteria	59	94	62	68	103	127

Results

Number of Isolates

During the period between 1983 and 1988, a subtotal (excluding strains isolated in culture of specimens from HIV-positive patients) of 513 NTM strains were isolated in our laboratory. Even though the number of specimens examined remained more or less constant (minimum per year, 11,220; maximum per year, 14,763; median, 12,768), there has been an increase of patients with NTM isolates since 1986 (table 2). From 1983 to 1988 the incidence of tuberculosis in Zurich declined from 16.2 to 13.2 per 100,000 population, which corresponds to an overall decrease of 17%. There were a total of 971 patients with TB; the number of isolates from these patients was 1.9 times the number of NTM isolates during this 6-year study period (table 3).

Epidemiological Data on NTM

Of 513 patients with NTM isolates, 34 (7%) had clinically significant disease. Of 17 different species isolated, only eight were shown to be clinically significant; thus, the pathogenic potential of different species varied greatly (table 4). Within the group of clinically relevant NTMs, the most important species were *Mycobacterium avium* complex (MAC), *Mycobacterium kansasii*, *Mycobacterium xenopi*, and *Mycobacterium malmoense*. *Mycobacterium gordonae* was the species most frequently isolated. Even though it accounted for 45% of all isolates, *M. gordonae* was never shown to be responsible for disease (table 4).

Demographic Data

The sex distribution differed between the group with clinically significant and the group with nonsignificant NTM isolates. Sixty percent of the group infected with nonsignificant isolates were male. The number of male and female patients with mycobacteriosis was the same. The median age of patients with nonsignificant isolates was 65 years. Among patients with any type of mycobacteriosis, the median age was 52 years. The number of patients with pulmonary mycobacteriosis found per age group who were screened for AFB is shown in figure 1. The mean attack rate was 0.12% (SD ± 0.15). The highest rate occurred in the group aged 41–50

Table 3. Incidence of mycobacterial diseases in the Zurich area, 1983–1988.

Year	Population of Zurich area (in millions)	No. of cases of mycobacteriosis	Incidence of mycobacteriosis (per 100,000 population)	No. of cases of tuberculosis	Incidence of tuberculosis (per 100,000 population)
1983	1.119	4	0.4	181	16.2
1984	1.120	3	0.3	181	16.2
1985	1.123	4	0.4	168	15.0
1986	1.128	7	0.6	140	12.4
1987	1.133	6	0.5	151	13.3
1988	1.140	10	0.9	150	13.2

years. The age distribution of patients with TB showed a bimodal distribution as was expected: one peak occurred in the group aged 21–30 years and the other in the group aged 71–80 years (data not shown).

Localization of Disease

The presentation of mycobacteriosis was found to be lung disease in 23 cases (68%), disseminated disease in one (3%), and soft-tissue disease in 10 (29%) (figure 2). There were more cases of lung mycobacterioses in the second half of the study period: five smear-positive and two smear-negative cases in the first half; nine smear-positive and seven smear-negative cases in the second half.

Lung disease. Pulmonary mycobacteriosis was caused by *M. kansasii* in eight patients and by MAC in eight patients.

Table 4. Pathogenic potential of nontuberculous mycobacterial isolates (n = 513).

Isolate	No. of strains associated with disease/ total no. of strains (%)
M. avium complex	13/61 (21)
M. kansasii	9/35 (26)
M. xenopi	4/25 (16)
M. malmoense	3/3 (100)
M. fortuitum	2/36 (6)
M. simiae	1/1 (100)
M. marinum	1/3 (33)
M. terrae	1/31 (3)
M. gordonae	0/229
M. scrofulaceum	0/22
M. nonchromogenicum	0/16
M. chelonae	0/14
M. flavescens	0/13
M. triviale	0/8
M. gastri	0/6
M. thermoresistibile	0/2
M. vaccae	0/2
Others*	0/6

* Three rapidly growing, two scotochromogenic, and one nonphotochromogenic species.

In the remaining cases, we found *M. xenopi* (four patients), *M. malmoense* (two patients), and *Mycobacterium simiae* (one patient).

Disseminated disease. Only one case showed generalized disease due to MAC.

Soft-tissue disease. Three of 10 cases of soft-tissue infection occurred in children with cervical lymphadenitis. These cases were caused exclusively by MAC. Two of these children had positive skin tests for *M. avium* (purified protein derivative test [PPD-A; Statens Serum Institute, Copenhagen]; skin testing for NTM antigens is not a common routine procedure in the Zurich area). In five of the 10 cases of soft-tissue infections, the following species were detected: *Mycobacterium terrae* (one, ulcer), *Mycobacterium fortuitum* (two, abscesses), MAC (four, cervical lymphadenitis), and *M. kansasii* (one, abscess). One case of swimming pool granuloma caused by *Mycobacterium marinum* was found in a young woman, and an additional case of a tenosynovitis due to *M. malmoense* was found in an elderly man. The number of cases of soft-tissue infections remained constant during the study period.

Medical Conditions

Table 5 shows associated medical conditions for patients infected with NTM isolates. There was no predominant underlying disease in the group of patients with mycobacteriosis compared with the group of patients with nonsignificant isolates (table 5). Clinically nonsignificant NTM isolates were found more frequently in patients with chronic lung diseases ($P < .01$) and especially in patients with a history of TB ($P < .001$) than were significant isolates.

Immunosuppression

Of 513 patients with NTM isolates, we identified 49 receiving immunosuppressive therapy, of whom only two had disease due to NTM. The causative species in a patient who had non-Hodgkin's lymphoma and who was receiving chemotherapy was MAC. The other patient who had chronic obstructive pulmonary disease (COPD) and who was receiv-

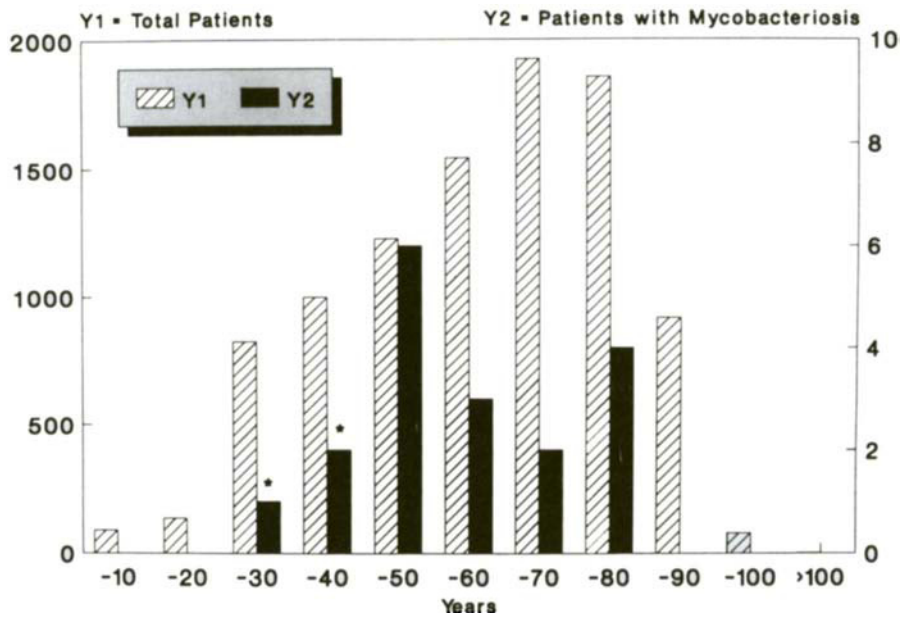

Figure 1. Age-dependent frequency of patients from whom respiratory tract specimens were submitted between 1986–1988 and of patients with pulmonary mycobacteriosis. (* = includes one HIV-positive patient).

ing steroids also had pulmonary mycobacteriosis due to *M. xenopi*.

Therapy

Antituberculous drug therapy was initiated in 22 (65%) of all cases caused by clinically relevant NTM and in 42 (9%) of all cases caused by nonsignificant isolates. In three of the 10 cases of soft-tissue infections, surgical treatment was combined with antituberculous drugs.

Interpretation of Results by Physicians

When evaluating a positive NTM culture and estimating the clinical relevance of this result according to the Yamamoto criteria [18], we observed an increasing conformity with the clinician's judgment over the study period. In 1983–1984, only 30% of patients with NTM isolates considered relevant by the responsible physician were actually significant; in 1985–1986, 53%; and in 1987–1988, 86%.

Specimens Examined for AFB

One hundred sixty-six specimens from 34 patients with mycobacteriosis were received (4.9 specimens per patient), whereas 1,911 specimens from 479 patients with nonsignificant NTM isolates were received (4.0 specimens per patient). The rate of positive cultures per submitted specimens was different for patients with mycobacteriosis and those with casual NTM isolates. Only one of 3.7 submitted specimens was positive among the patients with nonsignificant NTM isolates. Conversely, one of 1.9 submitted specimens was positive among patients with mycobacteriosis.

