REVIEW

The transmission of tuberculosis in the light of new molecular biological approaches

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This review briefly summarises the recent achievements in tuberculosis epidemiology associated with the introduction of molecular methods, and considers the implications of these methods for the understanding of occupational tuberculosis transmission. Special attention is paid to the relative contribution of recently transmitted tuberculosis; risk factors for recent transmission; and the occurrence and frequency of exogenous reinfection. There is a need for occupational epidemiological studies, which should combine the methods of ''classical'' epidemiology with those of molecular epidemiology.

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For a long time, about 90% of all tuberculosis cases in industrial countries were regarded as ''endogenous reactivation'' of previously acquired infections.[1 2] As a result of the implementation of molecular biological methods, the dominant significance of endogenous reactivation in the explanation of new cases of tuberculosis has been called into question, as many studies have found that a high percentage of the patients who were examined using molecular biology techniques gave identical DNA fingerprints. These patients whose strains exhibit an identical (or highly similar) fingerprint pattern during a defined period of time are included in a so-called cluster. It is assumed that cluster patients belong to a chain of recent transmission. The methods of molecular biology have also been used to show that new infection with a second tuberculosis pathogen occurs more frequently than stated before, possibly even in industrial countries with a low incidence of tuberculosis.[3 4]

If recently transmitted cases of tuberculosis, including exogenous reinfections, are considerably more frequent than previously thought, this would also indirectly be of significance for the assessment of the occupational risks of disease, as even if an employee is known to have had tuberculosis previously, recurrent tuberculosis could no longer be considered unreservedly to be endogenous reactivation. In this review, the following themes are discussed which could provide insights, some of them indirectly, into the frequency of recent occupational tuberculosis transmission.

- What is the proportion of recently transmitted tuberculosis cases in all new tuberculosis cases in countries with a low tuberculosis incidence?
- Is recent tuberculosis transmission only important in young patients?
- What is the proportion of exogenous reinfections in all new cases of tuberculosis in countries with low tuberculosis incidence?
- Is the proportion of recently transmitted tuberculosis dependent on occupation?

The above questions are clarified on the basis of a systematic analysis of the literature. A Medline search was performed in which all studies with the keywords ''tuberculosis'' and ''fingerprint'' (or ''fingerprinting'') and ''transmission'' were evaluated for their contribution towards answering the above questions.

Determination of the IS*6110* insertion sequence as a standard method of DNA ''fingerprinting'' analysis

DNA fingerprinting using the IS*6110* insertion sequence as basis of the analysis of restriction fragment length polymorphism (abbreviated as RFLP) is regarded as the standard method for the molecular typing of *Mycobacterium tuberculosis*.[5] Genetic fingerprinting analysis can only be used in patients with culture confirmed tuberculosis, not in patients with a latent infection. For fingerprinting analysis, the DNA from the bacterial culture is first extracted and then purified. The DNA is then treated with the restriction enzyme *PvuII*; this recognises a specific six-nucleotide palindromic sequence in the DNA and cleaves it at each occurrence of the sequence. The resulting restriction fragments are separated electrophoretically on the basis of their size, transferred onto a nylon membrane, and hybridised with an IS*6110* probe. This probe then specifically marks those fragments which contain the repetitive element IS*6110*. The insertion sequence IS*6110* possesses great variability with respect to the number of the IS*6110* copies (0 to ca. 25 copies) and their localisation in the *M tuberculosis* chromosome (cf. review articles[6–10]). The number of resulting bands corresponds to the number of IS*6110* copies, and the localisation of the bands reflects the molecular weight of the fragments containing the IS*6110* copies.

