Genotyping analyses of tuberculosis transmission among immigrant residents in Italy

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Abstract

We used DNA fingerprinting to analyse tuberculosis (TB) epidemiology in immigrant patients living in two major northern Italian urban areas. The study population included 1999 TB patients (1500 Italian-born and 499 immigrants). Univariate and multivariate logistic regression models were used to identify risk factors related to clustering similar proportions of immigrant and Italian-born patients (46%) had infection with TB strains that belonged to genetic clusters. This supports the hypothesis that the disease in foreign patients is more likely to have arisen from reactivation of latent infection acquired in the country of origin than from recent transmission. Gender, age, human immunodeficiency virus infection and drug resistance were not significantly linked to TB clustering. Risk factors associated with strain clustering were country of origin (Somalia, adjusted OR (AOR) 3.19, p 0.017; Peru, AOR 2.86, p 0.014; and Senegal, AOR 2.60, p 0.045) and city of residence. Immigrant status in the larger urban area was an independent risk factor for infection with clustered TB, as reinforced by a subanalysis of the Senegalese group. In conclusion, variations in TB transmission were observed among immigrants from different countries and even within national groups, where living conditions have been found to exert a profound impact. These results emphasize the importance of improving social integration of immigrant subjects in order to limit risks of TB transmission in developed countries.

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Introduction

The number of new cases of tuberculosis (TB) per capita appears to have been falling globally since 2003 [1]. However, the number of TB cases reported among immigrant people has continued to rise in Western countries [2]. The control of TB requires a greater understanding of disease transmission among immigrant patients, especially in major metropolitan areas with large foreign communities, where problems of integration abound.

Recent advances in molecular biology have provided new tools with which to better comprehend the epidemiology of the disease. DNA fingerprinting using the IS*6110* insertion sequence as the basis of analysis of restriction fragment length polymorphism (RFLP) [3] has been used in population-based studies to estimate the extent of ongoing TB transmission in various communities and to identify the subgroups at highest risk [4]. Strain typing has revealed that a larger percentage of TB cases than previously suspected is caused by recent strain transmission, accounting for about 40% of new TB cases [5–7]. This raises new questions regarding the epidemiology of TB in industrialized countries.

During recent years, the overall incidence of TB in Italy has been stable at approximately 8–9 per 100 000 persons annually [8], but the percentage of foreign-born TB patients is increasing. A similar trend has been described in Lombardy, northern Italy's most populous region, where the percentage of immigrant patients among TB cases rose from 21.7% in 1997 to 45.3% in 2002 (Guidelines for TB Control of Lombardy Region, *http://www.dgsan.lombardia.it/delibere/DGR2004_19767.pdf*).

In the present study, we describe the results of a 7-year population-based analysis of molecular TB epidemiology in immigrant patients living in two metropolitan areas with two different patterns of immigration. The first is a large urban area, with immigrant people coming from multiple geographical origins, with the typical problems of social integration in large metropolitan areas (Milan). The second is a highly industrialized province, with a few clusters of national immigration and higher rates of social integration and lower rates of unemployment (Brescia) [9]. The aims of the study were to estimate levels of ongoing TB transmission and to identify the subgroups most at risk, in order to facilitate the development of targeted TB-control interventions.

Materials and Methods

Lombardy is the most populous region in Italy, with approximately nine million residents. The two largest cities are Milan (about 1.3 million inhabitants) and Brescia (about 190 000 inhabitants). The provinces of Brescia and Milan have the highest estimated immigration levels in north-western Italy, with 93 and 78 immigrant people per 1000 inhabitants, respectively, in 2003 [10].

Setting

Our study was performed at the Infectious Diseases Departments of Milan and Brescia, and at the Regional Reference Centre for TB Control at the Villa Marelli Institute in Milan, designated for collection of all *Mycobacterium tuberculosis* strains isolated in the Milanese region. Because of this, virtually all *M. tuberculosis* isolates in the metropolitan areas of Milan [11] and Brescia are included in our database.

