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ORIGINAL PAPER

Exogenous reinfection of tuberculosis in a low-burden area

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Abstract
Purpose Recurrence of tuberculosis (TB) can be the consequence of relapse or exogenous reinfection. The study aimed to assess the factors associated with exogenous TB reinfection.
Methods Prospective cohort study based on the TB database, maintained at the Division of Infectious Diseases, Luigi Sacco Hospital (Milan, Italy). Time period: 1995–2010. Inclusion criteria: (1) ≥2 episodes of culture-confirmed TB; (2) cure of the first episode of TB; (3) availability of one *Mycobacterium tuberculosis* isolate for each episode. Genotyping of the *M. tuberculosis* strains to differentiate relapse and exogenous reinfection. Logistic regression analysis was used to assess the influence of risk factors on exogenous reinfections.
Result Of the 4682 patients with TB, 83 were included. Of these, exogenous reinfection was diagnosed in 19 (23 %). It was independently associated with absence of multidrug resistance at the first episode [0, 10 (0.01–0.95), $p = 0.045$] and with prolonged interval between the first TB episode and its recurrence [7.38 (1.92–28.32) $p = 0.004$]. However, TB relapses occurred until 4 years after the first episode. The risk associated with being foreign born, extrapulmonary site of TB, and HIV infection was not statistically significant. In the relapse and re-infection cohort, one-third of the patients showed a worsened drug resistance profile during the recurrent TB episode.
Conclusions Exogenous TB reinfections have been documented in low endemic areas, such as Italy. A causal association with HIV infection could not be confirmed. Relapses and exogenous reinfections shared an augmented risk of multidrug resistance development, frequently requiring the use of second-line anti-TB regimens.

Keywords Tuberculosis · Exogenous reinfection · Relapse · Fingerprinting

Introduction

Recurrence of tuberculosis (TB) is not infrequent after the completion of a full-course anti-tubercular treatment. Recurrence rate varies from 0.6 to 8.4 per 100 patients/year [1–5]. TB recurrence may be the consequence of either relapse or exogenous reinfection. In the last decades relapse and exogenous reinfections have been alternatively considered to be the leading cause of the recurrence of TB. The molecular characterization of *Mycobacterium tuberculosis* strains and the comparison of their genotypic profile posed an end to this long-lasting debate [6, 7]. The role played by exogenous reinfections and relapses in the recurrence of TB varies widely. Several factors account for this variability, while poor compliance to therapy, clinical presentation (e.g. pulmonary cavitation), and resistance to first-line anti-tuberculous drugs seems to favour relapses, high incidence of TB in the population and human immunodeficiency virus (HIV) infection are associated with an increased probability of TB reinfection [8–17]. At this regard, the results of numerous studies that have assessed the influence of HIV on the risk of exogenous reinfection

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are non-univocal: while Jasmer and Luzze showed similar recurrence rate of TB in HIV-infected and HIV-non-infected patients [1, 5], TB tended to recur more frequently in the HIV-infected population in other studies [2, 3, 7].

Our study was conducted in Lombardy, the most populated region of Italy, in which the occurrence of TB was characterized by a general increase in the early 1990s, due to the increasing flows of immigration and the diffusion of HIV epidemics. We have previously analysed recurrence in the years 1995–2010: during this period HIV infection played a significant role in the resurgence of TB [12], leading to nosocomial outbreaks of multidrug resistance (MDR)-TB [18, 19], that could favour relapse of TB, as previously described [10]. Now, the HIV-related immunosuppression is reduced due to the introduction of highly active antiretroviral therapy (HAART), widely available after 1997. Thus, a modification of HIV/TB interrelations is conceivable. In 2010, the approximate annual TB incidence rates for Italian-born and immigrant patients in the Lombardy region were about 6 per 100,000 Italian-born residents and 80 per 100,000 foreign-born residents [20, 21].

