Original article

RESP

Tuberculin Test Measurement at 48 and 72 hours: mismatch and clinical significance*

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ABSTRACT

Objective: To measure Tuberculin Skin Test (TST) at 48 and 72 hours and, if there are differences, to determine the concordance and whether it is of clinical significance (change in the TST score).
Material and method: Prospective study of those admitted to prison between March and June 2023, screened by means of TST read at 48 and 72 hours by trained personnel, assessed according to criteria in force in Spain. When the measurement varied, concordance was checked using the kappa index and its clinical significance. To determine variables associated with clinical significance, bivariate and mutivariate analysis was performed using logistic regression.
Results: 488 cases were studied. TST was positive in 20.1, 23.4 and 23.8% at 48 hours, 72 hours or any of them, respectively. There was more TST positive in foreigners or BCG vaccinated. The reading varied in 35.2% (mean deviation 5.5 +/–4.4 mm) and was higher at 72 hours (86% of different cases). Inter-reading concordance was excellent overall (κ = 0.892) and in BCG vaccinated (κ = 0.805), but moderate in immunocompromised (κ = 0.421). Discordance was clinically significant in 3.7% and was not associated with any variable.
Discussion: TST concordance at 48-72 hours is excellent, but in some patients (3.7% in this work) the score is incorrect, more so in readings at 48 hours. The proportion is low, but can be reduced if the reading is taken at 72 hours and Interferon Gamma Release Assay tests are used in some cases (immunocompromised, etc.).

Key words: tuberculin test; latent tuberculosis; mass screening; BCG vaccine; prisons; interferon-gamma release tests.

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INTRODUCTION

The incidence of tuberculosis (TB) has gone down in recent years, although the COVID pandemic changed this trend[1]. Notifications of TB in Spain in 2022 was 7.83 cases per 100,000 inhabitants, which places Spain amongst the countries with low incidence for the sixth year running, although a slight upswing in national levels was detected for the first time in over ten years. This has also been observed worldwide[2]. TB is still the infectious disease with the highest mortality levels, and one fourth of the world's population is infected by *M tuberculosis.* It is calculated that 5-10% of infected individuals will develop TB at some point in their lives, but this progression is not uniform and is larger in groups such as recently infected and immunosuppressed persons. To establish how to manage, care for and, where necessary, treat patients with latent tuberculosis infections (LTI) in countries with medium/high income levels and an incidence

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of TB <100 cases per 10^5, as is the case in Spain, the WHO published a series of directives in 2015[3].

The incidence of LTI and TB amongst inmates is higher because a large part of this group come from poor sectors of the population in large cities, where incidence is higher, or from countries with scarce economic resources and endemic levels of infection and disease. Furthermore, the risk of transmission in prisons is higher since they are enclosed institutions that are often densely populated. A recent meta-analysis calculated that the risk of developing TB in prison is between 6 and 30 times greater than in the community, especially in overcrowded prisons in African countries[4].

It is therefore a basic requirement for such centres to maintain a high level of suspicion in the face of respiratory sympthoms with no clear origins and provide protocols designed for early detection and active searching for cases. LTI screening when entering prison is a key factors in this regard[4,5] and is recommended by the WHO for countries with incidence levels like those of Spain, as long as resources are available[3,5]. Gamma interferon detection methods or IGRA present some advantages in diagnosing LTI, but their use is limited in population screening because they are more expensive than the Tuberculin Skin Test (TST), they require a blood sample and should be processed and transported within a fixed period. For these reasons the most widely used assay is the TST or Mantoux skin test, which is the benchmark technique and the one recommended by the Tuberculosis Prevention and Control Plan in Spain España (PPCT)[6]. The TST has been used for over 100 years and consists of the intradermal administration of the tuberculin in the proximal third of the anterior side of the forearm. It measures the delayed hypersensitivity reaction to more than 200 antigens of the mycobacteria contained in the PPD (purified protein derivative). The scores for positive or negative TST are measured according to the size of the subsequent induration. The main guides[3,5-8] recommend a period of 48-72 hours before measuring, although the reaction starts quickly and the size can vary over time. The **objective** of this study is to compare the reading of the TST after 48 and 72 hours, and if differences are found, determine the level of agreement and check if there is any clinical significance; i.e., if the differences lead to changes in the scores as a positive or negative TST.

MATERIAL AND METHOD

An observational, prospective and cross-sectional study, which included persons who had entered the Brians 1 remand centre in Sant Esteve de Sesrovires (Barcelona), between 13/03/2023 and 13/06/2023, and who were screened for LTI with TST in line with current criteria (not having a previous positive TST or TB previa, or having a negative TST >1 year. If negative TST <1 year was regarded as a non-infection). The tuberculin used was the PPD-RT 23, with a dose of two units of tuberculin (0.1 ml).