Study population

All cases of active TB observed during a 7-year prospective study period (July 1993 to June 2000) at the three institutions involved in the study were eligible for inclusion. At least one isolate for every culture-confirmed TB case patient was sent to the Institute of Infectious and Tropical Diseases of the University of Milan for fingerprinting analysis. Prospectively collected clinical and epidemiological information was obtained from clinical charts at the three institutions.

Molecular typing techniques

All *M. tuberculosis* strains were typed by RFLP DNA fingerprinting analysis, using an IS*6110* probe, following previously described standard methods [3]. As a secondary typing method, spoligotyping analysis was used to help differentiate strains with IS*6110* low copy number [12]. Isolates with identical IS*6110* patterns containing seven or more bands, or containing six or fewer bands with identical spoligotyping, were considered to be clonally related. A cluster was defined as two or more patients with clonally related TB strains. Clustered patients were assumed to belong to a chain of recent transmission; patients whose *M. tuberculosis* isolates displayed unique patterns were regarded as sporadic and assumed to have reactivated TB.

GelCompar software, version 4.1 (Applied Maths BVBA, Kortrijk, Belgium), was used to compare hybridization patterns obtained by RFLP DNA fingerprinting and spoligotyping. Following analysis, patients' isolates were compared with our database of more than 3000 TB DNA patterns in order to identify possible 'clusters' of matching isolates. This was performed by the unweighted pair group method using arithmetic averages [13].

Statistical analysis

We used SPSS for Windows (version 15.0; SPSS, Inc., Chicago, IL, USA) for calculation of summary statistics and comparisons. Pearson's chi-square test and Fisher's exact test were performed to check for differences between categorical variables. For univariate and multivariate analyses, logistic regression analysis was employed to identify independent factors related to clustering in the overall population. Risk factors in the initial model included: age; gender; *in vitro* susceptibility; human immunodeficiency virus (HIV) infection; city of residence; and country of origin. Estimates of crude ORs and adjusted ORs (AORs) were obtained from the models by comparing each category level with the reference, which was chosen according to clinical or epidemiological relevance. Specifically, in the case of country of origin, Philippine nationality was used as the reference, because this is the group with the longest history of consolidated immigration in Italy.

Results

A total of 2218 *M. tuberculosis* strains from culture-proven new TB cases were isolated during the 7-year study period. Of these, 219 were excluded because isolates were not available for analysis or failed to regrow after freezing, or because of incomplete relevant epidemiological and clinical data. In the final analysis, 1999 strains were included (Fig. 1).

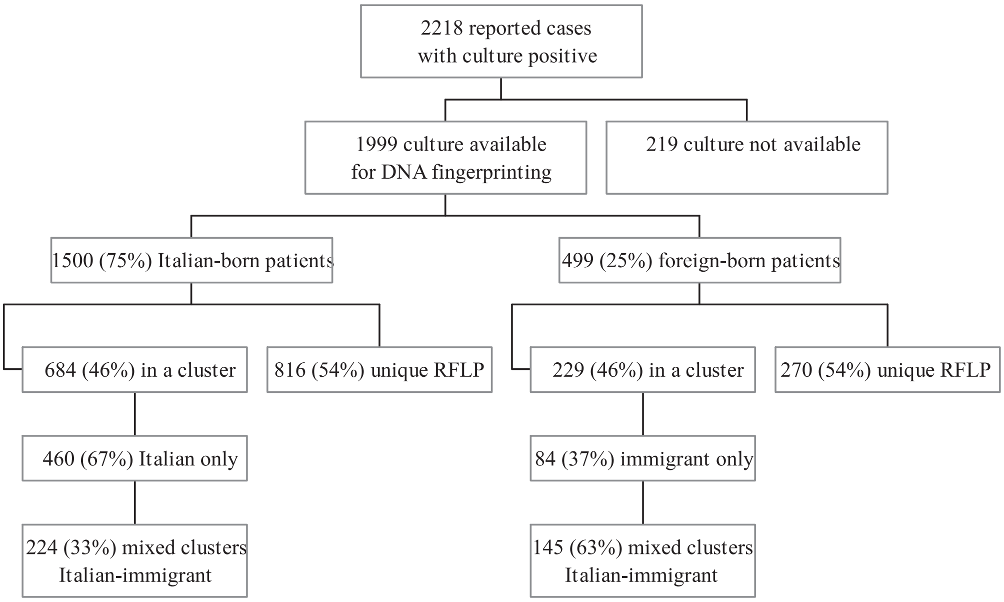

FIG. 1. Flow chart for tuberculosis cases in Milan and Brescia, Lombardy, Italy, 1993–2000. RFLP, restriction fragment length polymorphism.