In this new study, we have widened our investigation about recurrence of TB to the period 1996–2010, aiming to determine the characteristics of TB recurrences in our population and to find out whether recurrent disease was due to relapse or reinfection, and to analyse risk factors for these two mechanisms.

Methods

Setting and study population

This prospective cohort study was based on data within the TB database maintained at the III Division of Infectious Diseases, Luigi Sacco Hospital (Milan, Italy). The TB database contains prospective data collected between January 1, 1995 and December 31, 2010 on 4628 patients with culture-confirmed TB from three sites in Lombardy (Italy), Department of Infectious Diseases, Luigi Sacco Hospital (Milan) and Spedali Civili (Brescia), and the Regional TB Reference Center, Villa Marelli Institute, Niguarda Cà Granda Hospital (Milan), designated for collection of all *M. tuberculosis* strains isolated in the Milanese region. Because of this, virtually all *M. tuberculosis* isolates in the metropolitan areas of Milan and Brescia are included in our database.

Lombardy is the most populated region of Northern Italy (9.8 million inhabitants) and is characterized by a high prevalence of immigrants from countries where TB is still endemic. The region also has a high percentage of HIV-infected patients, accounting for more than one quarter of the Italian AIDS diagnoses (6504 out of 23,895 cases) [22]. The two largest cities are Milan (about 1.3 million inhabitants) and Brescia (about 190,000 inhabitants).

Eligible patients met all of the following criteria: (i) two or more episodes of TB diagnosed in the period 1995–2010 (with a minimum time interval of 2 months between the end of the first TB treatment and a new episode of TB); (ii) presumed cure of the first episode of TB based on clinical, radiological and microbiological findings. The outcome was assessed after completion of at least 6 months of anti-TB treatment; (iii) availability of at least one *M. tuberculosis* isolate for each TB episode in order to perform molecular analyses. For patients experiencing multiple recurrences, only the first recurrent episode was included in the study.

Clinical specimen collection and susceptibility testing

For each episode a single isolate was analysed. *M. tuberculosis* isolates were stored at the Infectious Diseases Laboratory, Luigi Sacco Hospital (Milan, Italy) and were cultured with BACTEC Mycobacterial Growth Indicator Tube (MGITTM69) (Becton–Dickinson, UK). The susceptibility profile to first-line anti-TB drugs was derived from the results of the susceptibility tests performed on the initially cultured strains at local laboratories. The following drug concentrations were used to test the susceptibility of *M. tuberculosis* to anti-TB molecules: isoniazid (1 mg/L), ethambutol (5 mg/L), rifampin (1 mg/L), and streptomycin (10 mg/L).

Genotyping

Mycobacterium tuberculosis DNA was extracted from mycobacterial colonies growing on Lowenstein-Jensen medium according to standard laboratory procedures. Two methods were used to genotype *M. tuberculosis* strains: Spacer Oligonucleotide Typing (Spoligotyping) as described by Kamerbeek et al. [23] and mycobacterial interspersed repetitive unit of variable number of tandem repeats (MIRU-VNTR) 12 *loci* genotyping as described by Supply et al. [24].

Definitions

Recurrence was defined as a culture-based diagnosis of TB in a patient with a previously cured episode of TB.

The confirmation of the cure of the first episode of TB was obtained by radiography, clinical assessment, and microbiological examination. Microbiological cure was attained when the patient had at least two negative sputum cultures during the course of treatment [25].

The definition of relapse or exogenous reinfection was based on the genotypic profile of the strains responsible for

each TB episode. An identical spoligotyping and MIRU-VNTR pattern was considered consistent with relapse, while a different profile was suggestive of exogenous reinfection. Patients diagnosed with relapse were assigned to 'Relapse Cohort', while patients diagnosed with exogenous reinfection were assigned to 'Re-infection Cohort'.

