EDITORIAL

Utility of molecular epidemiology of tuberculosis

D. van Soolingen

Just 10 yrs ago it was still generally accepted that bacteria of the *Mycobacterium tuberculosis* complex were so highly conserved, that phage typing, which could distinguish about 20 different phage types, was all one needed to study the epidemiology of tuberculosis [1–4]. From the late 1980s, repetitive deoxyribonucleic acid (DNA) sequences have been described, which are associated with different levels of genetic polymorphism [5–17]. In 1990, the highly similar insertion sequences IS986 and IS6110 were found in the genome of *M. tuberculosis* [10, 13, 14] and, when these elements were applied in restriction fragment length polymorphism (RFLP) typing, they provided an unprecedented degree of discrimination between *M. tuberculosis* complex isolates. When IS986-based RFLP typing was used in 1990 to confirm for the first time a suspected outbreak of tuberculosis in the Netherlands [8], interest in the molecular epidemiology of tuberculosis was awakened.

RFLP typing seemed ideal for studying small-scale transmission of *M. tuberculosis*, such as outbreaks in institutions, hospitals or among families [16, 18–22]. Within a remarkably short period of time the utility of fingerprinting for managing outbreaks was established. The RFLP typing technique was optimized and standardized to enable computer-assisted analysis of DNA fingerprints [23, 24]. This facilitated large-scale epidemiological studies. However, the utility and reliability of large-scale, or even routine, application of DNA fingerprinting in tuberculosis control is still debatable and hinges on whether clustering of DNA fingerprints among patients' isolates does truly reflect transmission of *M. tuberculosis*.

If clustering of cases on the basis of DNA fingerprinting does indeed reflect recent transmission, then the results from the population-based studies in San Francisco [25], New York [26], the Netherlands [27] and Denmark [28] would suggest that ongoing transmission contributes much more to the overall tuberculosis disease incidence in developed countries than has been assumed previously. However, how do the results of these studies fit with other findings of the transmission of *M. tuberculosis* between different groups? Both SMALL *et al.* [25] (in San Francisco, USA) and VAN DEUTKOM *et al.* [29] (in Amsterdam, the Netherlands) found that only 5–10% of the links indicated by DNA fingerprinting, were also found by conventional contact tracing. Furthermore, in the molecular epidemiological study in Bern, Switzerland, conducted by GENEWEIN *et al.* [30] cases in totally different social groups were in the same DNA fingerprint clusters.

To examine the utility of DNA fingerprinting in more detail, BRADEN *et al.* [31] chose another approach in the rural area of Arkansas, USA. In this study, extensive interviews with patients identified epidemiological connections for only 33 out of 78 (42%) of the clustered patients. It is unclear whether the conclusions from this study can be generalized to other (*e.g.* urban) populations, as it was conducted in a rural area in which a large proportion of individuals were elderly.

In contrast, in the study of BURMAN *et al.* [32] (in Denver, USA) 40 of the 51 patients (78%) with clustered isolates had definite or possible epidemiological links identified through contact tracing.

In the molecular epidemiological study in the metropolitan area of Zürich, Switzerland, presented by PFYFFER *et al.* [33] in this issue of the Journal, a very small percentage (20%) of cases were clustered. This reflects that in an area where tuberculosis control is organized in a "Swiss way", it is possible to have very few cases clustered and that DNA fingerprinting is a sensitive tool for monitoring the performance of a tuberculosis control programme. However, even in this area, with a well-organized network for contact tracing, only a small proportion of the epidemiological relations indicated by DNA fingerprinting were confirmed by conventional contact tracing. In the Netherlands, isolates from all culture-positive cases have been DNA fingerprinted since 1993. DNA fingerprinting results, reported to the tuberculosis control services since 1995, are used in contact tracing and source case finding. The routine application of DNA fingerprinting in tuberculosis control in the Netherlands is currently being evaluated. As can be expected, some of the epidemiological connections indicated by DNA fingerprinting confirm those suspected in conventional contact tracing; these are usually the links which are easy to identify on the basis of sociodemographic data. Other connections indicated by DNA fingerprinting results are less clear, or completely unexpected. For instance, there was recently a case of extra pulmonary tuberculosis in a 75 yr old woman, which was assumed to be attributable to the reactivation of a previous infection. She was socially isolated and did not leave her house during the manifestation of the disease. A home-visiting nurse, responsible for treating her tuberculous abscesses, became skin test positive and developed a pleural effusion, but otherwise no positive contacts were found. One year later, in the same village, pulmonary tuberculosis was diagnosed in a 16 yr old male. Contact tracing yielded no source case. To his best knowledge he had never contacted the female tuberculosis patient, as they were in completely different social groups and did not even know each other. It would have been impossible