TABLE 1. Clustered *Mycobacterium tuberculosis* isolates in immigrant patients by geographical region of origin

Geographical region	Total	Clustered isolates (%)	Non-clustered isolates (%)
Africa	220	101	119
North	70	29 (41.4)	41 (58.6)
East	51	28 (54.9)	23 (45.1)
West	95	42 (44.2)	53 (55.8)
Others[a]	4	2 (50)	2 (50)
Asia	71	23	48
China	17	7 (41.2)	10 (58.8)
Indian subcontinent	17	4 (23.5)	13 (76.5)
Philippines	31	10 (32.2)	21 (67.8)
Others[b]	6	2 (33.3)	4 (66.7)
South America	168	90	78
Peru	151	85 (56.3)	66 (43.7)
Others[c]	17	5 (29.4)	12 (70.6)
Europe	40	15	25
Eastern	38	13 (34.2)	25 (65.8)
Western[d]	2	2 (100)	0
Total	499	229 (45.9)	270 (54.1)

[a] Mauritius, Republic of South Africa, Uganda, Angola.
[b] Turkey (*n* = 3), Syria (*n* = 2), Thailand (*n* = 1).
[c] Brasil (*n* = 12), Ecuador (*n* = 4), Dominican Republic (*n* = 1).
[d] The Netherlands, Spain.

TABLE 2. Demographic characteristics of immigrant tuberculosis case patients in Milan and Brescia, Italy

Demographics	Immigrants in Milan (%), *n* = 419	Immigrants in Brescia (%), *n* = 80	Total (%), *n* = 499	p-value
Sex				
Male	267 (63.7)	71 (88.7)	338 (67.7)	**<0.01**
Female	152 (36.3)	9 (11.3)	161 (32.3)	
Age group (years)				
<36	302 (72.1)	40 (50)	342 (68.5)	**<0.01**
36–55	99 (23.6)	35 (43.7)	134 (26.9)	
>55	18 (4.3)	5 (6.2)	23 (4.6)	
HIV status				
Positive	56 (13.4)	6 (7.5)	62 (12.4)	0.10
Negative	363 (86.6)	74 (92.5)	437 (87.6)	
Country of origin				
Senegal	36 (8.6)	49 (61.2)	85 (17.0)	**<0.01**
Others	383 (91.4)	31 (38.8)	414 (83.0)	
In vitro susceptibility				
Susceptible	322 (76.8)	73 (91.3)	395 (79.2)	**0.03**
Multiresistant	23 (5.5)	0	23 (4.6)	
Resistance, others	74 (17.7)	7 (8.7)	81 (16.2)	
Time of stay in Italy (months)[a]				
<24	106	1	107	0.11
24–48	52	2	54	
>48	71	5	76	
Clustered isolates				
Yes	204 (48.7)	25 (31.2)	229 (45.9)	0.77
No	215 (51.3)	55 (68.8)	270 (54.1)	

HIV, human immunodeficiency virus.
[a] Data available for 237 patients only.
Bold characters indicate significant p values (<.05).

General characteristics of immigrant patients.

Of the 1999 TB strains included in the analysis, 499 originated from immigrant patients (25%). Among these, 220 (44.1%) were from African-born patients, 71 (14.2%) from Asian-born patients, 168 (33.7%) from Latin American-born patients, 38 (7.6%) from patients born in eastern Europe, and two from patients born in western Europe (0.4%). Overall, 38 different countries of origin were represented (Table 1).

Clinical and demographic data for these patient groups are presented in Table 2. Notably, the majority of patients were male (67.7%), with a median age of 33 years. Only five patients were over 64 years old. There were 62 patients (12.4%) with HIV–*M. tuberculosis* co-infection. This condition was particularly frequent among Latin American subjects (50% of patients from Brazil).