Microscopy

Four hundred forty-seven patients were found to have one or more positive smears from 1983 to 1988. In 424 patients (95%) TBC was the causative organism, in 15 patients (3%) NTM was found to be responsible for disease, and in eight patients (2%) a nonsignificant NTM isolate was identified. Fifteen (44%) of 34 patients with mycobacteriosis had smear-positive specimens. In the group of patients with nonsignificant isolates, only eight (2%) had a positive smear. Six of these eight patients were found to have chronic lung disease, including four patients with a history of tuberculosis, one with COPD, and one with bronchiectasis. One patient with previous and concurrent tuberculosis had a positive smear of a specimen from which *M. gordonae* was isolated. One of the remaining two patients was being treated for a prostate gland carcinoma, and one was found to have a Zenker's diverticulum. When analyzing the site of origin of specimens positive on microscopic evaluation, we found that all originated from the respiratory tract.

For 905 patients with pulmonary or extrapulmonary tuberculosis, one or more smears were performed. Smears for 424 (47%) of these patients showed AFB. Ninety percent of these microscopically positive specimens originated from the respiratory tract (1,160 of a total of 1,292 specimens).

Discussion

Among all mycobacteria, TBC remains the most important species that causes disease [14, 15, 19, 33]. As in other countries of the western world, a downward trend in the incidence of TBC disease had been observed in Switzerland over the past decades. In 1990 Rieder et al. [34] demonstrated in

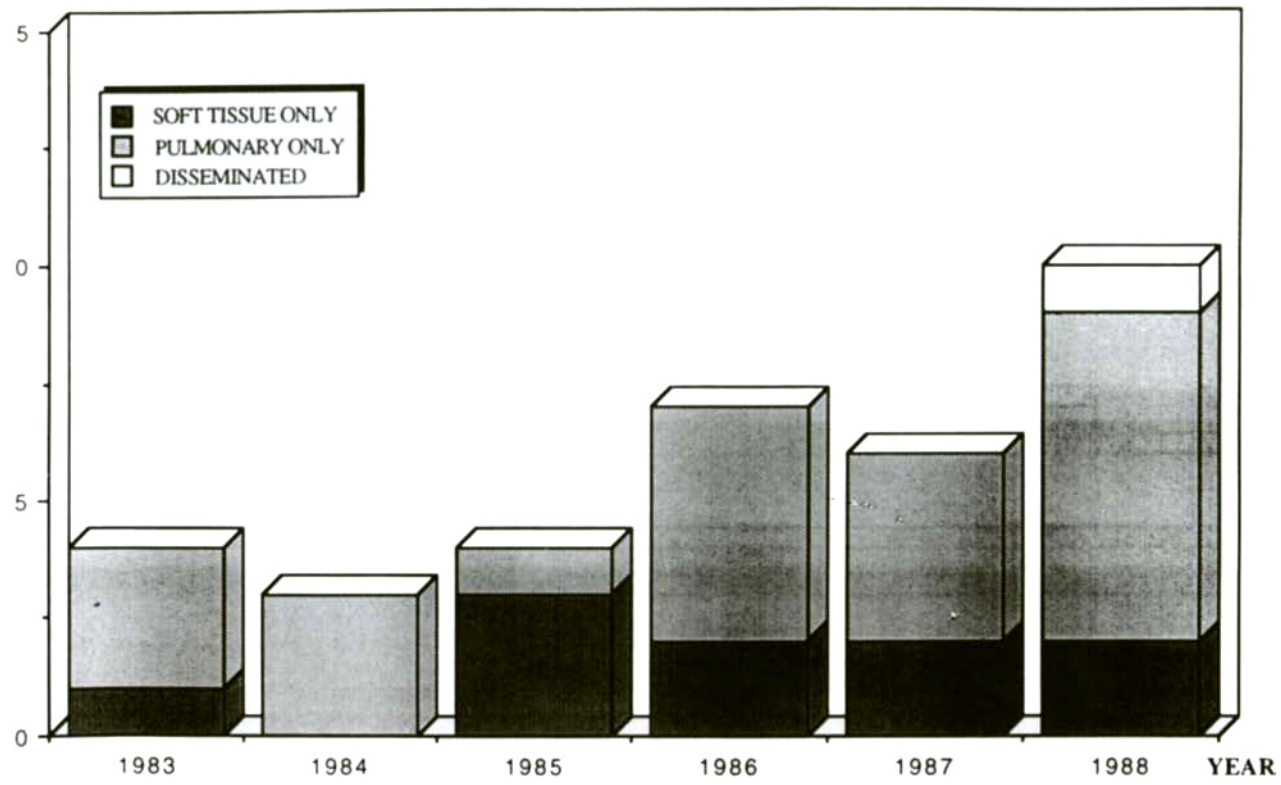

Figure 2. Sites of mycobacteriosis and patients recorded for each year from 1983 to 1988. Y axis = number of patients.

a Swiss survey a decline in the incidence of TBC disease from 108.1 cases per 100,000 inhabitants in 1945 to 17.4 cases per 100,000 inhabitants in 1988. A progressive increase in the number of NTM isolated at our laboratory was observed for approximately the last 10 years. In 1977 Nykänen [35] found that 2.2% of all cultures positive for AFB contained NTM; however, the technique at that time was not as good as it was in 1981. In 1981 NTM were found in 17% of all patients with mycobacterial isolates in the tuberculosis laboratory [36]. The present study shows an extension of this trend in terms of total NTM strains isolated as well as of clinically significant NTM isolates.

Geographic Differences

Geographic differences in the pattern of NTM species have been demonstrated in various studies. In 1969 Edwards et al. [37] found the highest reactivity to antigens prepared from *M. intracellulare* (PPD-B) and *M. scrofulaceum* (PPD-G) in the southeastern United States. Correspondingly, the frequency of recovery of isolates of these and the related species *M. avium* was also highest in that area [37]. Environmental studies [7] showed that these species are found in waters and soils of the Southeast rather than of the Northeast.

Comparing investigations from Europe, we found a different frequency in the isolation of species. In an extensive study over 22 years (1965–1986) from the eastern part of Germany [38], *M. intracellulare* was the species most frequently isolated from humans in the group of potentially pathogenic NTM. It was followed in order of decreasing frequency by *M. kansasii*, *M. xenopi*, *M. fortuitum*, *M. avium*, *Mycobacterium chelonae*, and *M. scrofulaceum*. *M. marinum* was isolated only from animals during the study period. In the group of NTM rarely considered as pathogens, *M. terrae* was found most frequently, followed by a group of scotochromogenic species (e.g., *M. gordonae*).

A bacteriologic survey from Sweden [39] between 1973 and 1981 showed MAC to be the species isolated most frequently. *M. xenopi* was found to be second, followed in order of decreasing frequency by *M. kansasii*, *M. marinum*, *Mycobacterium szulgai*, and a group of rapid growers. In another Swedish study [40] non-BCG-vaccinated schoolchildren underwent skin tests. For ~90% of the children, the sensitin reaction to *M. scrofulaceum* or to *M. avium* was larger than or equal to the tuberculin reaction. An environmental study from Wales (United Kingdom) disclosed an increase in the incidence of mycobacteriosis due to *M. kansasii* from 1953 to 1970; during the first 6 years of the study period there were 25 cases, the second 38 cases, and the third 52 cases [41]. The natural source of this microorganism, the most frequently isolated NTM species in this area, was not found. Other NTM species found less often than *M. kansasii* were *M. intracellulare* and *M. avium*.

Table 5. Medical conditions associated with isolation of nontuberculous mycobacteria.

Medical condition	No. (%) of isolates		
	Clinically significant (n = 34)	Clinically nonsignificant (n = 479)	Total (n = 513)
Chronic lung diseases	15 (44)	241 (50)*	256
Chronic obstructive pulmonary disease (COPD)	12 (35)	143 (30)	155
Bronchiectasis	2 (6)	19 (4)	21
History of tuberculosis	1 (3)	126 (26)†	127
Concurrent tuberculosis	2 (6)	19 (4)	21
Others	3 (9)	25 (5)	28
Malignancy	3 (9)	76 (16)	79
Radiotherapy	1 (3)	8 (2)	9
Chemotherapy	1 (3)	12 (3)	13
Surgery	0	21 (4)	21
None	1 (3)	5 (1)	6
Missing data	1 (3)	9 (2)	10
Tuberculin skin test			
Positive	8 (23)	64 (13)	72
Negative	5 (15)	47 (10)	52
Diabetes mellitus	5 (15)	36 (8)	41
Alcohol abuse	6 (18)	62 (13)	68
Immunosuppressive therapy	3 (9)	46 (10)	49
Connective tissue disease	2 (6)	15 (3)	17

* $P < .01$.
† $P < .001$

A study from Ontario, Canada [42] showed an increasing number of cases of mycobacteriosis due to *M. xenopi* from 1975 to 1985, now the second most commonly isolated NTM after MAC. In a report from northern India [43], 8% of patients with mycobacterial species harbored NTM isolates, mostly *M. fortuitum* (endometrial biopsy, five; sputum, three; CSF, two; bronchial washing, skin biopsy, and lymph node aspirate, one each). A similar result was found in a study from Jeddah, Saudi Arabia [44]: of the NTM isolates from 9% of patients, more than half belonged to the rapidly growing mycobacteria *M. fortuitum* (pulmonary specimen, nine; urogenital, 10; miliary, one; soft-tissue, one), *M. chelonae* (pulmonary specimen, four; urogenital, two; CSF, one), and *Mycobacterium smegmatis* (pulmonary specimen, three; urogenital, one). In the last two studies sputum specimens were decontaminated by the *N*-acetyl-L-cysteine/sodium hydroxide method.