For each patients enroled in the study, the following data were analysed: sex, age, HIV sero-status, country of origin, social risk factors for TB (recent institutionalization in prison; shelter or nursing home; homelessness; alcohol abuse; intravenous drug use; recent contact with active TB cases); the elapsing time from the two episodes of TB was calculated from the end of the first treatment and the date of the diagnosis of recurrence; in vitro susceptibility data of *M. tuberculosis* isolates (multidrug resistance was considered the contemporary lack of susceptibility to both isoniazid and rifampin); and pulmonary or extrapulmonary site of TB.

Statistical analysis

Continuous variables are presented as medians with ranges. Categorical variables are presented as frequencies and percentages of the specified group. Comparisons between groups were made with the χ^2 test, Fisher-exact test, and Kruskal–Wallis test as appropriate. A logistic regression model was used to compute the adjusted risk of exogenous reinfection. All variables found to have a univariable association with exogenous reinfection ($p < 0.05$) were entered in the model. Adjusted odds ratio (aOR) and 95 % confidence interval (95 % CI) were calculated. A two-sided p value of <0.05 was considered statistically significant. Statistical analyses were performed using SPSS software (version 17.0, Chicago, Illinois, USA).

Ethical standards

The study has been performed in accordance with the ethical standards laid down in the 1964 Declaration of Helsinki and its later amendments. The study has been submitted to the ethics committee and approved in May, 2014.

Results

Of the 4628 patients available in the TB database, 83 patients met the inclusion criteria. Sixty-four patients (77.1 %) were diagnosed with a relapse and were included in the 'Relapse Cohort'. The remaining 19 patients (22.9 %) formed the 'Re-infection Cohort'.

The majority of the patients were adult men [median (range): 41 (20–96) year-old]. Of the 83 subjects in the study, 48 (57.8 %) were born in Italy and 35 patients were born outside the country [Eastern Europe ($n = 9$), Southern America ($n = 9$), Asia ($n = 7$), Central Africa ($n = 6$), and Northern Africa ($n = 4$)].

The percentage of TB recurrences attributable to exogenous reinfections tended to increase over time but without a statistical significance [8/50 (16 %) in the period 1995–2002 vs. 11/33 (33.3 %) in the period 2003–2010; OR 2.62, 95 %CI 0.92–7.48, p value 0.07]. Demographic characteristics were similar in the relapse and reinfection groups except that patients with a diagnosis of exogenous reinfection were more frequently foreign born [12/19 (63.2 %) vs. 23/64 (35.9 %); p value 0.04]. No differences in the prevalence of HIV infection and of other risk factors for TB were observed in the two cohorts [4/19 (21.1 %) vs. 9/64 (14.1 %), p value 0.47; 2/16 (12.5 %) vs. 11/55 (20.0 %), p value 0.50]. The median time between the end of the treatment of the first episode and the recurrence of TB was 9 months (IQR 6–18 months) in the 'Relapse Cohort' and 30 months (IQR 9-57 months) in the 'Re-infection Cohort'. A prolonged interval (>24 months) between the first TB episode and its recurrence was more common among patients experiencing an exogenous reinfection than a relapse [11/19 (57.9 %) vs. 11/56 (19.6 %); p value <0.01]. Of the 64 relapses, 38 (59.4 %) occurred in the first 12 months following the end of the treatment, and ten after two years (15.6 %). No relapses were documented after the end of the fifth year. The prevalence of pulmonary infections was similar in both cohorts. MDR isolates were more frequently detected among patients who subsequently experienced a relapse of their initial TB episode compared to patients who developed an exogenous reinfection [20/32 (62.5 %) vs. 1/19 (5.3 %); p value 0.03] (Table 1). Of note, comparing the susceptibility profile of non-MDR strains isolated in the first episode with the corresponding strains isolated in the recurrent episode, we observed a similar propensity to develop MDR-TB in the 'Relapse and Re-infection Cohorts' [12/40 (30 %) vs. 7/17 (41.2 %); p value 0.49] (Table 2).