To standardise the TST reading, the nursing team who participated in the study underwent theoretical-practical training. The course included the technique for intradermal injection of the TST, and readings based on Sokal's technique[9]. The previous practical exercise consisted of observations of variations in readings amongst professionals, which were minimised with training. Groups of professionals were also organised to ensure that the reading between 48 and 72 hours was carried out by the same reader to avoid interpretation biases. The results of the TST were recorded in millimetres. The bases for determining the TST as positive or negative were the current criteria used in Catalonia[8], similar to the ones recommended by the PPCT in Spain[6].

The likelihood of prior vaccination with the bacillus Calmette-Guérin (BCG) was assessed as follows: a) anamnesis; b) checking for the vaccine scar; and c) BCG Atlas[10], according to the rate of vaccination of the country, the subject's age and time of residence in Spain.

The following variables were gathered: age, gender, origin, BCG vaccination, diabetes, HIV infection, other immunodeficiencies and treatment with antitumour or biological drugs.

Checks were carried out to see if the TST measurement varied according to the reading at 48 and 72 hours and to establish if this variation was clinically significant; i.e., if it changed the score as a positive or negative TST. To measure agreement, Cohen's kappa index (κ) was used, expressing in which measurement there was a match in the classification between observers in relation to the total number of cases examined. The limits proposed by Landis y Koch[11] were used to quantify the level of matches or agreement. The data was analysed using the Statistical Pack Age For The Social Sciences (SPSS)-PC V24

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statistical package. The descriptive dates was expressed in absolute numbers, percentages, means and standard deviation. The χ^2 and Fisher's exact test were used to study the associations between qualitative variables. The variables that were associated with the reading discordances in the bivariate analysis were included in the multivariate analysis of binary logistic regression, calculating the odds ratio (OR) and the confidence interval (CI) at 95%.

Ethical considerations

The study was carried out in accordance with the international ethical recommendations (Helsinki Declaration, Oviedo Convention and Nuremberg Code) and with the recommendations for best clinical practice (BPC) of the Spanish Government (Royal Decree 711/2002) and the Spanish Agency of Medicines and Health Care Products.

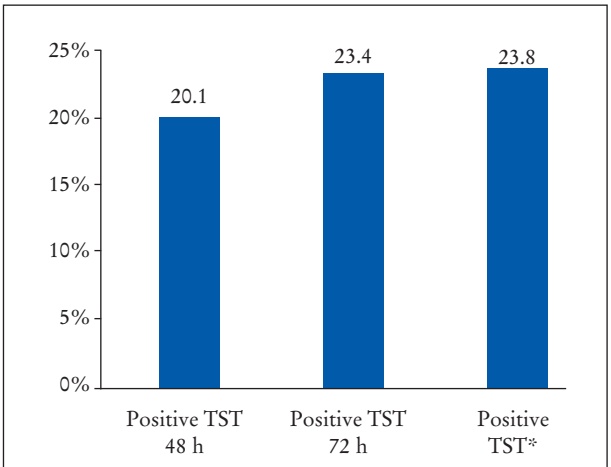
This study was evaluated and approved by the IDIAP Jordi Gol Research Ethics Committee under code number 23/110-P, as part of the project entitled Prevalence of Latent Tuberculosis Infection in the prison population of Catalonia.

RESULTS

827 inmates were admitted and 557 met the inclusion criteria. 488 (87.6%) were studied, as the TST could not be carried out or the reading could not be completed due to early release (release or transfer to another prison) in 69 cases. Data for the cases studied showed that the mean age was 36.3 ± 9.9 years (range 21-74), 99% were men and 69.9% were from other countries. Furthermore, 18 (3.7%) were infected with HIV, 4 (0.8%) presented other immunodeficiencies and 18 (3.7%) were diabetic. As regards vaccination with BCG, 315 (64.5%) were regarded as vaccinated, 169 (34.6%) unvaccinated and 4 could not be catalogued.

TST was positive at 48 hours amongst 20.1%, and 23.4% after 72 hours and 23.8% in either reading (Figure 1). The positive TST was more common amongst foreign inmates (28.4% vs 9.5% amongst Spanish inmates; p <0.001) and amongst persons vaccinated with BCG (27.9% vs 13.3% unvaccinated inmates; p <0.001), but it was not associated with other variables such as age, HIV infection, other immunodeficiencies or diabetes. The TST for foreigners was most often positive amongst inmates from North Africa (positive TST in 43.8%) and from Sub Saharan Africa (positive TST in 37.5%) with statistically significant differences (p <0.001) when compared to other groups.