Isolation of NTM from Healthy Persons

In the 1950s increasingly frequent reports of NTM associated with pulmonary disease [17] and in asymptomatic patients [45] prompted a study by Edwards and Palmer [46] to check for NTM in 122 apparently healthy persons of a small rural community in southwestern Georgia. Some of the specimens appeared to be sputum, but many of them were just saliva. After decontamination the sediment was inoculated onto one slant of an egg-based medium. Growth was detected in cultures of 30 specimens: *Nocardia* species from 12, *Streptomyces* species from two, scotochromogenic NTM from four, nonphotochromogenic NTM from eight (seven of them MAC), and rapid growers from five (three of them *M. fortuitum*). There were two mixed cultures, and one culture was lost. Chest roentgenograms for 29 persons with positive cultures were unremarkable. Edwards and Palmer concluded that the gastrointestinal tract may have been a frequent portal of entry for natural infection with some of the NTM.

In the early 1970s, the ratio of NTM to *M. tuberculosis* isolated in the mycobacteriology laboratory of the Hospital of the University of Pennsylvania was 3:2. A study [47] was therefore undertaken for determining if the oral cavity contributed to these large numbers of clinically insignificant NTM in sputum during collection. Each patient first used 20 mL of Todd-Hewitt broth as a mouth rinse without gargling and expectorated the fluid. Then the patient produced induced sputum by repeatedly inhaling a warmed aqueous solution consisting of 10% NaCl and 15% propylene glycol. From 19 (17%) of 113 patients attending the outpatient clinic, 32 NTM isolates were obtained. Examination of the data indicated that 24 of 32 organisms were isolated from cultures of either mouthwash or sputum. The following numbers of species were detected in the indicated numbers of

patients: MAC, 9 (9 patients); *Mycobacterium phlei*, 8 (3); *M. scrofulaceum*, 6 (5); *M. kansasii*, 5 (3); *M. smegmatis*, 3 (1); and *M. terrae*, 1 (1). Since two-thirds of these NTM were not found in paired specimens but were distributed about equally in mouthwashes and sputa, the author concluded that their presence was random and their acquisition may have been by ingestion.

Almost 15 years later, an editorial written about the high proportion of MAC infections in patients with AIDS by Collins [48] prompted another important study. Portaels and co-workers [49] examined the mycobacterial flora of the stools of 50 healthy European volunteers. After decontamination the pellet was inoculated onto four slants of Ogawa egg yolk medium and incubated at 30°C for 6 months. Cultures of 26 specimens were positive for NTM. The number of colonies varied between one and three per slant. Cultures of six specimens failed to yield growth in subcultures. From the 20 remaining positive specimens, 26 different strains were detected: *M. simiae* (14), MAC (5), *M. gordonae* (5), and *M. malmoense* (2). Six cultures revealed two different species. The finding of *M. simiae* and *M. malmoense*, both recognized as causes of human diseases, was particularly noteworthy.

Diagnostic Criteria for Mycobacteriosis

In the absence of specific diagnostic features, isolation of NTM in culture is essential for diagnosis. However, as these organisms are commonly found in nature, a single positive sputum culture is not sufficient for diagnosis of mycobacteriosis.

In 1990 the American Thoracic Society put forth the first official statement on diagnostic and therapeutic standards for pulmonary disease due to NTM [5]. There are different criteria for patients with cavitary lung disease, for patients with noncavitary lung disease, and for patients with cavitary or noncavitary lung disease for whom the sputum evaluation is "nondiagnostic or [for whom] another disease cannot be excluded." Additionally, failure to clear the organism after a course of bronchial hygiene supports the diagnosis of pulmonary mycobacteriosis. An investigation of 492 patients with pulmonary disease caused by *M. kansasii* and *M. intracellulare* by Ahn et al. in 1982 [50] demonstrated that criteria based on the presence or absence of cavitation and on the duration of sputum positivity after initiation of therapy enabled a more accurate distinction of colonization from invasive disease.

A localized soft-tissue lesion is a relatively uncommon form of mycobacteriosis. Cases of mycobacteriosis involving skin, soft tissue, tendon sheaths, joints, and bones have been reported previously [13]. The role of NTM in granulomatous synovitis was described by Sutker et al. [51] in 1979. To evaluate the significance of NTM in this localization, the authors used the following features: (1) general clinical features were reasonably characteristic; (2) many of the lesions were closed lesions, i.e., not open to surface contamination; and (3) many of the tissue sections showed granulomas containing AFB. In their study, NTM of the Runyon groups I, II, and III were very rarely isolated from surface lesions as "contaminants."

Attack Rate

There are no data published about attack rates in cases of pulmonary mycobacteriosis. In our study we found the highest rate of 0.49% in the group of patients 41–50 years old.

Incidence of Mycobacteriosis

In a comparison of studies from different countries on the incidence of mycobacteriosis and the species involved, a high degree of conformity can be found. In a survey from the Centers for Disease Control (CDC) in the United States (Atlanta, GA) [11, 52] from 1981 to 1983, the estimated prevalence of mycobacteriosis in the United States was 1.8 per 100,000 population. A study in Japan [53] from 1981 to 1984 showed an incidence of pulmonary mycobacteriosis of 1.73 cases per 100,000 population. In 1988 we observed 0.9 cases of mycobacteriosis per 100,000 population in the canton of Zurich. Considering the fact that we excluded all HIV-positive patients, these data are remarkably similar, even though different diagnostic criteria for defining mycobacteriosis were used in these three studies.

Despite certain geographic differences [52–55], MAC and *M. kansasii* represent the two clinically most important species of NTM. In the study from the CDC [52], 62% of the cases of mycobacteriosis were associated with MAC and 24% with *M. kansasii*. In Japan from 1983 to 1984, MAC was identified in ~70% of the patients with mycobacteriosis and *M. kansasii* in 25%. We found MAC in 13 (38%) of 34 patients and *M. kansasii* in nine patients (26%) to be responsible for disease. *M. gordonae*, the most frequently isolated species, was never shown to be responsible for disease. This species has been found in tap water in different parts of the world [56, 57]. We assume that transient colonization of a host is frequent, and therefore the overall chance of isolation is very high. Different NTM species have been observed as colonizing water systems, as described by Lockwood et al. [58] who registered an outbreak of *M. terrae* in clinical specimens associated with a hospital's potable water supply in 1986. The concentration of potentially pathogenic NTM (like MAC) in hospital water supplies is of interest as a possible source of infection for immunocompromised patients. Corresponding environmental studies have been provided by Du Moulin and co-workers in 1988 [59].

Obtaining specimens from the respiratory tract by fiberoptic bronchoscopy is a common procedure. A well-known problem is the possibility of cross-contamination of specimens by the bronchoscope, since in the process of steriliza-

tion of this instrument several possible sources of contamination may be involved. Therefore, it is not surprising that several reports on mycobacterial cross-contamination by NTM have been described [60–64].

Site of Mycobacteriosis

Lung disease. In our study, mycobacteriosis was localized exclusively in the respiratory tract of two-thirds of the patients. There were more cases of lung mycobacteriosis during the second half of the study period. During the first half of the study period, two of seven cases were smear negative vs. seven of 16 in the second half. Although we had changed our decontamination procedure, we do not believe that this change was responsible for the slight increase. The majority of the cases were smear positive and most probably occurred independently of the decontamination procedure.

In this group, *M. kansasii* was found to be responsible for eight of 23 cases. As described by Raleigh in 1988 [65], pulmonary localization is the most common manifestation of disease due to this species, even though various other clinical presentations have been reported [65, 66]. Clinical features and course of pulmonary mycobacteriosis due to *M. kansasii* were investigated in 1969 by Johanson and Nicholson [67]. This study demonstrated that patients with previously healthy lungs responded remarkably well to treatment, even though the extent of mycobacteriosis did not differ from that in patients with underlying pulmonary disease. A similar experience was reported by Lillo et al. in 1990 [68] who investigated 47 cases of pulmonary and eight of disseminated *M. kansasii* disease. For 5 years a systematic review was conducted of clinical, microbiological, and radiographic data on *M. kansasii* disease assembled from the records of 72 patients [69]. Twenty-three patients (32%) were found to be coinfected with HIV. This dual infection had increased from 0 to 58% over the study period. Twenty-two of the 23 dually infected patients had *M. kansasii* isolated from the respiratory tract. With use of criteria as defined by Ahn et al. [50], only five patients had persistently positive sputum or bronchial washings (suggesting mycobacteriosis) without colonization of the respiratory tract. An increase in the incidence of lung disease due to *M. kansasii* has been reported from different countries of the world [41, 70, 71]. During our study period, such an increase in incidence of mycobacteriosis due to *M. kansasii* was not observed.