Stability of the genetic fingerprint with time

IS*6110* RFLP patterns in actual transmission chains show a high degree of stability over time and may therefore be useful as ''epidemiological markers''.[11] On the other hand, the IS*6110* patterns must change rapidly enough so that isolates which are not connected by transmission can be differentiated. There are accordingly

Table 1 Molecular epidemiological studies on the transmission of tuberculosis

Study	Time	Site	Design	Study group	Availability/response	Part of a cluster	
						n method*	[n minus 1] method
USA studies							
Small *et al.* 1994[60]†	1991–92	San Francisco	IS6110; exclusion <2 bands	473 reported patients	69%	40%	31% (+PGRS: 29%, see comment)
Rhee *et al.* 2000[35] (Martinez, Rhee *et al.* 2000;[30] Borgdorff *et al.* 2000[53])†	1991–96	San Francisco	IS6110 (ident.)+PGRS for <6 bands (Borgdorff *et al.* 2000: exclusion of isolates with <5 bands)	1370 culture-pos. pat.;[30]: 1361;[53]: 1359; only including the 944 cases with pulmonary TB)	87% ([30]: 85%;[53]: 86%)	34% ([30]: 43%;[53]: 34% for pulmonary TB)	25% ([53]: 24% for pulmonary TB)
Alland *et al.* 1994;[54] Murray & Alland 2002[20]	1993	Bronx, New York	IS6110; additional analysis of preparations with <3 bands	104 of 130 pat. of a large hospital	80% of the hospital pat.; however only 10% of the assumed 1076 TB patients in New York City	40% (12 clusters)	30%
Sepkowitz *et al.* 1995[68]††	1992–2/94	New York City	IS6110 (ident.)	201 pat. from 6 hospitals (''TBNetwork'') with known occupational history	ca. 2% of the TB cases in NYC	43%	
Tornieporth *et al.* 1997[58]††	1992–94	New York City	IS6110 (ident.)+pTBN12 for <4 bands‡	302 pat. from 7 hospitals (''TBNetwork'')	47% of the hospital pat.; however only a very low proportion (<<10%) of all cases in NYC	31%	
Magnani *et al.* 2001[42]	1996–97; Corresponds to ''City Cluster'' library (4500 patterns)	New York City	IS6110 (ident.); no secondary typing‡	180 pat. from 8 hospitals (''TBNetwork'')	ca. 50% of the hospital pat.; ca. 13% of the cases in NYC	54% (2–17 pat.)	
McConkey *et al.* 2002[58]	1998–99	St Louis, Missouri	IS6110 (ident.)+PGRS (<6 bands or deviation ±1)	60 pat.	97%	38% (7 Cluster mit 2–6 Pat.)	27%
Geng *et al.* 2002[57]	1990–99	North Manhattan	IS6110 (ident.)+spoligotyping (<6 bands)	546 culture-pos. TB cases from the Columbia Presbyterian Medical Center	73.3% of the culture-positive hospital cases	48% (51 clusters with average. 5.2 pat.)	38.5%
Ellis *et al.* 2002[43]†††	1996–2000	7 Sentinel Regions: Arkansas, Maryland, Massachusetts, Michigan, New Jersey, Texas (partially), California (partially)	IS6110 (ident.)+spoligotyping (<=6 bands)	10,752 reported pat. with culture-pos. TB	90.2% of the culture-pos. cases	48.1% (1,018 clusters with 2–105 pat.)	38.6%
Sharnprapai etal. 2002[37]†††	1996–2000	Massachusetts	IS6110 (ident.)+spoligotyping (<6 bands)	983 reported patients	95% of the culture-positive cases	27.7% (82 clusters with 2–16 pat.)	19.3%
Cronin *et al.* 2002[24]	1996–2000	Maryland	IS6110 (dev. ±1?)+spoligotyping (<6 bands)	1172 reported patients	99% of the culture-positive cases	37.2% (111 clusters with 2–19 pat.)	27.7%
Burman *et al.* 1997[15]	1988–94	Denver, Colorado	IS6110 +pTBN12 for all isolates	189 reported patients	59% with inclusion of the culture-negative patients	15 clusters	19% (31% with exclusive use of the IS6110 technique)
Braden *et al.* 1997[55]	1992–93	Arkansas	IS6110 (dev. ±1)+pTBN12	235 reported pat. with both types	67%	33% (24 clusters with 2–7 pat.)	23%
Dobbs *et al.* 2001[26]	1994–98	Alabama	IS6110 +spoligotyping for <=5 bands (poss. Also other analyses)	253 reported pat.	ca. 80%	47% (119 clusters)	
Hernández-Garduño *et al.* 2002[29]	1995–99	Greater Vancouver	IS6110 (ident.)+spoligotyping (<6 bands)	793 reported pat.	100%	17.3% (46 clusters)	11.5%
Kulaga *et al.* 2002[41]	1997–98	Montreal	IS6110 (dev. ±1/ident.), exclusion <5 bands	243 reported pat.	80%	24.7% (dev. ±1)/7.0% (ident.)	18.1%/4.1%
European studies							
van Soolingen *et al.* 1999[49]††††	1993–97	Netherlands	IS6110 (ident.)+PGRS for <5 bands	4,266 pat. (pulmonary+extrapulmonary)	78%	46% (479 clusters with 2–47 pat.; 52% of the pat. in clusters with ⩽5 pat.)	35%
Borgdorff *et al.* 2001[46]††††	1993–98	Netherlands	IS6110 (ident.)+PGRS for <5bands	3,479 pat. with pulmonary TB (register-based)	ca. 83%	45% (390 clusters)	34%