While several variables were associated with exogenous reinfection in the univariable analysis (Table 1), logistic regression analysis demonstrated that exogenous reinfection was independently associated with elapsing time >24 months and absence of multidrug resistance. Moreover, in our setting exogenous reinfection did not have a statistically significant association with HIV infection [aOR 2.1 (0.3–12.9); p value 0.44] (Table 3).

Discussion

The possibility of persons previously infected with *M. tuberculosis* being exogenously reinfected is not negligible and depends on the prevalence of disease: where the prevalence is higher the likelihood of exogenous reinfection

Table 1 Demographic, clinical, and treatment data of patients with relapsing TB ('Relapse Cohort') and of patients with exogenous TB reinfection ('Re-infection Cohort')

	'Relapse Cohort' $n = 64$	'Re-infection Cohort' $n = 19$	OR (CI 95 %)	p value
Age >40 years, *n* (%)	30/56 (54 %)	8/17 (47 %)	0.77 (0.26–2.29)	0.638
Male sex, *n* (%)	47/64 (73 %)	14/19 (74 %)	1.01 (0.32–3.24)	0.983
HIV infection, *n* (%)	9/64 (14 %)	4/19 (21 %)	1.63 (0.44–6.03)	0.465
Foreign–born, *n* (%)	23/64 (36 %)	12/19 (63 %)	3.06 (1.06–8.85)	**0.039**
Social risk factors for TB[a]	11/55 (20 %)	2/16 (13 %)	0.57 (0.11–2.89)	0.499
Elapsing time from the episodes >24 months, *n* (%)	11/56 (20 %)	11/19 (58 %)	5.63 (1.83–17.31)	**0.003**
In vitro susceptibility (1st episode)				
Susceptible, *n* (%)	29/62 (47 %)	15/19 (79 %)	1	–
Resistance not MDR, *n* (%)	13/62 (21 %)	3/19 (16 %)	0.45 (0.11–1.81)	0.259
MDR, *n* (%)	20/62 (32 %)	1/19 (5 %)	0.10 (0.01–0.79)	**0.029**
Extrapulmonary site of TB, *n* (%)	8/55 (15 %)	5/17 (29 %)	2.45 (0.68–8.85)	0.172

Bold values indicate stastistical significance ($p < 0.05$)

CI confidence interval, *HIV* human immunodeficiency virus, *MDR* multidrug resistance, *OR* odds ratio

[a] Alcoholism, drug addiction, homelessness, incarceration, recent contact with TB case

Table 2 Antimicrobial susceptibility trends of *M. tuberculosis* strains isolated during the first TB episode and the recurrent TB episode in 'Relapse Cohort' and 'Re-infection Cohort'

	n	1st episode	2nd episode
'Relapse Cohort' $n = 64$	20	Susceptible	Susceptible
	8	Susceptible	Resistance non-MDR (3 H, 2 R, 1 E, 1 S, 1 Z)
	1	Susceptible	Na
	7	Resistance non-MDR (3 H, 2 S, 1 HS, 1 R)	Unmodified
	4	Resistance non-MDR (2 HS, 1 S, 1 R)	MDR
	1	Resistance non-MDR (Z)	Susceptible
	1	Resistance non-MDR (Z)	Na
	20	MDR	MDR
	1	Na	Resistance non-MDR (H)
	1	Na	MDR
'Re-infection Cohort' $n = 19$	9	Susceptible	Susceptible
	2	Susceptible	Resistance non-MDR (1 E, 1 S)
	4	Susceptible	MDR
	1	Resistance non-MDR (H)	Na
	1	Resistance non-MDR (H)	Susceptible
	1	Resistance non-MDR (HS)	MDR
	1	MDR	MDR

E ethambutol, *H* isoniazid, *R* rifampin, *S* streptomycin, *Z* pyrazinamide, *na* not available, *MDR* multidrug resistance

increases [15–17, 26]. In low- to moderate-incidence countries, studies have found the percentage of reinfection ranging from 10 % in Switzerland to 33 % in Spain [13, 27, 28]. In studies of high-burden countries, reinfection was common, ranging from 23 % in Uganda and India [14, 29] to 68–77 % in South Africa [3, 9, 30].