MAC was found as the causative agent in eight of our patients with pulmonary mycobacteriosis. Seven of these patients were considered immunocompetent, and one patient was receiving immunosuppressive therapy for the treatment of non-Hodgkin's lymphoma. Increasing numbers of pulmonary disease due to MAC in immunocompetent patients have been reported [39, 72]. Predisposing medical conditions observed in these patients were chronic pulmonary diseases such as COPD, bronchiectasis, previous tuberculosis, pneumoconiosis, or bronchogenic carcinoma [13].

Four patients in our study were found to have pulmonary mycobacteriosis due to *M. xenopi*. Three of them were men, and the ages ranged from 54 to 74 years. *M. xenopi* has been detected with different frequencies in different geographic areas. The first reports came mainly from Europe. In coastal regions of England, the isolation of *M. xenopi* was reported frequently, with many of these isolates interpreted as clinically significant [73]. A detailed bacteriologic study of this species, including case reports from Denmark, was published by Engbaek et al. in 1967 [74]. Colonization of water systems by *M. xenopi* was soon recognized as a possible source of contamination or even infection [75]. Smith and Citron [76], at Brompton Hospital in London, isolated *M. xenopi* from the sputum of 23 patients in a 6-year period; these isolates represented 56% of all NTM isolated at Brompton Hospital. In 15 of these patients, pulmonary disease due to this organism was suspected. Banks et al. [77] reviewed treatment and response in patients with pulmonary mycobacteriosis due to *M. xenopi* in 1984. Of the 47 patients, 39 (83%) were men, and the mean age of the group was 61.5 years (range, 36–84 years). Preexisting lung disease was present in 35 cases (74%), of which healed TBC disease accounted for 14, chronic bronchitis and emphysema for 12, and bronchiectasis for three; whereas three other patients had interstitial pulmonary fibrosis. In the Canadian province of Ontario, *M. xenopi* is a relatively common NTM isolate, and numerous cases of disease due to this species have been observed [42, 78, 79]. Until now, reports of *M. xenopi* cases from the United States have remained rather rare [52]. One of our patients affected by *M. xenopi* was receiving immunosuppressive therapy. A comparable case of pulmonary disease due to this species in a renal allograft recipient receiving immunosuppressive therapy was reported by Weber et al. in 1989 [80].

Two of our patients had evidence of pulmonary mycobacteriosis caused by *M. malmoense*. Both patients did not have pulmonary symptoms, and cavitary lesions were found by chance. *M. malmoense* was described and named by Schröder and Juhlin in 1977 [81]. This species was observed predominantly in northern Europe. Over the last years, an increasing number of isolates were reported from England [82–84], Scotland [85], and the Scandinavian countries [86]. Pulmonary mycobacteriosis represents the main manifestation of *M. malmoense* infection and is similar to pulmonary tuberculosis. Cavitary lesions are observed frequently [85].

There was one patient in our series who had pulmonary disease due to *M. simiae*. Results of biochemical tests were most likely compatible with *M. scrofulaceum*, which was confirmed by the reference laboratory in Borstel, Germany. The isolate was considered not to be clinically significant at that time. Retrospectively, it seems possible that this strain isolated in 1985 was *M. simiae*. Confusion of *M. simiae* with

MAC or with *M. scrofulaceum* may occur [87] because of similar biochemical properties as well as colonial morphology. In 1975 the first case of human mycobacteriosis due to *M. simiae* was reported by Krasnow and Gross [88]. The patient was an elderly woman with chronic cavitary lung disease and numerous positive sputum cultures. In the meantime, additional cases of pulmonary [89, 90] and even disseminated [90] disease due to *M. simiae* have been described. In 1989 Krümmel et al. [91] reported the first three pulmonary manifestations of disease due to this species observed in Europe. The source of this rarely isolated organism remains unclear.

Disseminated mycobacteriosis. In only one case of an 82-year-old man with pulmonary disease due to MAC was the same species isolated from both urine and sputum. This generalized form of disease is very rare in the immunocompetent host [13, 92]. In 1985 Horsburgh et al. [93] reported 13 cases with disseminated MAC infection.

Cervical lymphadenitis. MAC was also the species responsible for three of our cases of cervical lymphadenitis in children and in one case of a 59-year-old woman. First reports of NTM associated with this localization date back to the 1950s. In 1956 Prissick and Masson [94] found a scotochromogenic NTM species in 25 children with cervical lymphadenitis and proposed the name *M. scrofulaceum.* As reviewed by Lincoln and Gilbert [95], NTM was responsible for superficial lymphadenitis in children in an increasing number of cases. In 1963 a study from the Cleveland area [96] showed *M. scrofulaceum* to be the responsible agent in 35 of 43 children with cervical adenitis. In the remaining patients, MAC was found in six and *M. kansasii* and *M. fortuitum* in one each. Recent reports showed a preponderance of MAC in this localization [97–100]. Possible geographic differences or a changing pattern of infective agents has been proposed as explanations for these findings [98]. In contrast, in adults with cervical lymphadenitis TBC is still the most important causative agent, as reported by Huhti et al. in 1975 [101] and Levin-Epstein and Lucente in 1982 [102].

Skin and soft-tissue infections. Street et al. [103] reviewed the records of patients with NTM infections of the skin who were seen between 1963–1988 at the Mayo Clinic in Rochester, Minnesota. Only culture-positive cases were included. Fourteen patients were identified, and the following species were found: *M. marinum* (seven patients), MAC (three), *M. kansasii, M. chelonae, M. fortuitum,* and *Mycobacterium ulcerans* (one each). *M. ulcerans* infection occurred in a 5-year-old boy from Nigeria.

In our series, *M. marinum* was isolated from a swimming pool granuloma localized at the elbow of a 26-year-old woman. The patient was known to visit public indoor swimming pools frequently. *M. marinum* is the NTM species most commonly responsible for this kind of soft-tissue infection, which is also known by the name of "fish tank granuloma" [13, 104]. An outbreak of 80 cases of granulomatous skin lesions acquired in a swimming pool in Sweden was described by Linell and Nordén in 1954 [105]. The lesions were observed mostly on the elbows. The causative NTM species isolated from the soft-tissue lesion of these patients was named "*Mycobacterium balnei,*" later shown to be synonymous with *M. marinum.* A similar outbreak affecting more than 290 people in Colorado was reported by Mollohan and Romer in 1961 [106]. These people acquired the infection when they swam in a public pool of warm mineral water contaminated by *M. marinum.*

A 74-year-old man of our series developed tenosynovitis of the wrist months after decompression of the median nerve in the carpal tunnel. Reoperation was necessary. Macroscopically, a shaggy tenosynovitis with numerous "rice bodies" was found. The surgeon, who was experienced with the clinical appearance of tuberculous tenosynovitis, suspected mycobacterial infection. Tissue samples yielded a nonphotochromogenic NTM, *M. malmoense.* This species was first described in 1977 [81]. However, similar strains had already been isolated in 1954 [107]. The patterns observed on liquid chromatography were distinctive and supported recognition of the group as an entity named "provisional new species 2." In 1976 the original strains from Malmö, Sweden, and from Wales were compared and turned out to be identical [84]. In 1988 Prince et al. [108] published the first case of a *M. malmoense* infection of the hand that presented as carpal tunnel syndrome. There are a number of similarities between this case and ours. Both patients were in an older age group. Localization and clinical presentation were similar. NTM infections of the hand have been reported previously [39, 97, 109]. The species responsible for most of these cases was *M. marinum* [8, 110]. Less frequently found were *M. kansasii* [111], MAC [112], *M. terrae* [113], *M. fortuitum* [114], *M. chelonae* [115], and *M. szulgai* [116].

We observed cutaneous disease due to *M. fortuitum* in two patients. One of them was a 45-year-old woman who had a history of repeated injections for the treatment of rheumatic disease. She developed an abscess at the site of these injections and was treated by surgical debridement. The second case was seen in a 41-year-old man in whom the route of infection was not known. It is likely that he presented with a community-acquired form of disease. *M. fortuitum* belongs to the rapidly growing mycobacteria, a group that is most commonly involved in soft-tissue infections [13, 25]. Infections due to both *M. fortuitum* and *M. chelonae* (*M. fortuitum* complex) are generally associated with a penetrating injury of the skin [117]. Soft-tissue and wound infections are the common clinical manifestation of disease due to the *M. fortuitum* complex [118]. Reports of pulmonary mycobacteriosis due to *M. fortuitum* demonstrate that this form of disease does exist [119, 120].

In our series, *M. terrae* was detected both microscopically and in culture of specimens of a superficial, granulomatous, partly ulcerating lesion on the forearm of a 50-year-old man.