Table 1 Continued

Study	Time	Site	Design	Study group	Availability/response	Part of a cluster	
						n method*	[n minus 1] method
Diel *et al.* 2002[47]	1997–99	Hamburg	IS6110, exclusion <5 bands	423 reported (one of the 8 health offices) patients with TB confirmed by culture	77%; after exclusion of isolates with <5 bands 398 included in cluster analysis	33.9% (35 clusters with 2–23 pat.)	
Niemann *et al.* 1997[33]	1995	Germany	IS6110; exclusion ⩽4 bands (n = 4)	156 pat. with drug.-resistant TB (after exclusion of 7 pot. cross-contamin. isolates	ca. 32% (estimate)	29%	18%
Genewein *et al.* 1993[28]	1991–92	Canton Berne (Switzerland)	IS6110 (ident.?); evidently no ident. isolates with low band number	163 TB pat. of Canton Berne	61% of the culture- pos. cases	27.6% (9 clusters with 2–22 pat.)	22.1%
Pfyffer *et al.* 1998[34]	1991–93	Zurich	IS6110 (ident.); +PGRS for <6 bands or minimal deviations	361 culture-positive pat.	90%	17.5% (25 clusters with 2–6 pat.)	10.5%
Bauer *et al.* 1998 ;[44] (Yang *et al.* 1995[61])	1992–96	Denmark	IS6110 (ident.); exclusion <5 bands (9%; not with Yang *et al.* 1995)	1549 TB cases (only 1992: 245 pat. [Yang *et al.* 1995])	ca. 84% of the culture-TB pat. (with exclusion of isolates <5 bands)	49% (only 1992: 31% [Yang *et al.* 1995])	Not calculable
Bauer *et al.* 2000[23]	4–11/1998	Denmark (TB departments in Copenhagen and Jutland)	IS6110 (ident.)+spoligotyping with <5 bands	163 pat. from 5 TB dept. in Copenhagen and Jutland	49.5%	63%	
Lillebaek *et al.* 2001[45]	1992–99	Denmark	IS6110 +spoligotyping	763 TB cases in Somali immigrants; 1996–98: 391 cases in Somali immigrants, 470 cases in Danes	>97%	Somali immigrants: 59.1% (additional spoligotyping: 55.2%)	
Heldal *et al.* 2000[4]	1994–97	Norway	IS6110 (ident.); band number for all isolates >=5	51 for the year 1995 reported TB pat.; cluster membership compared with 555 TB pat. 1994–97	85% of the culture- pos. TB cases	15.7% (6 clusters with 2–6 pers.)	
Hayward *et al.* 2002[62]	1993	London (Inner)	spoligotyping+HIPCR (IS6110-based)	552 TB cases	80% of the reported culture-pos. TB cases (corr. 56% of all reported cases)	27% (42 clusters with 2–15 pat.)	19%
Maguire *et al.* 2002[48]	7/1995–1997	(Greater) London	IS6110 (ident.); exclusions: <5 bands (n = 448)	2.042 TB cases (diagnosed in one of 54 laboratories) with detection of ⩾5 bands	63% of the culture- pos. TB cases (assuming in all 3260 [?, text unclear] culture-pos. TB cases)	22.7% (169 clusters with 2–12 persons)	14.4%
Vachée *et al.* 1999[39]	1995	France (North Department)	IS6110 (dev. ±1)	154 pat., diagnosed in 38 laboratories	66% (comparison with regional survey)	18% (14 clusters)	9%
Gutiérrez *et al.* 1998[52]	1995	Paris and surroundings	IS6110 +DR sequences, exclusion: 1 band (n = 10)	272 hospital-based TB cases		35.7% (26 clusters with 2–12 pat.)	
Codecasa *et al.* 1999[25]	1993–96	Milan City	IS6110 (ident.?)+spoligotyping for <3 bands	247 of the pat. reported to the TB Reference Center for Lombardy (Villa Marelli Institute)	9% (247 of 2616 TB cases); however the degree of completeness for the time window (not given) may be higher	30.5% for immigrants, 53% for patients born in Italy	
Moro *et al.* 2002[31]	1995–97	Milan City	IS 6110+spoligotyping for <5 bands or dev. ±1	581 culture-pos. pat., reported by Public Health offices and labs	71%	41.1% (76 clusters with 2–11 pat.)	28.1%
Nastasi u. Mammina 1999[32]	1994–98	Palermo (Sicily)	IS6110 (ident.)	95 in-patients in 5 hospitals in Palermo	35% of the reported (culture-pos. and negative) pat.	12.6% (6 clusters with 2–4 pat.)	6.3%
Garzelli *et al.* 1997[40]	1993–95	Pisa (Region)	IS6110 (ident.)	60 pathogen strains in 3 hospitals	Hospital	15% (4 clusters with 2–3 pat.)	8%
Safi *et al.* 1997[36]	1993–95	Seville, Spain	IS6110 (dev. ±1)	175 culture-pos. TB pat. from the University Hospital (176 isolates and 1 reinfection)	85%	38% (19 clusters with 2–7 pat.)	27%
Samper *et al.* 1998[59]	1993	Saragossa Region, Spain	IS6110 (ident.), exclusion <5 bands	226	83%	39% (28 clusters with 2–10 pat.)	27%
Martínez, Sanchez *et al.* 2000;[50] Fernandez de la Hoz *et al.* 2001[27]	1997–98	South. Madrid	IS6110 (ident.)+spoligotyping (<6 bands)	148 register-based TB cases	71.5% of all pat. with TB	42% (18 clusters with 2–12 pat.)	30%
Elizaga *et al.* 2002[56]	1995–99	Segovia (Spain, rural region)	IS6110+spoligotyping	87 TB cases diagnosed in the only microbiology lab		27.6% (8 clusters with 2–6 pat.)	18.4%