More than three-quarters of the isolates (395/499, 79.2%) were susceptible to all tested anti-TB drugs. Overall, 69 isolates (13.8%) were resistant to at least isoniazid; rifampin resistance was detected in 28 isolates (5.6%), ethambutol resistance in 35 (7.0%), and streptomycin resistance in 69 (13.8%). A total of 23 patients (4.6%) had infection with multidrug-resistant isolates.

Data regarding the duration of stay in Italy at the time of TB diagnosis were available for only 237 of 499 patients (47.4%), and indicated that 61 (25.7%) patients were diagnosed within 1 year of arrival in Italy, 100 (42.2%) within 1–4 years after arrival, and 76 (32.1%) more than 4 years after arrival. Significant differences were detected between different nationalities.

Table 2, showing data comparing immigrants in Brescia with those in Milan, highlights the prevalence of Senegalese among immigrants with TB in Brescia (61.2% vs. 8.6%, p <0.001), whereas in Milan, the Peruvian immigrant community was the most affected (33.6%).

Risk factors for clustering in immigrant patients

During the 7-year study period, 45.9% (229/499) of the immigrant TB patients were infected with a strain that was clonally related to ≥ 1 other strains, as compared with 45.6% (684/1500, p 0.951) of Italian-born cases.

Youth (<36 years) was not an independent risk factor for belonging to a cluster: the mean age for patients with clustered isolates was similar to the mean age of those infected with unique isolates (33.7 and 33.6 years, respectively). Nor was infection by a strain that was resistant to isoniazid and rifampicin an independent risk factor (p 0.823). Other factors not significantly associated with clustering were gender (p 0.703) and HIV infection (p 0.249) (Table 3). Median duration of stay in Italy was equal (36 months) for patients with clustered and non-clustered strains, but these data were available for less than half of the population and were therefore not included in the statistical model.

We compared the various national groups to determine whether they differed significantly in terms of clustering rates. Higher proportions of clustered isolates were observed in patients from East Africa (overall rate of 54.9%) and from Peru (56.3%), whereas clustering rates in patients from other areas were generally below 45%. In particular, the multivariate analysis showed that birth in either Somalia (AOR 3.19, p 0.017), Peru (AOR 2.86, p 0.014) or Senegal (AOR 2.60, p 0.045) was a significant risk factor for belonging to a cluster.

We observed a significant difference in clustering between immigrant patients in Milan and those in Brescia (48.7% and 31.2%, respectively), confirmed by multivariate analysis (AOR 0.42; p 0.006).

TABLE 3. Logistic regression analysis of being part of a cluster in 499 immigrant patients with tuberculosis

Patient characteristics	AOR (95% CI)	p-value
Gender		
Male	1	
Female	1.087 (0.707–1.673)	0.703
Age (years)		
<36	1	
36–55	1.202 (0.783–1.843)	0.400
>55	1.002 (0.41–2.429)	0.996
In vitro susceptibility		
Susceptible	1	
Multidrug resistance	1.109 (0.450–2.728)	0.823
Resistance, others	0.717 (0.431–1.195)	0.202
HIV infection		
Yes	1	
No	0.715 (0.404–1.264)	0.249
City of residence		
Milan	1	
Brescia	**0.421 (0.229–0.777)**	**0.006**
Country of origin		
Philippines	1	
Somalia	**3.186 (1.227–8.271)**	**0.017**
Peru	**2.860 (1.238–6.608)**	**0.014**
Senegal	**2.597 (1.023–6.590)**	**0.045**
Morocco	1.729 (0.688–4.346)	0.244
Others	1.292 (0.534–3.131)	0.570

AOR, adjusted OR; HIV, human immunodeficiency virus.
Bold characters indicate significant p values (<.05).

We performed a subsequent comparison between the two different Senegalese groups in Milan and Brescia to determine whether they differed significantly in terms of clustering rates. In Milan, 20 of 36 patients (55.5%) were infected with clustered isolates, as compared with 17 of 49 Senegalese patients in Brescia (34.7%).