To our knowledge, this is one of the largest studies investigating the recurrence of TB in Europe and the influence of HIV infection on this public health issue. A preliminary analysis regarding TB recurrence in the time period 1995–1999 has already been published by our group [12]. The current study confirmed that recurrence of TB is primarily due to the relapse of a previous episode and that exogenous reinfection may occur despite a low incidence of TB, in a proportion similar to previous European studies [13, 27, 28]. Finally, the study showed that there is no demonstrable association between HIV and exogenous TB reinfections in a low-burden country for TB, like Italy.

Table 3 Logistic regression analysis to evaluate risk factors for exogenous TB reinfection

	aOR (CI 95 %)	*p* value
HIV infection	2.06 (0.33–12.92)	0.439
Foreign born	2.27 (0.61–8.36)	0.219
Elapsing time from the episodes >24 months	7.38 (1.92–28.32)	**0.004**
MDR isolate at the first episode	0.10 (0.01–0.95)	**0.045**

Bold values indicate stastistical significance ($p < 0.05$)

aOR adjusted odds ratio, *CI* confidence interval, *HIV* human immunodeficiency virus, *MDR* multidrug resistance

Recurrence of TB is a frequent event among HIV population [10–30]. The role of HIV infection in favouring a TB recurrence due to exogenous TB reinfection has been emphasized in several studies, with estimated recurrence rates due to exogenous reinfection ranging from 4.1 to 9.4 patients/year among HIV-infected persons [1–3, 5, 7, 10–30]. This variability depends on the prevalence of TB in the study population (the higher the prevalence of TB, the greater the likelihood of exogenous reinfection) [1–3, 5, 7, 15–17, 27]. Although the majority of studies from Central and Southern Africa have reinforced the opinion that HIV is a relevant risk factor for exogenous TB reinfection [aOR 21.9 (7.8–61.4)] [11, 31], rates of exogenous TB reinfection were similar among HIV-infected and HIV-non-infected patients [aOR 1.2 (0.6–2.2)] in several European cohorts [1, 4, 12, 13, 32]. HIV infection seems to favour TB reinfections in regions where TB is endemic, which may be due to an increased risk of exposure to *M. tuberculosis* and/or to an impairment of T-cell response. As a consequence, public health strategies to prevent the spread of HIV infection may also reduce the incidence of TB recurrences. The experience of Luzze et al. [5, 33] corroborates the idea that extensive efforts to warrant antiretroviral coverage may be an indirect TB control measure in areas where TB is endemic, such as Uganda.

Previous research has under- or over-represented MDR strains in the study population [15], which has made it difficult to assess the influence of multidrug resistance on the recurrence of TB. However, our study has documented an association between MDR *M. tuberculosis* and increased risk of relapse of TB. Similar results have already been published by our group and by other authors [12, 31]. Furthermore, in this study, the *M. tuberculosis* strains isolated during the second episode were characterized by a more extensive spectrum of drug-resistance compared to the strains isolated during the first TB episode. The variation in the susceptibility pattern during the recurrent episode of TB required a second-line anti-TB regimen in one-third of the patients in both relapsed and reinfected patients. On the contrary, a more severe clinical presentation during the second episode (either new extrapulmonary *foci* or disseminated disease) was not significantly more frequent in the setting of exogenous reinfections.

Despite the firm association of exogenous reinfection with an elapsing time greater than 24 months, this study confirmed that TB relapses are possible even 4 years after the first episode. Thus, a prolonged follow-up after the conclusion of the anti-TB treatment may be needed [4].