MDR, multiple drug resistance; ident., identical fingerprint pattern; dev., deviation with respect to the number or size of the IS*6110* insertions.
*The n method starts with all (n) patients in a cluster, the [n minus 1] method subtracts an index case from each cluster. The [n minus 1] method therefore appears to be more appropriate for the calculation of the recent or reactivated cases.
†, ††, †††, ††††Indicates studies with identical or partially identical study populations.
‡Whereas IS*6110* is the most commonly used probe internationally, pTBN12 can be used as a secondary probe for confirmation or comparison of IS*6110* negative strains. Furthermore, the pTBN12 probe (but not IS*6110*) allows typing of other mycobacteria.

conflicting demands on genetic fingerprinting. In order to clarify the stability of the genetic fingerprint, sequential molecular genetic studies have been performed on patients with repeated positive tuberculosis cultures, at intervals of between days and years. In studies in San Francisco, the Netherlands, and South Africa, the half-life of the DNA fingerprint was calculated to be 2.0, 3.2, or 8.7 years respectively.[12–14] It can be assumed that the half-life of the DNA fingerprint depends on regional factors, such as the efficiency of the therapy, the time interval between manifestation of the disease and the start of treatment and possibly also the regional dominance of stable or less stable pathogen strains. Niemann *et al* refer to the stability of the DNA fingerprint in chains of infection which had been established on the basis of medical histories.[11] This approach focuses on the stability of the DNA fingerprint in recently transmitted tuberculosis and *not* on the ''intracorporal'' constancy of the DNA fingerprint in patients with repeated positive tuberculosis cultures, as in the studies described above. This study revealed a rate of change of only 1.9% per transmission. We therefore regard DNA fingerprinting as a suitable method to evaluate the proportion of recently transmitted tuberculosis cases.