There were differences in the TST readings between 48 and 72 hours amongst 172 (35.2%) cases (mean deviation: 5.5 ± 4.4 mm, range 1-20). Where there were differences, the induration was generally larger at 72 hours (86% of cases). However, the degree of agreement between both readings was excellent (K= 0.892). The difference between the readings was discordant (Table 1) and was also clinically significant (change of TST score) amongst 18 (3.7%) of patients (Figure 2). 16 (88.9%) of them showed a change of the TST from negative at 48 h to positive after 72 h. None of the variables (age, origin, immunodeficiency or BCG vaccination) had any statistically significant associations with the clinically significant data.



TST: tuberculin skin test; *Positive TST in reading after 48 or 72 hours.

Figure 1. Distribution of cases with positive TST at 48 hours, 72 hours or in any of the two readings.

Table 1. Distribution of outcome of tuberculin test according to reading after 48 and 72 hours and level of agreement in the population analysed.

		TST test after 48 hours	
	Result	Positive (%)	Negative (%)
TST after 72 hours	Positive (%)	98 (20.1)	16 (3.3)
	Negative (%)	2 (0.4)	372 (76.2)

Note: Mismatched results: 18 (3.7%); Kappa index: 0.892 (excellent); TST: tuberculin test.

The level of agreement between readings in the group vaccinated with BCG (Table 2) was also excellent (K = 0.805) and mismatches were 7.9%. Finally, only 22 of the subjects analysed presented immunodeficiency (Table 3) and the level of agreement in this group was moderate (K = 0.421) and mismatches between readings was 18.2%

DISCUSSION

23.8% of the patients in this study presented a positive TST after 48 or 72 hours, with a significantly higher frequency amongst foreign inmates, especially those from North and Sub-Saharan Africa and patients vaccinated with BCG. However, there were mismatches between readings at 48 and 72 hours in 35.2% of the cases studied. TST is a delayed hypersensitivity reaction that reflects cellular immunity to TB, made visible by the appearance of a dermal infiltrate in the place of the injection with numerous polymorphonuclear leukocytes[12]. This infiltrate appears early (6-12 hours post-administration), it may vary in time and is persistent, sometimes remaining for up to a week afterwards. In fact, studies carried out to establish the value of this induration over time have shown that measurement of the induration after 24 hours is highly predictive of the findings after 48-72 hours[13]. The readings for some subjects who oTST to not go to the programmed appointment are found to be reliable even after 168

Table 2. Distribution of outcome of tuberculin test according to reading after 48 and 72 hours and level of agreement in the population vaccinated with BCG.

		TST test after 48 hours	
	Result	Positive (%)	Negative (%)
TST test after 72 hours	Positive (%)	76 (24.1)	25 (7.9)
	Negative (%)	0 (0)	214 (67.9)

Note: Mismatched results: 25 (7.9%); Kappa index: 0.805 (excellent); TST: tuberculin test.

Table 3. Distribution of outcome of tuberculin test according to reading after 48 and 72 hours and level of agreement in the population analysed.

		TST test after 48 hours	
	Result	Positive (%)	Negative (%)
TST test after 72 hours	Positive (%)	2 (9.1)	0 (0)
	Negative (%)	4 (25)	16 (72.7)

Note: Mismatched results: 4 (18.2%); Kappa index: 0.421 (moderate); TST: tuberculin test.