Only few cases of disease due to *M. terrae* have been reported (septic arthritis and synovitis [121, 122]).

From the last of our 10 patients with soft-tissue diseases, *M. kansasii* was cultured from pus. The 78-year-old woman suffered from an abscess in the psoas region and had no history of trauma. The patient was immunocompetent. Cutaneous lesions have been seen occasionally in this disease [103, 123, 124].

Other Mycobacterial Species with a Pathogenic Potential

M. szulgai. *M. szulgai* was first recognized in 1972 [125] and superficially resembled *M. gordonae.* The distinct characteristic of this species is pigment production: when grown at 37°C, the organism is scotochromogenic; when grown at room temperature, the colonies are photochromogenic. Maloney et al. [126] published three cases of infection with *M. szulgai* and reviewed 24 previous cases reported before 1987. Most of the cases were of chronic pulmonary disease with chest roentgenograms commonly showing cavitary lesions. Other presentations included olecranon bursitis, tenosynovitis [116], other soft-tissue infections, and localized cutaneous disease [126].

M. ulcerans. This organism was first isolated in 1948 from a child who had chronic skin ulcers in Australia [127]. Most cases have been seen in Africa, Malaysia, and other countries with tropical or temperate climates. *M. ulcerans* grows very slowly even at its optimal temperature of 30°C–33°C. Up to 12 weeks may be required for colonies to develop, which tend to resemble those of tubercle bacilli. This is also the only species in the genus for which production of a soluble toxin has been proved [128]. Recently, Hayman and McQueen [129] reviewed the pathology of cutaneous ulcers resulting from *M. ulcerans.*

Mycobacterium haemophilum. This organism grows optimally at temperatures <37°C and is unique in requiring hemoglobin or hemin for growth. *M. haemophilum* was first recovered in 1978 in Israel from the subcutaneous lesion of a patient with Hodgkin's disease [130]. Later, cases were reported of patients receiving immunosuppressive therapy for kidney allografts [131] or patients with AIDS suffering from tenosynovitis [132]; however, there are also reports of *M. haemophilum* causing cervical lymphadenitis in otherwise healthy children [133, 134].

Mycobacterium asiaticum. In a routine diagnostic laboratory, *M. asiaticum* may be misidentified as *M. gordonae.* There is only one easily determined attribute: *M. gordonae* is scotochromogenic and *M. asiaticum* is photochromogenic. *M. asiaticum* was first described by Weiszfeiler et al. in 1971 [135]. So far, only a few cases of pulmonary mycobacteriosis due to this species have been reported [136].

M. smegmatis. *M. smegmatis* is traditionally regarded as a frequent contaminant of the lower end of the urethra, but in fact it is more commonly cited in medical textbooks than it is isolated from clinical specimens. Collins et al. [137] encountered it less than a dozen times among some 5,000 NTM isolates examined in 10 years. In 1986 Vonmoos et al. [138] reported the first case of pleuropulmonary disease caused by *M. smegmatis.* Wallace and co-workers [139] identified 22 additional human isolates of *M. smegmatis* from Australia and the southern United States: 19 were from skin or soft-tissue infections, and none were from urine or the male genital tract.

Mycobacterium shimoidei. The original description of this species was published in 1975 by Tsukamura et al [140]. The acid phosphatase distinguishes *M. shimoidei* (positive) from the closely related *M. malmoense* (negative). Clinically significant isolates from the respiratory tract were reported from Japan [140] and from Germany [141].

Mycobacterium genavense. Recently, Hirschel et al. [142] reported a case of a patient who was seropositive for HIV and whose disease clinically resembled infection with MAC. Numerous acid-fast rods were found in nearly all tissues examined, but the cultures remained negative. In a subsequent publication from the same group, a second similar case was identified [143]. Ribosomal RNA (rRNA) sequences of isolates extracted from infected tissues of patient 1 contained conserved sequences common to all mycobacteria. While other parts of the sequences differed from those of all known mycobacteria, they were identical to the rRNA sequences of an isolate from a second specimen from patient 2. For this previously unknown species they proposed the name *M. genavense* [143]. Additionally, Nadal et al. [144] reported three cases in pediatric patients with AIDS.

Species of Mycobacteria Generally Considered To Be Nonpathogenic

A few species, such as *M. gordonae, Mycobacterium nonchromogenicum, Mycobacterium triviale, Mycobacterium gastri,* and almost all rapidly growing mycobacteria (*M. fortuitum* and *M. chelonae* excluded) are rarely associated with human disease [13, 25, 145].

Medical Conditions

In our study we explored predisposing medical conditions similar to those observed in former studies on NTM. When comparing patients who were infected with nonsignificant isolates with those affected by mycobacteriosis, we found similar occurrences of underlying diseases (table 5). A possible explanation is that these predisposing factors also facilitate the colonization of a patient. More than one-fourth of the patients infected with nonsignificant NTM isolates were found to have a history of tuberculosis. Possible explanations for this observation may be the colonization of preformed cavities by NTM or the mere fact that former TB patients are screened for AFB more frequently [146]. The chance of iso-

lating an ubiquitous organism is therefore much higher. In contrast with TB patients, for whom the young age group was found to be predominantly foreign (non-Swiss) and the older age group of Swiss nationality [34], no preferential ethnic group could be determined in the group with NTM. This finding can be explained by the different mode and sources of infection in TB and disease due to NTM, respectively. Transmission of TB is facilitated by crowded housing. In contrast, person-to-person transmission of NTM disease through aerosols may not occur and, therefore, no ethnic stratification can be found.

Specimens

The examination of sputum for AFB remains the diagnostic procedure most frequently requested by the physician. When analyzing the ratio of the number of positive culture results to the number of submitted specimens, we found a striking difference between the ratio of clinically relevant NTM isolates (1 positive culture per 1.9 submitted specimens) and the ratio of nonsignificant isolates (1 per 3.7). This finding confirmed the value of the practice of obtaining at least three specimens for the diagnosis of NTM disease. Large numbers of submitted specimens increase the chance of finding nonsignificant isolates. This fact should always be considered when positive culture results are being interpreted [46, 47, 49, 147].

It is important to emphasize that 44% of all clinically significant isolates of NTM were positive for AFB in the direct smear. This finding contrasts sharply with that for the group of nonsignificant isolates where only 2% were smear positive. It seems likely that these positive microscopic findings with no clinical significance were caused by colonization of the respiratory tract of these patients. All of the patients had severe medical problems; six of eight had chronic lung disease, which is known to be a predisposing factor for colonization.

In the immunocompetent host, the respiratory tract specimens had the highest diagnostic yield. This finding can be explained by differences of mode and site of disease. As was shown before, the major site of NTM infection in immunocompetent hosts was the respiratory tract. In contrast, patients who have both AIDS and mycobacteriosis have mostly disseminated disease, the route of infection possibly being oral, as postulated by O'Brien [11].

We conclude that the direct smear, which is one of the first clinical procedures performed with submitted specimens, provides important evidence for diagnosis of AFB-related diseases. TBC was found to be responsible for 424 of 447direct smears positive for AFB. Fifteen smears positive for AFB resulted from clinically significant NTM, and only eight were caused by nonsignificant NTM.

Diagnostic and Prognostic Value of an NTM Isolate

When evaluating the diagnostic value and therefore the clinical relevance of a NTM isolate, we referred to the Yamamoto criteria, which were established in 1967 [18]. Because these criteria are not specific for a given NTM species, they cannot be applied unconditionally. Basically, Yamamoto's evaluation combines clinical and microbiological aspects that support the clinical diagnosis of disease due to NTM.

For patients with extrapulmonary mycobacteriosis, the Yamamoto criteria can be used with certain modifications. We recommend that the following conditions be added to the minor criteria: (1) two or more positive direct smears of a specimen considered not to have come from a normally sterile area and resulting in a positive culture with moderate to heavy growth; and (2) isolation of the same NTM species from two or more specimens normally considered sterile (e.g., blood, bone marrow, tissue, CSF). The identification of mycobacteria in the routine laboratory has become more efficient and faster [148, 149]. While the number of TB cases is decreasing, the number of NTM isolates is increasing. These developments underscore the importance of proper interpretation of positive results of culture.