Alternative or complementary typing methods

In up to 30% of the DNA fingerprints, fewer than five IS*6110* copies could be found, so that unambiguous distinction between different chains of infection is then no longer possible.[15] Additional procedures must be used for it in order to assign isolates with no or only few IS*6110* copies to possible chains of infection. The most frequently used alternatives are the ''polymorphic guanine-cytosine-rich repetitive sequence'' (PGRS)[16] and the so-called spoligo-(= spacer-oligonucleotide-) typing.[17] Some of the alternative procedures are based on the multiplication of specific DNA segments using the polymerase chain reaction (PCR) in vitro; this method is rapidly performed and is moreover not dependent on the presence of mycobacteria which are capable of replicating. Some PCR supported methods, such as the ''mixed linker PCR'',[10] may achieve a degree of discriminating ability and reproducibility which is comparable to that of the IS*6110* RFLP standard method.[6] Another PCR based assay which may prove to be a powerful tool is tandem repeat typing, based on variable number tandem repeats (VNTRs) of genetic elements named mycobacterial interspersed repetitive units (MIRUs) in 12 human minisatellite-like regions of the *M tuberculosis* genome.[18]

Methodical limitations to DNA typing

It should be pointed out that an identical fingerprint pattern may indicate that patients belong to the same chain of infection, but does not prove that there is a close connection between the patients in question. Kulaga *et al* underscore that the following explanations are in principle possible if two individuals in the same place exhibit an identical fingerprint:[9 19]

- A recently transmitted infection
- The simultaneous reactivation of an infection with the same organism which has been acquired earlier (coincidence of time)
- The regional dominance of a bacterial strain spreading over a long time
- A chance localisation of the insertion sequence at the same site
- A laboratory error.

The probability of finding a unique DNA fingerprint and to interpret this wrongly as an endogenous reactive case increases with decreasing study duration and decreasing completeness of the degree of case recruitment. The incompleteness of the cluster found is due to geographical reasons (migration), reasons of time (study duration), and reasons connected with the sample. In recent years, some of the above mentioned methodical problems have been analysed in empirical studies.[20–22]

WHAT IS THE PROPORTION OF RECENTLY TRANSMITTED TUBERCULOSIS IN ALL NEW CASES OF TUBERCULOSIS?

Molecular biological studies permit the estimate of the proportion of clustered patients, which is the same as the proportion of patients who can be ascribed to a chain of infection. Table 1 summarises the molecular epidemiological studies on the transmission of tuberculosis separately for American and European studies. In table 1, some studies are included that are not further mentioned in this review article.[23–40] The proportion of clustered patients varies in the more or less population based studies between 7% (Montreal, 1997–98[41]) and 54% (New York City, 1996–97[42]). If one index patient per cluster is regarded as an endogenously reactivated source case ([n minus 1] method), this gives the proportion of recently transmitted cases as lying between 4% (Montreal[41]) and 38.6% (USA, 1996–2000[43]). The great differences between these study results are essentially due to differences in methodology. Thus, studies based on a poorly defined population, low completeness of case collection, and short duration tend to considerably underestimate the proportion of recently transmitted cases. Another reason for the different study results on the proportion of clustered cases can be seen in the regional differences in the proportion of immigrants. In general, the proportion of cluster membership is clearly lower in immigrants in industrial countries than in the native population. A comparatively accurate estimate of the proportion of recently transmitted tuberculosis should be expected in Denmark and the Netherlands, because of the molecular biological investigation of almost all culture positive tuberculosis cases in the whole population.

About 57% of the tuberculosis cases born in Denmark can be ascribed to a cluster.[44] About 30% of the immigrants to Denmark belong to a cluster;[44] however, this is partially a case of endogenous reactivation of tuberculosis pathogens which were endemic in the land of origin in the distant past.[45]

In the Netherlands, about 48% of the patients with Netherlands citizenship (42% of the patients with non-Netherlands citizenship) can be ascribed to a cluster (1993–98);[46] application of the [n minus 1] method gives the proportion of recently transmitted tuberculosis cases as being 37% in the patients with Netherlands citizenship and 30% in the patients with non-Netherlands citizenship.

