

Comparison of Tuberculin Skin test and Quantiferon immunological assay for latent Tuberculosis infection

L.R. Codecasa¹, M. Ferrarese¹, V. Penati¹, C. Lacchini¹, D. Cirillo²,
C. Scarparo³, P. Piccoli³, C. Piersimoni⁴, G.B. Migliori⁵

ABSTRACT: *Comparison of Tuberculin Skin test and Quantiferon immunological assay for latent Tuberculosis infection. L.R. Codecasa, M. Ferrarese, V. Penati, C. Lacchini, D. Cirillo, C. Scarparo, P. Piccoli, C. Piersimoni, G.B. Migliori.*

Background. Correct identification of individuals with latent tuberculosis infection (LTBI) is a crucial element of the elimination strategy, allowing their adequate treatment. In addition to tuberculin skin test (TST), the Quantiferon test (QFT, based on whole blood γ -interferon release) had been recently proposed.

Aim of the study is to compare this test to TST for identification of LTBI in a non-selected population, in order to verify their value in identifying truly infected individuals (entitled to receive preventive chemotherapy), and to exclude from treatment those having a positive TST for other reasons (e.g. after BCG vaccination).

Methods. 136 consecutive persons (78 males, mean age 34 \pm 9 years) referred to the clinic for TST were re-

cruited (78 born in low - or middle - income countries). Based on their history, the cases were divided into 4 groups: 1) recently traced contacts of whom 18 TST negative and 28 TST positive; 2) 22 screening subjects, all TST negative; 3) BCG vaccinated subjects (14); and 4) 54 subjects already undergoing treatment of LTBI for exposure to TB.

Results. The overall agreement between TST and QFT was 72% (64% in TST positive and 88.4% in TST negative subjects). The proportion of TST positive/QFT negative BCG vaccinated individuals was 23.1%. The K coefficient was 0.474 in recently traced contacts, 0.366 in BCG vaccinated individuals and 0.451 overall.

Conclusions. The study results suggest that agreement between TST and QFT is lower in TST positive than in negative subjects, being lower in individuals treated for LTBI. Quantiferon does not seem to have brought significant improvement in the diagnosis of LTBI.

Monaldi Arch Chest Dis 2005; 63: 3, 158-162.

Keywords: Tuberculosis, latent tuberculosis infection, quantiferon test, tuberculin.

¹ Lombardia Region TB Reference Clinic and Laboratory, Villa Marelli Institute, Niguarda Hospital, Milano,

² Emerging and Re-emerging Bacterial Infections Unit, S. Raffaele Hospital, Milano,

³ Veneto Region TB Reference Laboratory, Vicenza,

⁴ Department of Clinical Microbiology, United Hospitals of Ancona,

⁵ WHO Collaborating Centre for TB and Lung Diseases, Clinical Epidemiology of Respiratory Diseases Service/ Dpt of Pnumonology, Fondazione S. Maugeri, Care & Research Institute, Tradate, Italy.

Correspondence: Dr G.B. Migliori, WHO Collaborating Centre for TB and Lung Diseases, Clinical Epidemiology of Respiratory Diseases Service/ Dpt of Pnumonology, Fondazione S. Maugeri, Care & Research Institute, Tradate, Italy; e-mail: gbmigliori@fsm.it

Introduction

Latent tuberculosis infection (LTBI) is a widespread condition involving about one third of the world population [1, 2]. It is characterized by the presence of "silent" mycobacteria in individuals who had been exposed to contagious tuberculosis (TB) cases. Approximately one out of ten of them will develop active TB during life [3, 4]. Identifying those persons is a crucial step towards TB elimination, because the development of active disease can be prevented with appropriate LTBI treatment [5-8]. Tuberculin skin test (TST) has been widely used as a screening tool, but the test is prone to significant individual variations [5, 9]. Furthermore, interferences due to cross reaction with environmental mycobacteria and BCG vaccination have been described, lead-

ing to a drastic reduction of the predictive value of the test [9]. Detection of serum level of IgG and IgM antibodies against several Mycobacterial antigens have also been proposed, but at the present time none of them could appropriately distinguish between LTBI and active or cured TB disease [8, 9].

The ideal LTBI detection test should be sensitive and specific (in order to reduce inappropriate preventive treatment), easy to perform (requiring a single office visit) and cost-effective.