Immigrants from countries where TB is still endemic account for most of the TB reinfections in Western Europe [4]. The increased migrations from endemic regions together with the precarious and overcrowded housing conditions of immigrants may explain the uptrending incidence of exogenous reinfections in the present study as in previous studies [12]. Although foreign origin was not an independent risk factor of exogenous reinfection in this analysis, further studies are needed to validate our findings.

Finally, logistic regression analysis did not show any significant association between age >40 year-old, sex, comorbidities (like diabetes and cancer) and social risk factors (as imprisonment; homelessness; alcohol abuse; intravenous drug use; and recent contact with active TB cases) for TB and exogenous reinfections. Previous studies by Bang et al. [4] and Sonnenberg et al. [11] showed that patients with pulmonary cavitation are at risk factor for relapse, suggesting a prolonged duration of treatment in this population. Our data are insufficient to further evaluate this association.

This study, which assessed patients not only reinfected but showing also a disease progression (excluding reinfected subjects without clinical active TB), has several noteworthy limitations. The relatively small number of patients with TB reinfection limits our ability to detect significant differences between groups. The high incidence of loss at follow-up in the study population made the calculation of the recurrence rate unreliable, and as a consequence evaluation of the recurrence rate was not included as a study objective. The genotyping techniques used have several inherent limitations. First, despite the high discrimination capacity of MIRU-VNTR, MIRU-VNTR cannot differentiate a relapse from a reinfection if the reinfection is due to a strain with the same pattern of the strain that caused the first TB episode. This event, although improbable, is possible in a low endemic setting. Second, an initial TB infection caused by multiple strains may lead to the misclassification of the recurrent TB episode [34]. Third, we used the same definition of cure for both MDR and non-MDR-TB. This choice has avoided differences in the criteria of inclusion of the patients; nevertheless it would have allowed the inclusion of some MDR-TB patients judged as cured. With a stricter definition of cure the latter ones could have differently classified if repeatedly negative cultures (at least five) for an adequate period of observation were warranted [35].

Finally, the external validity of our findings is not universal. Our results must be related to the particular setting in which this study was developed (*e.g.* migrations from high-endemic areas, spreading of nosocomial MDR strains during the first period of the study, and improved care of HIV infection).

In conclusion, relapse of a previous infection remains the most common cause of TB recurrence in low endemic areas like Italy. TB relapse is associated with multidrug resistance and with a short elapsing time between the first TB diagnosis and the recurrent episode. Reinfections are possible but not as frequent as documented in high-endemic areas. Outside these areas, HIV infection seems to exert a less relevant role in the development of exogenous reinfections. Nonetheless changes in the migration flows may modify the contribution of HIV infection on TB recurrence.

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Conflict of interest The authors declare that they have no conflict of interest.