References

1. Wayne LG, Kubica GP. The mycobacteria. In: Sneath PHA, Mair NS, Sharpe ME, Holt JG, eds. Bergey's manual of systematic bacteriology. Vol 2. Baltimore: Williams & Wilkins, **1986**:1435–57.
2. Wayne LG. The "atypical" mycobacteria: recognition and disease association. Crit Rev Microbiol **1985**;12:185–222.
3. Collins CH, Yates MD, Grange JM. Names for mycobacteria. BMJ **1984**;288:463–4.
4. Grange JM. Nomenclature of mycobacterial disease [letter]. Am Rev Respir Dis **1989**;140:561.
5. Wallace RJ Jr, O'Brien R, Glassroth J, Raleigh J, Dutt A. Diagnosis and treatment of disease caused by nontuberculous mycobacteria. Am Rev Respir Dis **1990**;142:940–53.
6. Runyon EH. Whence mycobacteria and mycobacteriosis? [editorial]. Ann Intern Med **1971**;75:467–8.
7. Falkinham JO III, Parker BC, Gruft H. Epidemiology of infection by nontuberculous mycobacteria. I. Geographic distribution in the eastern United States. Am Rev Respir Dis **1980**;121:931–7.
8. Lacy JN, Viegas SF, Calhoun J, Mader JT. *Mycobacterium marinum* flexor tenosynovitis. Clin Orthop **1989**;238:288–93.
9. Feld R, Bodey GP, Gröschel D. Mycobacteriosis in patients with malignant disease. Arch Intern Med **1976**;136:67–70.
10. Wolinsky E, Rynearson TK. Mycobacteria in soil and their relation to disease-associated strains. Am Rev Respir Dis **1968**;97:1032–7.
11. O'Brien RJ. The epidemiology of nontuberculous mycobacterial disease. Clin Chest Med **1989**;10:407–18.
12. Alvarez E, Tavel E. Recherches sur le bacille de Lustgarten. Arch Physiol Norm Pathol **1885**;3:303–21.
13. Wolinsky E. Nontuberculous mycobacteria and associated diseases. Am Rev Respir Dis **1979**;119:107–59.
14. Kochi A. The global tuberculosis situation and the new control strategy of the World Health Organization. Tubercle **1991**;72:1–6.
15. Good RC. Isolation of nontuberculous mycobacteria in the United States, 1979. J Infect Dis **1980**;142:779–83.
16. Timpe A, Runyon EH. The relationship of "atypical" acid-fast bacte-

ria to human disease—a preliminary report. J Lab Clin Med 1954;44:202–9.

17. Runyon EH. Anonymous mycobacteria in pulmonary disease. Med Clin North Am 1959;43:273–90.
18. Yamamoto M, Ogura Y, Sudo K, Hibino S. Diagnostic criteria for disease caused by atypical mycobacteria. Am Rev Respir Dis 1967;96:773–8.
19. Rieder HL, Cauthen GM, Kelly GD, Bloch AB, Snider DE Jr. Tuberculosis in the United States. JAMA 1989;262:385–9.
20. Grosset J, Boisvert H, Truffot-Pernot C, Rica C. Organization of laboratory services in low prevalence countries, our personal view. Bull Int Union Tuberc Lung Dis 1990;65:60–3.
21. Sathe SS, Reichman LB. Mycobacterial disease in patients infected with the human immunodeficiency virus. Clin Chest Med 1989;10:445–63.
22. Snider DE Jr, Hopewell PC, Mills J, Reichman LB. Mycobacterioses and the acquired immunodeficiency syndrome. Am Rev Respir Dis 1987;136:492–6.
23. Modilevsky T, Sattler FR, Barnes PF. Mycobacterial disease in patients with human immunodeficiency virus infection. Arch Intern Med 1989;149:2201–5.
24. Portaels F. Le SIDA et les mycobactéries atypiques. Ann Soc Belg Med Trop 1987;67:93–116.
25. Woods GL, Washington JA II. Mycobacteria other than *Mycobacterium tuberculosis:* review of microbiologic and clinical aspects. Rev Infect Dis 1987;9:275–94.
26. Davidson PT. The diagnosis and management of disease caused by *M. avium* complex, *M. kansasii*, and other mycobacteria. Clin Chest Med 1989;10:431–43.
27. Runyon EH, Karlson AG, Kubica GP, Wayne LG. Mycobacterium. In: Lennette EH, Balows A, Hausler WJ Jr, Truant JP, eds. Manual of clinical microbiology. 3rd ed. Washington, DC: American Society for Microbiology, 1980;150–79.
28. Sommers HM, Good RC. Mycobacterium. In: Lennette EH, Ballows A, Hausler WJ Jr, Shadomy HJ, eds. Manual of clinical microbiology. 4th ed. Washington, DC: American Society for Microbiology, 1985:216–48.
29. Vestal AL. Procedures for the isolation and identification of mycobacteria. Publication no. (CDC) 76-8230. Atlanta: Department of Health, Education and Welfare, 1975.
30. Salfinger M, Kafader FM. Comparison of two pretreatment methods for the detection of mycobacteria of BACTEC and Lowenstein-Jensen slants. J Microbiol Methods 1987;6:315–21.
31. Gruft H. Isolation of acid-fast bacilli from contaminated specimens. Health Lab Sci 1971;8:79–82.
32. Salfinger M, Kafader FM. Mycobacteriaceae. In: Burkhardt F, ed. Mikrobiologische Diagnostik. Stuttgart, Germany: Thieme Verlag, 1992:269–89.
33. Rieder HL, Cauthen GM, Comstock GW, Snider DE Jr. Epidemiology of tuberculosis in the United States. Epidemiol Rev 1989;11:79–98.
34. Rieder HL, Zimmermann H, Zwahlen M, Billo NE. Epidemiologie der Tuberkulose in der Schweiz. Schweiz Rundsch Med Prax 1990;79:675–9.
35. Nykänen H. 13 Fälle atypischer Mykobakteriosen im Jahre 1977 im Kanton Zürich [dissertation]. Zürich: University of Zürich, 1981:29.
36. Salfinger M. Atypische Mykobakterien (MOTT): Häufigkeit und Bedeutung. Schweiz Med Wochenschr 1983;113:100–1.
37. Edwards LB, Acquaviva FA, Livesay VT, Cross FW, Palmer CE. An atlas of sensitivity to tuberculin, PPD-B and histoplasmin in the United States. Am Rev Respir Dis 1969;99(no 4, part 2):1–132.
38. Käppler W, Kalich R. Ergebnisse der Identifizierung von Mykobakterien während 22 Jahren. Z Erkr Atmungsorgane 1989;173:23–30.
39. Wickman K. Clinical significance of nontuberculous mycobacteria: a bacteriological survey of Swedish strains isolated between 1973 and 1981. Scand J Infect Dis 1986;18:337–45.
40. Lind A, Larsson LO, Bentzon MW, et al. Sensitivity to sensitins and tuberculin in Swedish children. I. A study of schoolchildren in an urban area. Tubercle 1991;72:29–36.
41. Paull A. An environmental study of the opportunist mycobacteria. Med Lab Tech 1973;30:11–9.
42. Thomas P, Liu F, Weiser W. Characteristics of *Mycobacterium xenopi* disease. Bull Int Union Tuberc Lung Dis 1988;63:12–3.
43. Chakrabarti A, Sharma M, Dubey ML. Isolation rates of different mycobacterial species from Chandigarh (north India). Indian J Med Res 1990;91:111–4.
44. Zaman R. Tuberculosis in Saudi Arabia: epidemiology and incidence of *Mycobacterium tuberculosis* and other mycobacterial species. Tubercle 1991;72:43–9.
45. Edwards LB, Palmer CE. Epidemiologic studies of tuberculin sensitivity. I. Preliminary results with purified protein derivatives prepared from atypical acid-fast organisms. Am J Hyg 1958;68:213–31.
46. Edwards LB, Palmer CE. Isolation of "atypical" mycobacteria from healthy persons. Am Rev Respir Dis 1959;80:747–9.
47. Mills CC. Occurrence of *Mycobacterium* other than *Mycobacterium tuberculosis* in the oral cavity and in sputum. Appl Microbiol 1972;24:307–10.
48. Collins FM. *Mycobacterium avium*–complex infections and development of the acquired immunodeficiency syndrome: casual opportunist or causal cofactor? Int J Lepr 1986;54:458–74.
49. Portaels F, Larsson L, Smeets P. Isolation of mycobacteria from healthy persons' stool [letter]. Int J Lepr 1988;56:468–71.
50. Ahn CH, McLarty JW, Ahn SS, Ahn SI, Hurst GA. Diagnostic criteria for pulmonary disease caused by *Mycobacterium kansasii* and *Mycobacterium intracellulare*. Am Rev Respir Dis 1982;125:388–91.
51. Sutker WL, Lankford LL, Tompsett R. Granulomatous synovitis: the role of atypical mycobacteria. Rev Infect Dis 1979;5:729–35.
52. O'Brien RJ, Geiter LJ, Snider DE Jr. The epidemiology of nontuberculous mycobacterial diseases in the United States. Results from a national survey. Am Rev Respir Dis 1987;135:1007–14.
53. Tsukamura M, Kita N, Shimoide H, Arakawa H, Kuze A. Studies on the epidemiology of nontuberculous mycobacteriosis in Japan. Am Rev Respir Dis 1988;137:1280–4.
54. Martin EC, Parker BC, Falkinham JO III. Epidemiology of infection by nontuberculous mycobacteria. VII. Absence of mycobacteria in southeastern groundwaters. Am Rev Respir Dis 1987;136:344–8.
55. Lavy A, Rusu R, Shaheen S. *Mycobacterium avium-intracellulare* in clinical specimens: etiological factor or contaminant? Isr J Med Sci 1990;26:374–8.
56. Scarlata G, Pellerito AM, DiBenedetto M, Massenti MF, Nastasi A. Isolation of mycobacteria from drinking water in Palermo. Boll Ist Sieroter Milan 1985;64:479–82.
57. DuMoulin GC, Stottmeier KD. Waterborne mycobacteria: an increasing threat to health. ASM News 1986;52:525–9.
58. Lockwood WW, Friedman C, Bus N, Pierson C, Gaynes R. An outbreak of *Mycobacterium terrae* in clinical specimens associated with a hospital potable water supply. Am Rev Respir Dis 1989;140:1614–7.
59. DuMoulin GC, Stottmeier KD, Pelletier PA, Tsang AY, Hedley-Whyte J. Concentration of *Mycobacterium avium* by hospital hot water systems. JAMA 1988;260:1599–601.
60. Dawson DJ, Armstrong JG, Blacklock ZM. Mycobacterial cross-contamination of bronchoscopy specimens. Am Rev Respir Dis 1982;126:1095–7.
61. Wheeler PW, Lancaster D, Kaiser AB. Bronchopulmonary cross-co-

Ionization and infection related to mycobacterial contamination of suction valves of bronchoscopes. J Infect Dis **1989**;159:954–8.