Recently, a new test based on whole blood interferon gamma (γ INF) detection has been proposed as an alternative to TST [10]. The assays measures the γ INF in whole blood after challenging with *M tuberculosis* tuberculin (PPD). Among them, Quantiferon test or QFT (Cellestis Limited, Carnegie, Victoria, Australia) is the only one cur-

rently approved in USA (by Food and Drug Administration) and is now commercially available in Italy [11].

The aim of the study is to compare this test to TST for identification of LTBI in a non-selected population referred to the Lombardia Region TB Reference Centre, in order to offer a better identification of truly infected individuals (entitled to receive preventive chemotherapy), excluding from treatment those having a positive TST for other reasons (e.g. after BCG vaccination) [12].

Materials and Methods

Study design

This is a prospective, observational, open study.

Population

The study was conducted at the Lombardia Region TB Reference Centre (Villa Marelli Institute, Milano, Italy), managing over 400 TB cases per year. 136 consecutive persons (58 females, 78 males, mean age 34±9 years) referring to the Centre for TST testing were recruited for the study. All were 18 years or older [11] and all provided written informed consent. Reasons for TST testing were exposure to contagious TB case (100 persons), pre-employment or other work or immigration-related reasons (22 persons), and BCG post-vaccination screening (done in health care workers (HCW), according to the guidelines in force, in order to assess their baseline tuberculin skin reactivity for comparison in case of future exposure to contagious TB cases (14 persons) [13]. Countries of origin were: Italy or Western Europe for 58 persons and low- or middle- income Countries for 78 (majority from South America and North Africa). Data was collected on person's age, race, country of birth and residence, previous TST status, BCG vaccination and recent contact with active TB (sputum smear positive case). All subjects, except those belonging to the post-vaccination BCG group, reported that they had not been vaccinated with BCG in the last 10 years. Still, many of them being immigrants may have received the BCG vaccination in the past and perhaps more than once even if the post-vaccination scar is not visible. Blood for γ INF assay was drawn at the time of TST evaluation for 82 patients, while for the 54 patients known as TST positive and already on Isoniazid preventive treatment the test was performed at the end of treatment.

TST

The TST was administered by the Mantoux method using 5 TU of tuberculin (Biocine Test-PPD Chiron Vaccine, Siena, Italy) and interpreted according to international guidelines [6-8].

TST was measured 72 hours after administration by a trained HCW who registered the transverse diameter of induration. TST was considered

negative between 0-4 mm of diameter, weakly positive between 5-9 mm and positive if > 10 mm. The threshold to initiate treatment of LTBI for close contacts was established at 5 mm of induration according to guidelines in force [13].

Gamma Interferon assay

Blood for γ INF assay was drawn in 7ml heparinized blood tubes when patients were returning for TST reading or at the end of treatment. The assay was performed and interpreted according to manufacturer instructions [11]. In brief, 1ml aliquots of heparinized whole blood are incubated with antigens provided in the test for 14-24h. Antigens included a negative control (saline), PPD from *M. tuberculosis* (human PPD), PPD from *M. avium* (avian PPD) and a positive control (mitogen). 200 μ l of challenged blood were then collected and the amount of γ INF was determined by an ELISA assay. QFT results are based on the proportion of INF released in response to human PPD compared to a maximal response induced by mitogen.

A computer programme provided by the test manufacturer performed the calculations and interpreted the test results. The responsiveness to *M. tuberculosis* tuberculin for the Quantiferon test (% of Human Response) is expressed as a percentage of the subject's response to a non specific mitogen stimulus. According to the manufacturer the cut-off for a positive value for individual with no identified risk factors of TB exposure has been established at 30% [11].

Statistical analysis

Personal information was collected and entered onto an Excel spreadsheet (Microsoft Corporation, Redmond, Washington, USA). The TST status was determined for all of the patients.

Based on their history, the cases were divided into 4 groups: 1) recently traced contacts of whom 18 TST negative and 28 TST positive; 2) 22 screening subjects, all TST negative; 3) BCG vaccinated subjects [14]; and 4) 54 subjects already undergoing treatment of LTBI for exposure to TB cases.

For 82 of them, and precisely those belonging to group 1, 2 and 3 the γ INF assay was performed at the time of TST reading. The 54 already in course of Isoniazid treatment for LTBI (group 4) were tested upon completion of the treatment.