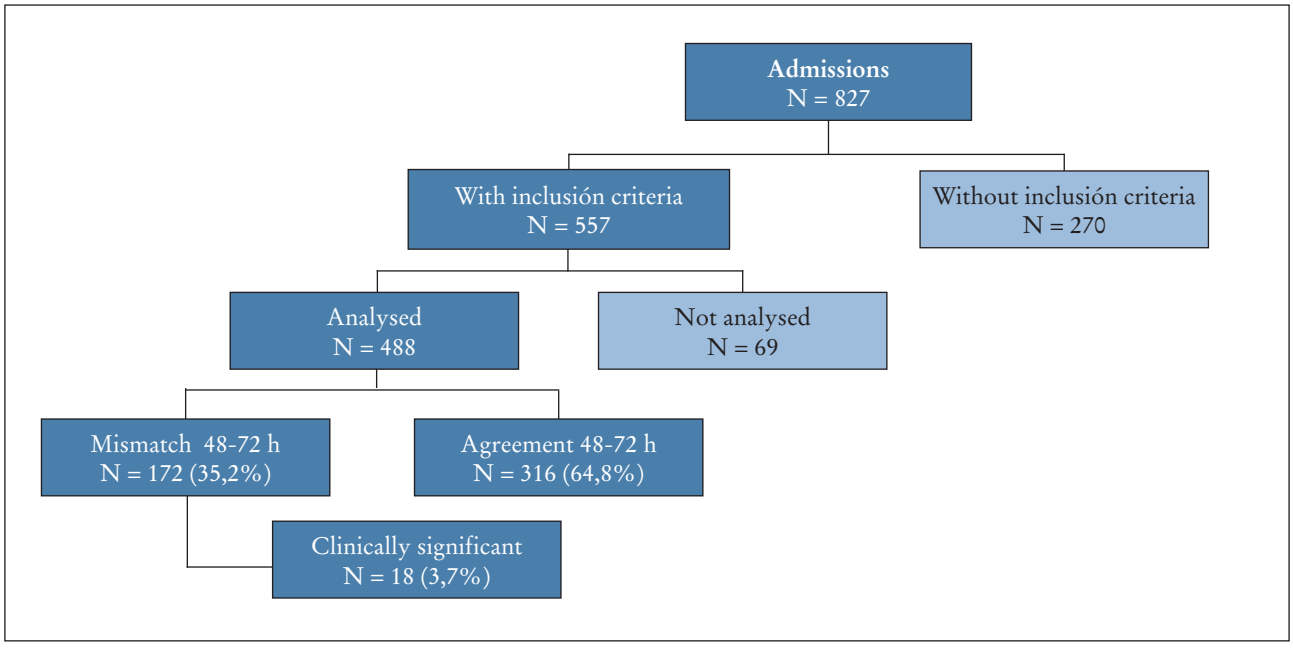


Figure 2. Distribution of admissions, inmates analysed and cases with mismatches and clinical significance.

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10.18176/resp.00094

hours[14]. Despite this data, the guides by experts and agencies[3,5-8] recommend that decisions should be based on TST results after 48-72 hours, which is the estimated time period for the effect of the reaction to be at its highest, and therefore the moment when the reading is most reliable. However, measurement of the induration at 48 and 72 hours does not always match. Our study shows that the mean deviation between readings was 5.5 ± 4.4 mm and the mismatched results affected 35.2% of the cases. Furthermore, the induration in 86% of the patients with discrepancies was larger after 72 hours; this phenomenon has been mentioned in other studies[14-16] and led to a situation in which the TST for 16 cases changed from being negative at 48 hours to being positive at 72. For this reason, some researchers have recommended that the induration should preferably be measured after 72 hours[15,16].

The global deviation between TST readings was clinically significant, i.e., there was a change in the TST score, in 3.7% of the cases studied. This result is somewhat lower than the one shown in the study by Sting *et al*[15], where it was 8.5%. However, the level of agreement (K = 0.892) obtained between both readings was excellent. As far as we know, only on other study carried out on children has established the level of agreement between readings. It obtained a slightly lower result (K = 0.73), but the authors considered that there were greater variations due to different readers taking the measurements[17]. It is also worth noting that none of the variables analysed in our study (age, origin, immunodeficiency and vaccination with BCG) were associated with clinical significance, making it impossible to specify variables that enable changes in the TST score to be predicted.

A rather larger proportion of mismatched results (7.9%) was obtained in the sub-analysis of subjects vaccinated with BCG, but there was also an excellent level of agreement (K= 0.805) between both readings. Special mention should be made of patients with immunodeficiencies. In this group, the level of inconsistent results was much greater (18.2%) and the level of agreement was only moderate (K = 0.421). The interpretation of TST in patients infected with HIV or with other immunodeficiencies is a complex matter, since the reaction may be adequate, limited of even non-existent, depending on the degree of immunodeficiency. The immunological reasons for the reduced reactivity are many, complex and not well known[12], and may affect inter-reading deviation. In any case, the low number of patients with immunodeficiencies in the study (n = 22) means that we have had to exercise extreme caution when presenting this data. At the same time, it should be pointed out that LTI screening for this population, and for subjects vaccinated with BCG along with other groups recommended by the PPCT in Spain[6] should be carried out with IGRA tests. This type of test can also be affected by immunosuppression, but its impact is lower than the one presented by TST[18], and it generally eliminates the risk of mismatches between readings, and the possible clinical significance, which were the subject of this study.

The most frequent limitations of the work in which TSTs are used are related to incorrect measurement or interpretation of the reaction, often related to the presence of more than one reader[19] or to the fact that they have not been trained or are inexperienced[20]. In this study: a) the same professional carried out the readings at 48 and 72 hours; b) the Sokal technique was used for measurement[9]; and c) previously trained personnel were used, in line with recommendations[20,21]. Therefore, and despite the TST reading not always being precise, we feel that the necessary measures have been taken to minimise the risks associated with measuring and interpreting the test.

In short, LTI screening when entering prison in Catalonia is mainly carried out with the TST test given that it is a screening for populations, and IGRA tests are only performed occasionally. TST readings can be carried out at 48-72 hours since the level of agreement between them is excellent, as we found in this study. However, it should be remembered that a proportion of patients (3.7% in this study) experienced a change in the TST score. Such a proportion is low, but it could be further reduced if IGRA tests are integrated into screening of some groups (cases with immunodeficiencies, etc.) and if the reading is carried out after 72 hours by expert staff with sufficient training in the process.

CONFLICTS OF INTEREST

The authors declare no conflicts of interest.

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