References

1. Jasmer RM, Bozeman L, Schwartzman K, Cave MD, Saukkonen JJ, Metchock B, et al. Recurrent Tuberculosis in the United States and Canada. Am J Respir Crit Care Med. 2004;170:1360–6.
2. Picon PD, Bassanesi SL, Avancini Caramori ML, Targa Ferreira RL, Jarczewski CA, Rodrigues de Borba Vieira P. Risk factors for recurrence of tuberculosis. J Bra Pneumol 2007; 33:572–578.
3. Charalambous S, Grant AD, Moloi V, Warren R, Day JH, van Helden P, et al. Contribution of reinfection to recurrent tuberculosis in South African gold miners. Int J Tuberc Lung Dis. 2008;12:942–8.
4. Bang D, Andersen AB, Thomsen VO, Lillebaek T. Recurrent tuberculosis in Denmark: relapse vs re-infection. Int J Tuberc Lung Dis. 2010;14:447–53.
5. Luzze H, Johnson DF, Dickman K, Mayanja-Kizza H, Okwera A, Eisenach K, et al. Relapse more common than reinfection in recurrent tuberculosis 1-2 years post treatment in urban Uganda. Int J Tuberc Lung Dis. 2013;17:361–7.
6. Das S, Chan SL, Allen BW, Mitchison DA, Lowrie DB. Application of DNA fingerprinting with IS986 to sequential mycobacterial isolates obtained from pulmonary tuberculosis patients in Hong Kong before, during and after short-course chemotherapy. Tuberc Lung Dis. 1993;74:4751.
7. Mallory KF, Churchyard GJ, Kleinschmidt I, De Cock KM, Corbett EL. The impact of HIV infection on recurrence of tuberculosis in South African gold miners. Int J Tuberc Lung Dis. 2000;4:455–62.
8. Das S, Paramasivan CN, Lowrie DB, Prabhakar R, Narayanan PR. IS6110 restriction fragment length polymorphism typing of clinical isolates of Mycobacterium tuberculosis from patients with pulmonary tuberculosis in Madras, south India. Tuber Lung Dis. 1995;76:550–4.
9. van Rie A, Warren R, Richardson M, Victor TC, Gie RP, Enarson DA, et al. Exogenous reinfection as a cause of recurrent tuberculosis after curative treatment. N Engl J Med. 1999;341:1174–9.
10. Crampin AC, Mwaungulu JN, Mwaungulu FD, Mwafulirwa DT, Munthali K, Floyd S, et al. Recurrent TB: relapse or reinfection? The effect of HIV in a general population cohort in Malawi. AIDS. 2010;24:417–26.
11. Sonnenberg P, Murray J, Glynn JR, Shearer S, Kambashi B, Godfrey-Faussett P. HIV-1 and recurrence, relapse, and reinfection of tuberculosis after cure: a cohort study in South African mineworkers. Lancet. 2001;358:1687–93.
12. Bandera A, Gori A, Catozzi L, Degli Esposti A, Marchetti G, Molteni C, et al. Molecular epidemiology study of exogenous reinfection in an area with a low incidence of tuberculosis. J Clin Microbiol. 2001;39:2213–8.
13. Garcia de Viedma DG, Marin M, Hernangomez S, Diaz M, Serrano MJ, Alcala L, et al. Tuberculosis recurrences: reinfection plays a role in a population whose clinical/epidemiological characteristics do not favor reinfection. Arch Intern Med. 2002;162:1873–9.
14. Fitzpatrick LK, Okwera A, Mugerwa R, Ridzon R, Ehiner J, Onorato I. An investigation of suspected exogenous reinfection in tuberculosis patients in Kampala, Uganda. Int J Tuberc Lung Dis. 2002;6:550–2.
15. Lambert ML, Hasker E, Van Duen A, Roberfroid D, Boelaert M, Van der Stuyft P. Recurrence in tuberculosis: relapse or reinfection. Lancet Infect Dis. 2003;3:282–7.
16. Chiang CY, Riley LW. Exogenous reinfection in tuberculosis. Lancet Infect Dis. 2005;5:629–36.
17. Wang JY, Lee LN, Lai HC, Hsu HL, Liaw YS, Hsueh PR, et al. Prediction of the tuberculosis reinfection proportion from the local incidence. J Infect Dis. 2007;196:281–8.
18. Franzetti F, Gori A, Iemoli E, Meraviglia P, Mainini F, Quirino T, et al. Outcome of multidrug-resistant tuberculosis in human immunodeficiency virus-infected patients. Clin Infect Dis. 1999;29:553–60.
19. Moro ML, Gori A, Errante I, Infuso A, Franzetti F, Sodano L, et al. An outbreak of multidrug-resistant tuberculosis involving HIV-infected patients of two hospitals in Milan, Italy. AIDS. 1998;12:1095–102.
20. Codecasa LR, Porretta AD, Gori A, Franzetti F, Degli Esposti A, Lizioli A, et al. Tuberculosis among immigrants from developing countries in the province of Milan, 1993–1996. Int J Tuberc Lung Dis. 1999;3:589–95.
21. CCM, ISS e Regione Emilia-Romagna. La tubercolosi in Italia. Rapporto 2008. http://www.salute.gov.it/imgs/c_17_pubblicazioni_1472_allegato.pdf.
22. Istituto Superiore di Sanità. Notiziario ISS. 2014;27(9):16–22.
23. Kamerbeek J, Schouls L, Kolk A, van Agterveld M, van Soolingen D, Kuijper S, et al. Simultaneous detection and strain differentiation of *Mycobacterium tuberculosis* for diagnosis and epidemiology. J Clin Microbiol. 1997;35:907–14.
24. Supply P, Lesjean S, Savine E, Kremer K, Van Soolingen D, Locht C. Automated high-throughput genotyping for study of global epidemiology of Mycobacterium tuberculosis based on mycobacterial interspersed repetitive units. J Clin Microbiol. 2001;39:3536–71.
25. World Health Organization; International union Against Tuberculosis and Lung Disease and Royal Netherlands Tuberculosis Association. Revised international definitions in tuberculosis control. Int J Tuberc Lung Dis. 2001;5:213–5.
26. Fine PEM, Small PM. Exogenous reinfection in tuberculosis. N Engl J Med. 1999;341:1226–7.
27. Sudre P, Pfyffer GE, Bodmer T, Prod'hom G, Furrer H, Bassetti S, et al. Molecular epidemiology of tuberculosis among HIV-infected persons in Switzerland: a countrywide 9-year cohort study: swiss HIV Cohort Study. Infection. 1999;27:323–30.