The analysis was done separately for persons referred for contact tracing (group 1) and persons referred for other reasons. In addition the analysis repeatedly excluded BCG vaccinated subjects.

Statistical analysis was performed using SPSS statistical software (version 7.5.1, SPSS Inc, Chicago, Ill). Concordance between TST and QFT test results was assessed using k coefficients (k > 0.75: excellent agreement; K between 0.4 to 0.75: fair to good agreement; K < 0.4 poor agreement) in recently traced contacts (group 1) and in BCG vaccinated individuals (group 2) [14].

Results

Between June and December 2002, 136 persons were enrolled in this study. In Groups 1, 2, 3, respectively, one case was removed from the analysis as the sample was not processed correctly for technical reasons.

The agreement between the two tests ranged from 95.2% in group 2 to 62.2% in group 4 (table 1). The overall agreement found between the 2 tests was 72% (95/132).

Table 1. - Agreement between Tuberculin skin test (TST) and Quantiferon test (QFT) in detecting Latent Tuberculosis Infection per group

	Group 1	Group 2	Group 3	Group 4
TSTpos/QFTpos	18	0	6	33
TSTpos/QFTneg	9	0	3	20
TSTneg/QFTneg	15	20	3	0
TSTneg/QFTpos	3	1	1	0
Total	45*	21*	13*	53
Agreement	33/45 (73.3%)	20/21 (95.2%)	9/13 (62.9%)	33/53 (62.2%)
K value	0.474°	–	0.366	–

* 1 case was removed from analysis for technical reasons.

The concordance was 64% in TST positive and 88.4% in TST negative subjects. Out of 32 individuals with discordant tests (TST positive/QFT negative), 28 (87.5%) have a TST induration ≥ 10 mm and 20 (62.5%) ≥ 15 mm. The proportion of TST positive/ QFT negative BCG vaccinated individuals was 23.1% (3 out of 13).

The K coefficient was 0.451 overall, 0.474 in recently traced contacts (group 1) and 0.439 in persons referred for other reasons (groups 2,3,4). The K coefficient, removing BCG vaccinated individuals from persons referred for other reasons was 0.451 (being 0.366 in BCG vaccinated individuals). TST and QFT test results are summarised in table 1. Five subjects resulted TST negative/QFT positive: in particular 3 belonged to group 1, and 1 respectively to group 2 and 3. No certain explanation of these cases can be proposed for them as, the study was designed such that the results were received after the sample collection and therefore many patients including these 5, could not be traced and retested for both TST and QFT. However, at least in the 3 subjects in group 1, a possible earlier positivity to QFT after contact could be presumed.

Individual results of TST induration and blood test values (QFT) are summarised in figure 1.