62. Steere AC, Corrales J, von Graevenitz A. A cluster of *Mycobacterium gordonae* isolates from bronchoscopy specimens. Am Rev Respir Dis **1979**;120:214–6.
63. Pappas SA, Schaaf DM, DiCostanzo MB, King FW Jr, Sharp JT. Contamination of flexible fiberoptic bronchoscopes [letter]. Am Rev Respir Dis **1983**;127:391–2.
64. Gubler JGH, Salfinger M, von Graevenitz A. Pseudoepidemic of nontuberculous mycobacteria due to a contaminated bronchoscope cleaning machine. Chest **1992**;101:1245–9.
65. Raleigh JW. Disease due to *Mycobacterium kansasii*. Sem Respir Med **1988**;9:498–504.
66. McGeady SJ, Murphey SA. Disseminated *Mycobacterium kansasii* infection. Clin Immunol Immunopathol **1981**;20:87–98.
67. Johanson WG Jr, Nicholson DP. Pulmonary disease due to *Mycobacterium kansasii*. An analysis of some factors affecting prognosis. Am Rev Respir Dis **1969**;99:73–85.
68. Lillo M, Orengo S, Cernoch P, Harris RL. Pulmonary and disseminated infection due to *Mycobacterium kansasii:* a decade of experience. Rev Infect Dis **1990**;12:760–7.
69. Valainis GT, Cardona LM, Greer DL. The spectrum of *Mycobacterium kansasii* disease associated with HIV-1 infected patients. J Acquir Immune Defic Syndr **1991**;4:516–20.
70. Tsukamura M, Shimoide H, Kita N, et al. Rapid increase of the incidence of lung disease due to *Mycobacterium kansasii* in Japan. Chest **1983**;83:890–2.
71. Kubin M, Švandová E, Medek B, Chobot S, Olšovský Ž. *Mycobacterium kansasii* infection in an endemic area of Czechoslovakia. Tubercle **1980**;61:207–12.
72. Prince DS, Peterson DD, Steiner RM, et al. Infection with *Mycobacterium avium* complex in patients without predisposing conditions. N Engl J Med **1989**;321:863–8.
73. Beck A, Stanford JL. *Mycobacterium xenopei:* a study of sixteen strains. Tubercle **1968**;49:226–34.
74. Engbaek HC, Vergmann B, Baess I, Will DW. *M. xenopei*. A bacteriological study of *M. xenopei* including case reports of Danish patients. Acta Pathol Microbiol Scand **1967**;69:576–94.
75. Bullin CH, Tanner EI, Collins CH. The isolation of *Mycobacterium xenopi* from water taps. J Hyg (Lond) **1970**;68:97–100.
76. Smith MJ, Citron KM. Clinical review of pulmonary disease caused by *Mycobacterium xenopi*. Thorax **1983**;38:373–7.
77. Banks J, Hunter AM, Campbell IA, Jenkins PA, Smith AP. Pulmonary infection with *Mycobacterium xenopi:* review of treatment and response. Thorax **1984**;39:376–82.
78. Simor AE, Salit IE, Vellend H. The role of *Mycobacterium xenopi* in human disease. Am Rev Respir Dis **1984**;129:435–8.
79. Contreras MA, Cheung OT, Sanders DE, Goldstein RS. Pulmonary infection with nontuberculous mycobacteria. Am Rev Respir Dis **1988**;137:149–52.
80. Weber J, Mettang T, Staerz E, Machleidt C, Kuhlmann U. Pulmonary disease due to *Mycobacterium xenopi* in a renal allograft recipient: report of a case and review. Rev Infect Dis **1989**;11:964–9.
81. Schröder KH, Juhlin I. *Mycobacterium malmoense* sp. nov. Int J Syst Bacteriol **1977**;27:241–6.
82. Connolly MJ, Magee JG, Hendrick DJ. *Mycobacterium malmoense* in the northeast of England. Tubercle **1985**;66:211–7.
83. Jenkins PA. *Mycobacterium malmoense*. Tubercle **1985**;66:193–5.
84. Jenkins PA, Tsukamura M. Infections with *Mycobacterium malmoense* in England and Wales. Tubercle **1979**;60:71–6.
85. France AJ, McLeod DT, Calder MA, Seaton A. *Mycobacterium malmoense* infections in Scotland: an increasing problem. Thorax **1987**;42:593–5.
86. Katila M-L, Brander E, Viljanen T. Difficulty with *Mycobacterium malmoense*. Lancet **1989**;2:510–1.
87. Boisvert H, Truffot C. Relations entre *Mycobacterium simiae* et le complexe *M. avium-intracellulare-scrofulaceum*. Ann Microbiol **1979**;130B:457–66.
88. Krasnow I, Gross W. *Mycobacterium simiae* infection in the United States. Am Rev Respir Dis **1975**;111:357–60.
89. Bell RC, Higuchi JH, Donovan WN, Krasnow I, Johanson WG Jr. *Mycobacterium simiae*. Clinical features and follow-up of twenty-four patients. Am Rev Respir Dis **1983**;127:35–8.
90. Rose HD, Dorff GJ, Lauwasser M, Sheth NK. Pulmonary and disseminated *Mycobacterium simiae* infection in humans. Am Rev Respir Dis **1982**;126:1110–3.
91. Krümmel A, Schröder KH, von Kirchbach G, Hirtzel F, Hövener B. *Mycobacterium simiae* in Deutschland. Zentralbl Bakteriol Mikrobiol Hyg [A] **1989**;271:543–9.
92. Falk GA, Hadley SJ, Sharkey FE, Liss M, Muschenheim C. *Mycobacterium avium* infections in man. Am J Med **1973**;54:801–10.
93. Horsburgh CR Jr, Mason UG III, Fahri DC, Iseman MD. Disseminated infection with *Mycobacterium avium-intracellulare*—a report of 13 cases and a review of the literature. Medicine (Baltimore) **1985**;64:36–48.
94. Prissick FH, Masson AM. Yellow-pigmented pathogenic mycobacteria from cervical lymphadenitis. Can J Microbiol **1957**;3:91–100.
95. Lincoln EM, Gilbert LA. Disease in children due to mycobacteria other than *Mycobacterium tuberculosis*. Am Rev Respir Dis **1972**;105:683–714.
96. Wolinsky E. The role of scotochromogenic mycobacteria in human disease. Ann N Y Acad Sci **1963**;106:67–71.
97. Taha AM, Davidson PT, Bailey WC. Surgical treatment of atypical mycobacterial lymphadenitis in children. Pediatr Infect Dis J **1985**;4:664–7.
98. Lai KK, Stottmeier KD, Sherman IH, McCabe WR. Mycobacterial cervical lymphadenopathy. Relation of etiologic agents to age. JAMA **1984**;251:1286–8.
99. Alessi DP, Dudley JP. Atypical mycobacteria-induced cervical adenitis. Arch Otolaryngol Head Neck Surg **1988**;114:664–6.
100. Romanus V. First experience with BCG discontinuation in Europe. Experience in Sweden 15 years after stopping general BCG vaccination at birth. Bull Int Union Tuberc Lung Dis **1990**;65:32–5.
101. Huhti E, Brander E, Paloheimo S, Sutinen S. Tuberculosis of the cervical lymph nodes: a clinical, pathological and bacteriological study. Tubercle **1975**;56:27–36.
102. Levin-Epstein AA, Lucente FE. Scrofula: the dangerous masquerader. Laryngoscope **1982**;92:938–43.
103. Street ML, Umbert-Millet IJ, Roberts GD, Su WPD. Nontuberculous mycobacterial infections of the skin. J Am Acad Dermatol **1991**;24:208–15.
104. Collins CH, Grange JM, Nobel WC, Yates MD. *Mycobacterium marinum* infections in man. J Hyg (Camb) **1985**;94:135–49.
105. Linell F, Nordén Å. *Mycobacterium balnei:* a new acid-fast bacillus occurring in swimming pools and capable of producing skin lesions in humans. Acta Tuberc Scand **1954**;33(suppl):1–84.
106. Mollohan CS, Romer MS. Public health significance of swimming pool granuloma. Am J Public Health **1961**;51:883–91.
107. Birn KJ, Schaefer WB, Jenkins PA, Szulga T, Marks J. Classification of *Mycobacterium avium* and related opportunist mycobacteria met in England and Wales. J Hyg (Camb) **1967**;65:575–89.
108. Prince H, Ispahani P, Baker M. A *Mycobacterium malmoense* infection of the hand presenting as carpal tunnel syndrome. J Hand Surg [Br] **1988**;13B:328–30.
109. Horsburgh CR Jr, Selik RM. The epidemiology of disseminated non-

tuberculous mycobacterial infection in the acquired immunodeficiency syndrome (AIDS). Am Rev Respir Dis **1989**;139:4–7.