In an own molecular epidemiological study which was performed in Hamburg (period 1997–99), about 40% of the tuberculosis patients born in Germany and 25% of the immigrants to Germany were ascribed to a cluster.[47] As the duration of the study was relatively short in comparison to the Danish and Netherlands studies and the study population smaller, the actual extent of recent transmission in the Hamburg study was presumably underestimated.

There is a particularly high proportion of recently transmitted tuberculosis cases in the so-called high risk groups, such as alcoholics and drug addicts[15 47–52] and the homeless.[15 52] There is, however, no linear relation between the incidence of tuberculosis in any population group and the danger of infection to the whole population from this group.[53] The evaluation of the risk of infection for hospital workers can accordingly not exclusively be based on the proportion of patients who belong to certain high risk groups for the occurrence of an episode of tuberculosis. The methods of

molecular biology can contribute more by helping to identify high risk groups for the transmission of tuberculosis.

IS RECENT TRANSMISSION OF TUBERCULOSIS ONLY IMPORTANT IN YOUNG PATIENTS?

Most molecular epidemiological studies find that the proportion of recently transmitted cases of tuberculosis sinks with increasing age;[15 43 48–50 54–61] in some studies no significant correlation between age and the cluster membership of the patients could be established.[42 47 51 52 62] The following analysis of age distribution will also deal in more detail with the Danish and Netherlands results, because of the high quality of the data.

Bauer and colleagues[44] found that the proportion of clustered cases was above 50% for tuberculosis patients born in Denmark in all age groups under 60. The proportion of clustered cases in the patients of Danish origin of over 60 was under 50%. The proportion of clustered patients in the over 80s alone was under 20%.

The analysis of the Netherlands tuberculosis cases gives the result that the proportion of clustered cases in all younger and intermediate age groups including the 45–54 year olds came to about 50% (49–53%).[46] The proportion of clustered cases was 42% for the 55–64 year olds and 21% for the patients of 65 years and above. In relation to these results, van Soolingen and colleagues[49] pointed out that although the relative proportion of cluster members decreased with age, the incidence of cases belonging to clusters exhibited no age dependency in the Netherlands population. Thus the absolute number of recently transmitted tuberculosis cases in the Netherlands remains roughly constant with increasing age. These molecular epidemiological studies therefore allow the conclusion that involvement in a chain of infection rather than endogenous reactivation becomes relatively less important with increasing age. However, a clear reduction in the proportion of clustered tuberculosis patients can only be established for pensioners.

There is a limitation to this conclusion which should be pointed out; namely that, according to Vynnycky and colleagues,[22] with increasing age cluster membership may not unreservedly be interpreted as implying recent transmission of tuberculosis. However, this effect appears to be of limited significance, at least for the older working population. On the basis of their model calculations based on the Netherlands data, Vynnycky and colleagues concluded that the proportion of recently transmitted tuberculosis cases is clearly greater than 25% in women of all age groups, including the 55–64 year olds, and in men of all age groups, up to the 45–54 year olds.[22]

Taken together, the results indicate that the age dependency of recent transmission of tuberculosis is of limited significance in the assessment of the occupational risk of tuberculosis, as recent transmission of tuberculosis is important even for people within the upper ranges of the working population.

WHAT IS THE PROPORTION OF EXOGENOUS REINFECTIONS IN ALL NEW CASES OF TUBERCULOSIS?

Molecular epidemiological studies indicate that exogenous reinfections are more frequent than used to be thought, even in regions with a low or relatively low incidence of tuberculosis. Most studies which have been carried out permit only a conclusion on the proportion of exogenous reinfections in patients with two clinically manifest episodes of tuberculosis, and this is restricted by the low number of cases. Thus a South African study found exogenous reinfection in 12 of 16 (75%) patients with two episodes of tuberculosis.[63] However, the incidence of tuberculosis in South Africa is very high. In a study performed in Lombardy (North Italy), five of 32 (16%) patients with two episodes of tuberculosis were attributed to exogenous reinfection,[64] and in a study performed in Grand Canaria, eight of 18 (45%) patients were found to be reinfected.[65] In a Dutch study, tuberculosis in 29 of 183 (16%) patients who had reported tuberculosis infection or disease years ago was attributed to recent reinfection.[66] A study performed in Norway, a country with a very low incidence of tuberculosis, concluded that six of 45 (13%) patients notified to the National Tuberculosis Register in 1995 were due to exogenous reinfection; the majority (six of eight) of cluster patients was thought to have been exogenously reinfected.[4] More detailed interpretation of these percentages must be cautious because of low numbers.