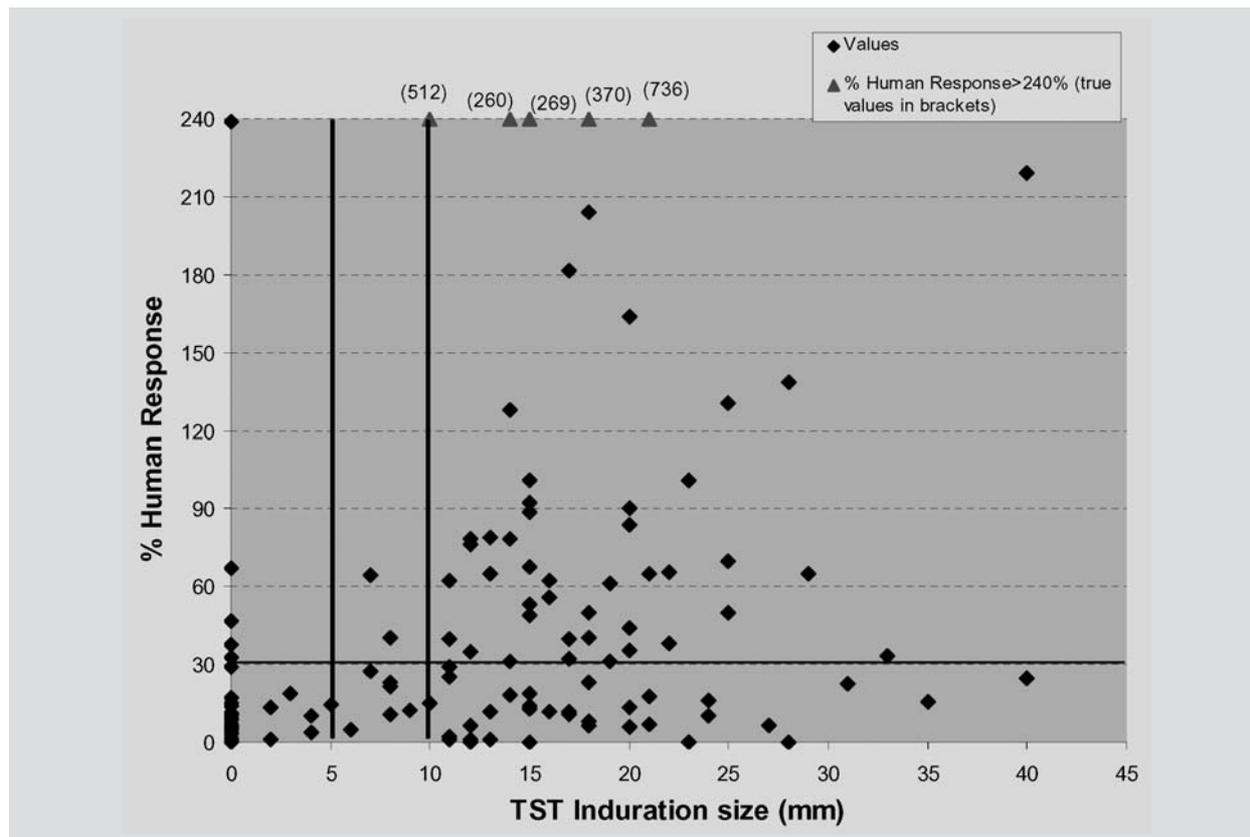


Fig. 1. - Individual results of TST induration sizes and Quantiferon blood test values (% Human Response). 5 results exceeding 240% had been reported on the 240% Quantiferon blood test values line with exact values in brackets. A horizontal line corresponding to 30% Human Response is considered the cut-off for positive Quantiferon results. A vertical line corresponding to 5 mm is considered the cut-off for weak positivity (and treatment of LTBI), while the line corresponding to 10 mm identifies a positive TST induration.

Discussion

Aim of the study is to compare TST and QFT tests for identification of LTBI in a non-selected population referred to the Lombardia Region TB Reference Centre in Milan. The agreement between the two tests was slightly lower than the 83.1% agreement found by Mazurek [11]. In our study the concordance was 64% in TST positive and 88.4% in TST negative subjects (being, respectively, 65 and 90% in Mazurek's study). Similar levels of agreement were found in HCW and immigrants in New Zealand [15]. Concordance over 90% had been detected by Streeton (90% for individuals not exposed and negative TST and for TST positive individuals not undergoing treatment of LTBI [16]. The proportion of TST positive/QFT negative BCG vaccinated individuals (23.1%) was consistent with Mazurek's findings. Differences in the study population are probably the main reason for the difference in agreement among the different studies. According to the results of a recent meta-analysis by Wang *et al* [12], patients who received BCG vaccination were more likely to have a positive TST and indurations > 15 mm were more likely to be due to LTBI. As described in table 1, all Group 3 individuals with discordant TST/QFT results had less than 15 mm induration.

The key problem in evaluating studies comparing TST and γ INF assays is the lack of a well established "gold standard". Although arguments suggesting that γ INF assays might be more sensitive than TST in detecting LTBI had been raised, (Intravenous Drug Users with or without HIV infection [17, 18], cattle model [19]), further evidence is necessary to consider γ INF assays as the reference tests [11]. In the absence of an agreed "gold standard" it is not possible to calculate sensitivity and specificity of both tests in diagnosing LTBI.

Focusing on the discrepancies found in our study, and assuming that QFT is more sensitive than TST, there are two groups of individuals that should be speculated: a) Potential TST false negative subjects (TST negative/ QFT positive) of which there are five. Three are known contacts of a sputum smear positive case, one recently immigrated from Congo and one (BCG vaccinated) recently immigrated from Peru. As all of them have risk factors, they might be candidates for treatment of LTBI, representing the potential gain in diagnostic accuracy of QFT over TST; b) Potential TST false positive (TST positive/ QFT negative) of which there are 32. Three of them are BCG vaccinated, (2 HCW and one immigrant from Peru), 9 are recent contacts of a sputum smear positive TB case (5 being immigrants from high and middle income countries and 2 experiencing professional exposure) and 20 underwent treatment of LTBI (10 being immigrants and 6 HCW). Out of those 32 individuals, 26 have additional risk factors for LTBI.