Correspondence: D. van Soolingen, RIVM (LIS), Mycobacterial Dept, Postbus 1, 3720 BA Bilthoven, The Netherlands. Fax: 31 3027 44418.

to link the two cases using conventional contact tracing. However, the DNA fingerprints of the isolates of these two patients were identical and had not yet been seen in the database of Dutch fingerprints.

Given that these fingerprints were observed in a very restricted geographical area, this finding cannot be attributed to any errors in the fingerprinting technique. This anecdote itself illustrates the fact that in the Netherlands clustered cases are frequently found in restricted geographic areas.

The strong negative correlation between age and clustering in population-based studies in the Netherlands and Cuba [27] (and DIAZ *et al.*, unpublished data) provides further evidence that clustering of DNA fingerprints does reflect recent transmission. In both studies, the percentage of cases clustered on the basis of DNA fingerprinting steadily decreased with age, reflecting that the proportion of disease attributable to endogenous reactivation increases with age.

In summary, the question arises as to whether we can assess the utility of DNA fingerprinting by comparing its findings with the results of conventional contact tracing, as this will only highlight the (lack of) correlation between the new and the classical approach. We may have to face the fact that transmission of *M. tuberculosis* occurs in ways that we have not previously imagined and that it cannot be predicted using sociodemographic data. Therefore, it is time to work from the opposite end of the spectrum and to use DNA fingerprinting results to start "open minded" investigations. All possible short, incidental contacts between clustered patients during a long-term period, which in current contact tracing policies would never be recorded, should be considered as possible routes of transmission. This may well change our ideas on the way a significant proportion of *M. tuberculosis* transmission occurs.

The utility of RFLP typing has been restricted by the need for large quantities of pure DNA; this is itself hampered by the slow growth rate of mycobacteria. RFLP typing results often only become available after the conventional contact tracing has been completed. Therefore, several polymerase chain reaction (PCR)-based methods have been developed recently [34–37], including one designated "spoligotyping", which simultaneously detects and types *M. tuberculosis* complex bacteria [34]. In theory, this method can be performed directly on clinical specimens (e.g. sputum), because it is based on the PCR. This circumvents a culture delay of 2–5 weeks. Unfortunately, the state of the art only allows a limited application of this technique, as this typing method is far less discriminatory than RFLP typing (DIAZ *et al.*, unpublished data).

Despite great advances in the development of molecular laboratory methodologies for the diagnosis of infectious diseases in recent years, the little genetic information that we do obtain comes only through laborious and demanding laboratory techniques. However, this situation will change shortly because of the developments in chip technology. It is already possible to generate approximately 20,000 oligonucleotide probes on a surface of 1 cm² of high-density arrays with the aid of light-directed chemical synthesis [38]. The hybridization to the array can be read using epifluorescence confocal scanning. This means that, in principle, in one automated test the genetic information of an infectious agent with regard to evolutionary lineage at different levels, including strain-identification, resist-

ance-related mutations, and possibly genetically defined virulence factors can be disclosed. This will certainly provide unprecedented knowledge to be used in our long-standing battle against tuberculosis and other infectious diseases.

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