28. El Sahly HM, Wright JA, Soini H, Bui TT, Williams-Bouyer N, Escalante P, et al. Recurrent tuberculosis in Houston, Texas: a population-based study. Int J Tuberc Lung Dis. 2004;8:333–40.
29. Sahadevan R, Narayanan S, Paramasivan CN, Prabhakar R, Narayanan PR. Restriction fragment length polymorphism typing of clinical isolates of *Mycobacterium tuberculosis* from patients with pulmonary tuberculosis in Madras, India, by use of direct-repeat probe. J Clin Microbiol. 1995;33:3037–9.
30. Murray J, Sonnenberg P, Shearer SC, Godfrey-Faussett P. Human immunodeficiency virus and the outcome of treatment for new and recurrent pulmonary tuberculosis in African patients. Am J Respir Crit Care Med. 1999;159:733–40.
31. Caminero JA, Pena MJ, Campos-Herrero MI, Rodríguez JC, Afonso O, Martin C, et al. Exogenous reinfection with tuberculosis on a European island with a moderate incidence of disease. Am J Respir Crit Care Med. 2001;163:717–20.
32. Martín A, Herranz M, Navarro Y, Lasarte S, Ruiz Serrano MJ, Bouza E, et al. Evaluation of the inaccurate assignment of mixed infections by Mycobacterium tuberculosis as exogenous reinfection and analysis of the potential role of bacterial factors in reinfection. J Clin Microbiol. 2011;49:1331–8.
33. Houben RM, Crampin AC, Ndhlovu R, Sonnenberg P, Godfrey-Faussett P, Haas WH, et al. Human immunodeficiency virus associated tuberculosis more often due to recent infection than reactivation of latent infection. Int J Tuberc Lung Dis. 2011;15:24–31.
34. Glynn JR, Murray J, Bester A, Nelson G, Shearer S, Sonnenberg P. High rates of recurrence in HIV-infected and HIV-uninfected patients with tuberculosis. J Infect Dis. 2010;201:704–11.
35. Laserson KF, Thorpe LE, Leimane V, Weyer K, Mitnick CD, Riekstina V, Zarovska E, Rich ML, Fraser HS, Alarcón E, Cegielski JP, Grzemska M, Gupta R, Espinal M. Speaking the same language: treatment outcome definitions for multidrug-resistant tuberculosis. Int J Tuberc Lung Dis. 2005;9:640–5.