In the absence of a "gold standard", the perspective that QFT is wrong cannot be ruled out. In the first group of subjects (QFT positive/TST neg-

ative), specificity of QFT may be lower, and the second group of subjects (QFT negative/ TST positive) may represent false negatives. Since tuberculosis is a disease that may onset years after infection, only long perspective studies on γ INF tests will be able to rule out the possibility that a negative patient may instead develop active disease in the future.

However, under this perspective, the TST being too sensitive and too little specific (and QFT even more sensitive), there is the concrete risk of unnecessarily treating even more individuals. We cannot exclude, in our series, that at least some of the individuals treated "per guidelines" were not truly infected, although their number does not seem to be relevant (out of 37 individuals with discordant tests, TST positive/QFT negative, 28 have a TST induration ≥ 10 mm and 20 ≥ 15 mm) [12].

Further studies based on larger samples and long-term follow-up are necessary to define the "gold standard". The main limitations of our study are represented by the sample size and the absence of double blindness (not allowed by the resources available). A possible source of bias, represented by the execution of QFT at the time of TST reading (boosting effect), can be ruled out as only 5 cases in the overall sample (see potential TST false negatives) experienced a bias-compatible disagreement between the tests.

With the selection of group 4 being arbitrary (defined on the basis of a medical decision to treat derived from guidelines in force), it is possible that results from other settings (e.g. where guidelines recommend higher cut-off for positivity) may differ.

The major point to note from a clinical point of view is the risk of progression to active tuberculosis. This has been recently determined for different TST levels [20], including concomitant risk factors for developing TB and the opportunity of prescribing LTBI preventive treatment

Data was calculated on a meta-analysis of published reports in order to estimate the lifetime risk of TB among tuberculin skin test positive persons with specific medical conditions, but these aspects remains completely unclear for immunological blood tests.

Considering that since TB may develop many years after exposure, even though this generally occurs more frequently in the early stages, the absence of long-term experiences render far premature to advance similar hypotheses on interferon gamma test results. Still, at time of writing this paper, new developments are in progress and it looks certain that promising information will be available in the near future.

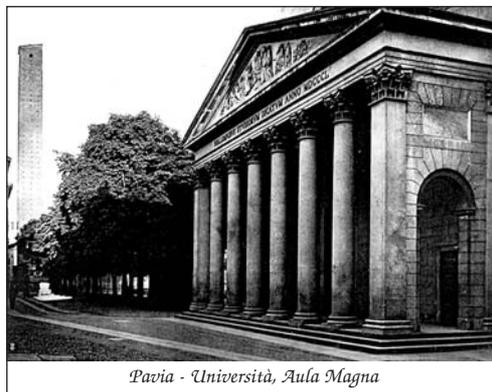
In conclusion, 1) QFT offers advantages (one single visit; scar resulting from Mantoux test avoided, etc), although its cost is still high compared with that of TST; 2) the results of our study substantially confirm the results of previous studies [11] on the agreement of TST and QFT test in detecting LTBI; 3) the study results indicate that the larger proportion of disagreement is found in individuals undergoing treatment of LTBI with

Isoniazid; 4) as evidence on γ INF assay potentialities in the clinical setting is increasing over time [21] we expect that their definite role in the diagnosis of LTBI will be stated in the next future. The new generation of γ INF tests (using ESAT-6 and/or culture filtrate protein 10- CFP-10) will further increase the test's potentialities as higher specificity will be obtained and less interferences from other mycobacteria will be confirmed.

Acknowledgements: The Authors wish to acknowledge Cellestis Limited, Carnegie, Victoria, Australia for providing the QFT reagents, Rosella Centis (WHO Collaborating Centre, Tradate, Italy) for data analysis and Luca Richeldi (University of Modena) for the useful comments on the manuscript. The study had been partially supported by the AIPO Grant N°TBC1.