110. Chow SP, Ip FK, Lau JHK, et al. *Mycobacterium marinum* infection of the hand and wrist. Results of conservative treatment in twenty-four cases. J Bone Joint Surg (Am) **1987**;69A:1161–8.
111. Kelly BJ, Karlson AG, Weed LA, Lipscomb PR. Infection of synovial tissues by mycobacteria other than *Mycobacterium tuberculosis*. J Bone Joint Surg (Am) **1967**;49A:1521–30.
112. Gunther SF, Elliot RC, Brand RL, Adams JP. Experience with atypical mycobacterial infection in the deep structures of the hand. J Hand Surg (Am) **1977**;2:90–6.
113. Love GL, Melchior E. *Mycobacterium terrae* tenosynovitis. J Hand Surg (Am) **1985**;10A:730–2.
114. Crick JC, Vandevelde AG. *Mycobacterium fortuitum* midpalmar space abscess: a case report. J Hand Surg (Am) **1986**;11A:438–40.
115. Stern PJ, Gula DC. *Mycobacterium chelonei* tenosynovitis of the hand: a case report. J Hand Surg (Am) **1986**;11A:596–9.
116. Stratton CW, Phelps DB, Reller LB. Tuberculoid tenosynovitis and carpal tunnel syndrome caused by *Mycobacterium szulgai*. Am J Med **1978**;65:349–51.
117. Subbarao EK, Tarpay MM, Marks MI. Soft-tissue infections caused by *Mycobacterium fortuitum* complex following penetrating injury. Am J Dis Child **1987**;141:1018–20.
118. Wallace RJ Jr. The clinical presentation, diagnosis, and therapy of cutaneous and pulmonary infections due to the rapidly growing mycobacteria, *M. fortuitum* and *M. chelonae*. Clin Chest Med **1989**;10:419–29.
119. Griffith DE, Wallace RJ Jr. Pulmonary disease due to rapidly growing mycobacteria. Sem Respir Med **1988**;9:505–13.
120. Rolston KVI, Jones PG, Fainstein V, Bodey GP. Pulmonary disease caused by rapidly growing mycobacteria in patients with cancer. Chest **1985**;87:503–6.
121. Rougraff BT, Reeck CC Jr, Slama TG. *Mycobacterium terrae* osteomyelitis and septic arthritis in a normal host. A case report. Clin Orthop **1989**;238:308–10.
122. Dijkmans BAC, Mouton RP, Macfarlane JD, et al. Bacterial arthritis caused by *Mycobacterium terrae*. Infection **1981**;9:204–7.
123. Beyd BE Jr, Ortbals DW, Santa Cruz DJ, Kobayashi GS, Eisen AZ, Medoff G. Cutaneous mycobacteriosis: analysis of 34 cases with a new classification of the disease. Medicine (Baltimore) **1981**;60:95–109.
124. Santa Cruz DJ, Strayer DS. The histologic spectrum of the cutaneous mycobacterioses. Hum Pathol **1982**;13:485–95.
125. Marks J, Jenkins PA, Tsukamura M. *Mycobacterium szulgai*—a new pathogen. Tubercle **1972**;53:210–4.
126. Maloney JM, Gregg CR, Stephens DS, Manian FA, Rimland D. Infections caused by *Mycobacterium szulgai* in humans. Rev Infect Dis **1987**;9:1120–6.
127. MacCallum P, Tolhurst JC, Buckle G, Sissons HA. A new mycobacterial infection in man. J Pathol Bacteriol **1948**;60:93–122.
128. Hockmeyer WT, Krieg RE, Reich M, Johnson RD. Further characterization of *Mycobacterium ulcerans* toxin. Infect Immun **1978**; 21:124–8.
129. Hayman J, McQueen A. The pathology of *Mycobacterium ulcerans* infection. Pathology **1985**;17:594–600.
130. Sompolinsky D, Lagziel A, Naveh D, Yankilevitz T. *Mycobacterium haemophilum* sp. nov., a new pathogen of humans. Int J Syst Bacteriol **1978**;28:67–75.
131. Mezo A, Jennis F, McCarthy SW, Dawson DJ. Unusual mycobacteria in 5 cases of opportunistic infections. Pathology **1979**;11:377–84.
132. Males BM, West TE, Bartholomew WR. *Mycobacterium haemophilum* infection in a patient with acquired immune deficiency syndrome. J Clin Microbiol **1987**;25:186–90.
133. Dawson DJ, Blacklock ZM, Kane DW. *Mycobacterium haemophilum* causing lymphadenitis in an otherwise healthy child. Med J Aust **1981**;2:289–90.
134. Saubolle MA, Rudinsky M, Merritt ES, Williams J, Raines JM, Dimler M. *Mycobacterium haemophilum* infection in two otherwise normal paediatric patients (abstract no C-298). In: Proceedings of the annual meeting of the American Society for Microbiology. Washington, DC: American Society for Microbiology, **1991**:391.
135. Weiszfeiler G, Karasseva V, Karczag E. A new mycobacterium species: *Mycobacterium asiaticum* N. Sp. Acta Microbiol Acad Sci Hung **1971**;18:247–52.
136. Blacklock ZM, Dawson DJ, Kane DW, McEvoy D. *Mycobacterium asiaticum* as a potential pulmonary pathogen for humans. A clinical and bacteriologic review of five cases. Am Rev Respir Dis **1983**;127:241–4.
137. Collins CH, Grange JM, Yates MD. Organization and practice in tuberculosis bacteriology. London: Butterworths, **1985**.
138. Vonmoos S, Leuenberger P, Beer V, de Haller R. Infection pleuro-pulmonaire à *Mycobacterium smegmatis*. Description d'un cas et revue de la litérature. Schweiz Med Wochenschr **1986**;116:1852–6.
139. Wallace RJ Jr, Nash DR, Tsukamura M, Blacklock ZM, Silcox VA. Human disease due to *Mycobacterium smegmatis*. J Infect Dis **1988**;158:52–9.
140. Tsukamura M, Shimoide H, Schaefer WB. A possible new pathogen of group III mycobacteria. J Gen Microbiol **1975**;88:377–80.
141. Rüsch-Gerdes S, Wandelt-Freerksen E, Schröder K-H. Occurrence of *M. shimoidei* in the Federal Republic of Germany. Zentralbl Bakteriol Mikrobiol Hyg [A] **1985**;259:146–50.
142. Hirschel B, Chang HR, Mach N, et al. Fatal infection with a novel unidentified mycobacterium in a man with acquired immunodeficiency syndrome. N Engl J Med **1990**;323:109–13.
143. Böttger EC, Teske A, Chang HR, Hirschel B. Molecular identification of a novel, uncultured *Mycobacterium* causing fatal infections in patients with AIDS. Lancet (in press)
144. Nadal D, Caduff R, Kraft R, Salfinger M, Bodmer T, Schaad UB. Invasive infection with the novel nontuberculous *Mycobacterium genovense* in children with the acquired immunodeficiency syndrome. Clinical Microbiology and Infectious Diseases (submitted).
145. Wayne LG, Sramek HA. Agents of newly recognized or infrequently encountered mycobacterial diseases. Clin Microbiol Rev **1992**;5:1–25.
146. Schraufnagel DE, Leech JA, Pollak B. *Mycobacterium kansasii:* colonization and disease. Br J Dis Chest **1986**;80:131–7.
147. Wolinsky E. When is an infection disease? [editorial]. Rev Infect Dis **1981**;3:1025–7.
148. Presslich J, Lahounik E, Kraus G. The Bactec-system in the diagnosis of tuberculosis—comparison of a conventional and the radiometric method (Bactec) for culturing, differentiation and susceptibility testing of mycobacteria. Zentralbl Bakteriol Mikrobiol Hyg [A] **1989**;270:487–91.
149. Musial CE, Tice LS, Stockman L, Roberts GD. Identification of mycobacteria from culture by using the Gen-Probe rapid diagnostic system for *Mycobacterium avium* complex and *Mycobacterium tuberculosis* complex. J Clin Microbiol **1988**;26:2120–3.