On the other hand, most cases of exogenous reinfection are to be expected in patients who were infected with *M tuberculosis* in the past, without developing the clinical picture of tuberculosis. With patients of this sort, *M tuberculosis* isolates for two different episodes of the disease are not available, so that the methods of molecular biology cannot be used to distinguish between endogenous reactivation and exogenous reinfection. In an attempt to classify the extent of exogenous reinfection in spite of this, Murray and Nardell proposed estimating the later membership of a cluster in a cohort of persons who had given a positive tuberculin test in earlier investigations.[67] This study design would however necessitate a long follow up period of the infected cohort. A subgroup with documented tuberculin tests in the past (such as a subgroup of health workers) could be particularly suitable for a study of this sort.

IS THE PROPORTION OF RECENTLY TRANSMITTED TUBERCULOSIS CASES DEPENDENT ON OCCUPATION?

Only two molecular epidemiological studies permit the separate calculation of the proportion of clustered cases for medical and non-medical occupations.[68 69] In the study performed by Sepkowitz *et al*, the cases of tuberculosis were identified in the years 1992 to 1994 on the basis of a surveillance network (''TBNetwork'') in six hospitals in New York City. This surveillance network included about 8% of all tuberculosis cases in New York City. Because of the restricted identification of the population and the high proportion of missing values, biased selection in this study cannot be excluded. Twenty of the 142 tuberculosis cases included were in medical professions: eight nurses, seven doctors, one social worker, one watchman, one medical auxiliary, one nutritionist, and one caretaker. Sixty five per cent of the tuberculosis patients in medical occupations, but only 41% of the tuberculosis patients in non-medical occupations were clustered cases. In a multivariate analysis, the odds ratio for recent tuberculosis transmission (versus endogenous reactivation) for persons in medical occupations was 2.77 (95% CI 1.19 to 6.41). Sepkowitz *et al* conclude that many of the apparently sporadic cases of tuberculosis in members of medical professions could be due to unrecognised cases of occupational infection.[68] In a population based study performed by van Deutekom and colleagues,[69] residents of Amsterdam with culture proven tuberculosis (diagnosed between 1992 and 1995) were included. Multivariate analysis revealed that among Dutch patients, health care workers were significantly less likely to be part of a cluster. Among patients of foreign ethnicity, health care work was not significantly associated with clustering. The authors do not have a sufficient explanation for the reduced risk of Dutch health care workers of being clustered. However, the results of van Deutekom *et al* are not adjusted for socioeconomic factors and for neighbourhood incidence of tuberculosis.

The search for the source case of patients with potentially occupationally related tuberculosis was traditionally performed with conventional, non-molecular biological, methods of contact evaluation. However, several molecular epidemiological studies have shown that conventional contact tracing is an unreliable procedure, even with standardised instruments and trained personnel. In the study of Small and colleagues,[60] case pairs could only be established by conventional methods for 10% of cluster members before DNA typing had been performed; the corresponding value in the Hamburg study was 18%.[47] A possible reason for the low reliability of conventional methods of evaluation which should be pointed out is that brief contacts are often the actual source case. The evaluation of the source case by contact tracing is even an uncertain procedure when both the index person and the contact person are suffering from tuberculosis at the same time.[70] There has as yet hardly been any work on the extent to which this low reliability of contact evaluation by conventional methods also applies to occupational transmission of tuberculosis. In this respect, the study of Bennett *et al* indicates that the unreliability of conventional methods in discovering transmission relationships in the workplace is possibly greater than in the domestic context.[71]

To summarise, the molecular epidemiological study of Sepkowitz *et al* indicates that the risk of occupational tuberculosis transmission may possibly be increased in health care workers.[68] However there is a real deficiency in the amount of research which has been carried out on the occupational risk of recently transmitted tuberculosis. Further prospective studies are necessary, which combine classical epidemiological methods and the methods of molecular biology.

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