References

1. Raviglione MC, Snider DE jr, Kochi A. Global epidemiology of tuberculosis: morbidity and mortality of a worldwide epidemic. *JAMA* 1995; 273: 220-226.
2. Raviglione MC, Dye C, Schmidt S, Kochi A. Assessment of worldwide tuberculosis control. *Lancet* 1997; 350: 624-629.
3. Nardell EA. Pathogenesis of tuberculosis. In: Reichman LB, Hershfield ES, eds. Tuberculosis: A comprehensive International Approach. NEW York, NY: Marcel Dekker Inc; 1993: 103-122.
4. Selwyn PA, Sckell BM, Alacbes P, Friedland GH, Klein RS, Schoenbaum EE. High risk of active tuberculosis in HIV-infected drug users with cutaneous anergy. *JAMA* 1992; 268: 504-509.
5. Clancy L, Rieder HL, Enarson DA, Spinaci S. Tuberculosis elimination in the countries of Europe and other industrialized countries. *Eur Respir J* 1991; 4: 1288-1295.
6. Migliori GB, Raviglione MC, Schaberg T, et al. Tuberculosis in Europe. Recommendations of a Task Force of the European Respiratory Society (ERS), the World Health Organization (WHO) and the International union Against Tuberculosis and Lung Diseases (IUATLD), Europe Region). *Eur Respir J* 1999; 70: 149-159.
7. Broekmans JF, Migliori GB, Rieder HL, et al. European framework for tuberculosis control and elimination in countries with a low incidence. *Eur Respir J* 2002; 19: 765-775.
8. Bass JB Jr, Farer LS, Hopewell PC, et al. Treatment of tuberculosis and tuberculosis infection in adults and children. *Am J Respir Crit Care Med* 1994; 149: 1359-1374.
9. Rieder HL. Methodological issues in the estimation of the tuberculosis problem from tuberculin surveys. *Tuberc Lung Dis* 1995; 76: 114-121.
10. Rothel JS, Jones SL, Corner LA, Cox JC, Wood PR. A sandwich enzyme immunoassay for bovine interferon-gamma and its use for detection of tuberculosis in cattle. *Aust Vet J* 1990; 67: 134-137.
11. Mazurek GH, LoBue PA, Daley CL, et al. Comparison of a whole-blood interferon- γ assay with tuberculin skin testing for detecting latent *Mycobacterium tuberculosis* infection. *JAMA* 2001; 286: 1,740-1,747.
12. Wang L, Turner MO, Elwood RK, Schulzer M, FitzGerlad JM. A meta-analysis of the effect of Bacille Calmette Guérin vaccination on tuberculin skin test measurements. *Thorax* 2002; 57: 804-809.
13. Codecasa LR, Besozzi G. Acceptance of isoniazid preventive treatment by close contacts of tuberculosis cases: a 692-subject Italian study. *Int J Tuberc Lung Dis* 1998; 2: 208-212.
14. Fleiss JL. Statistical Methods for Rates and Proportions. Second Edition. New York, NY: John Wiley & Sons Inc; 1981: 212-236.
15. Pottumarthy S, Morris AJ, Harrison AC, Wells VC. Evaluation of the tuberculin γ interferon assay: potential to replace the Mantoux skin test. *J Clin Microbiol* 1999; 37: 3229-3232.
16. Streeton JA, Desem N, Jones SL. Sensitivity and specificity of a γ interferon blood test for tuberculosis infection. *Int J Tuberc Lung Dis* 1998; 2: 443-450.
17. Converse PJ, Jones SL, Astemborki J, Vlahov D, Graham NM. Comparison of a tuberculin interferon- γ assay with the tuberculin skin test in high risk adults: effect of human immunodeficiency virus infection. *J Infect Dis* 1997; 176: 144-150.
18. Kimura M, Converse PJ, Astemborki J, et al. Comparison between a whole blood interferon- γ release assay and tuberculin skin testing for the detection of tuberculosis infection among patients at risk for tuberculosis exposure. *J Infect Dis* 1999; 179: 1297-1300.
19. Wood PR, Corner LA, Rothel JS, et al. Field comparison of the interferon- γ assay and the intradermal tuberculin test for the diagnosis of bovine tuberculosis. *Aust Vet J* 1991; 68: 286-290.
20. Horsburg CR. Priorities for the treatment of latent tuberculosis infection in the United States. *N Engl J Med* 2004; 350: 2060-2067.
21. Lalvani A. Spotting latent infection: the path to better tuberculosis control. Editorial. *Thorax* 2003; 58: 916-918.



Pavia - Università, Aula Magna