Multivariate analysis of clustering risk factors in the Senegalese TB population demonstrated that Milanese residence was related to clustering (AOR 2.18, p 0.09), but age, gender, drug susceptibility or HIV infection were not.

Discussion

In recent years, multiple molecular epidemiological studies have been employed to clarify the epidemiology of TB among immigrants in countries with low rates of the disease. DNA fingerprinting has been used to distinguish between epidemiologically related and unrelated strains of *M. tuberculosis*. People with clustered isolates are assumed to belong to a transmission chain, whereas those with unique isolates are assumed to have reactivated disease.

In both European [14–17] and US [18,19] studies, molecular investigations of this type have shown that immigrants are not more likely to have clustered isolates than unique ones. Our data from the present Italian study support this conclusion: most TB cases in immigrants were shown to have been probably caused by reactivation of old or recent infections that occurred in the person's country of origin rather than by new infections acquired in Italy. However, the percentage of clustered isolates among immigrants in Italy appeared to be higher than in other European countries [14,15,20], with wide variability among country of origin.

In our study, age, gender, HIV-seropositivity and drug resistance were not independent predictors of strain clustering. The most relevant factor related to clustering seemed to be country of origin. In particular, Peruvian patients (the most numerous community of immigrant patients in Milan) were at significant risk of belonging to clusters, and this could explain our higher percentage of clustered isolates among immigrants as compared with other European studies. Patients from Somalia and Senegal were also at significant risk of clustering.

In light of these differences between immigrant populations, it would seem inadvisable to group all immigrant patients together when analysing TB transmission patterns [21]. The contrasting situation that we described in Peruvian vs. Philippino patients is of particular interest: Peruvians have the highest rate of clustering (56%) and Philippinos the lowest (32%).

This may merely reflect the different social habits of these two national groups. Philippinos, with a history of consolidated integration dating from the 1970s (about 50% had been resident in Italy for more than 10 years), had the highest levels of legalized immigration, remittance rates, and long-term employment rates [10,22]. In contrast, Peruvians make up a relatively recent immigrant group (only 20% of Peruvians had been resident in Italy for more than 10 years) and had the least favourable remittance rate per person among the groups statistically analysed [22]. A negative social stability index thus correlated with a tendency to belong to clusters of recent TB transmission. These data concur perfectly with previous findings indicating that Peruvians in Italy have ten-fold higher rates of TB than Peruvians in their country of origin, whereas Philippinos residing in Italy have lower rates than those reported in the Philippines [23]. The most likely explanation for these phenomena is the substantially varied history of social integration among different national communities. The probable influence of these factors on TB spread is further demonstrated by results for Senegalese immigrants studied in two different cities. We found that the OR of infection with a clustered strain among Senegalese patients living in the large urban area was double that of those living in the smaller town, and that city of residence was the sole significant risk factor for clustering. The different rates of TB transmission are probably associated with the contrasting social conditions of immigrant patients in the two cities during the study period. Living and working conditions in the smaller town were considered to be generally satisfactory in comparison with the more precarious living conditions of many immigrant residents in the larger city.

The role of social factors has been identified in previous studies. Low income, lack of high school diploma, alcohol dependence and homelessness were independent predictors of strain clustering, among both US-born patients [5–7,18,24] and European-born patients [17,25,26]. Although we did not analyse these factors in our study, variations in clustering rates observed within national groups, depending on city of residence, suggests that living conditions, rather than provenance from a country with high incidence of the disease, is the key factor favouring TB transmission.

In order to fully examine the extent to which Italian and immigrant TB patients transmit TB, a longitudinal investigation of contacts would be necessary. Moreover, results from one particular setting cannot always be extrapolated to others, owing to the diversity of characteristics in local populations. Nevertheless, our observation that in Italy the proportion of TB isolates in clusters among immigrant patients was similar to that observed in indigenous patients supports the widely accepted hypothesis that the disease in foreign patients is more likely to have arisen from reactivation of latent infection acquired in the country of origin than from recent transmission. These findings emphasize yet again the importance of improving living conditions of immigrant subjects in order to limit the risks of TB transmission in developed countries.

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Transparency Declaration

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