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Title: Avoiding the effect of BCG-vaccination in detecting MTB infection with a blood test

Short title: MTB contact investigation by TST and ELISPOT

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Abstract

Background. BCG-vaccination can confound tuberculin skin test (TST) reactions in the diagnosis of latent TB-infection (LTBI).

Methods. We compared the TST with a *Mycobacterium tuberculosis* (MTB)-specific ELISPOT-assay during an outbreak of MTB infection at a police academy in Germany.

Results. Participants were grouped according to their risk of LTBI in close (*n*=36) or occasional (*n*=333) contacts to the index case. For the TST the positive response rate was 53% (19/36) among close and 16% (52/333) among occasional contacts. Fifty-six TST-positive contacts (56/71=78.9%) and 27 TST-negative controls (27/298=9.1%) underwent ELISPOT testing. The OR of a positive test result across the two groups was 29.2 (95% CI 3.5–245.0) for the ELISPOT and 19.7 (95% CI 2.0 –190.2) for the TST with a 5 mm cut-off. Of 369 contacts, 158 (42.8%) had previously received BCG-vaccination. The overall agreement between the TST and the ELISPOT was low (κ=0.16), and positive TST-reactions were confounded by BCG-vaccination [OR 4.8 (95% CI 1.3–18.0)]. In contrast, use of a 10-mm induration cut-off for the TST among occcasional contacts showed strong agreement between TST and ELISPOT in non-vaccinated persons (κ=0.61).

Conclusion. In BCG-vaccinated individuals the MTB-specific ELISPOT-assay is a better indicator for the risk of LTBI than the TST.

Introduction

Approximately 1/3 of mankind is currently infected with Mycobacterium tuberculosis (MTB), causing significant morbidity and mortality with more than 2 million deaths per year [1]. Tuberculosis (TB) control programmes aim to decrease the incidence of TB by interruption of the transmission of MTB. Contact tracing and treatment of individuals with latent TB infection (LTBI) are the key components of TB control programmes. In addition, other important key components of TB control are the improvement of case finding among persons presenting with symptoms of TB, especially in HIV-infected individuals, by providing better access to quality-assured TB sputum microscopy as well as the DOTS strategy in TB treatment, the latter achieving higher cure rates by an uninterrupted supply of antituberculotic drugs in a standardized short-course chemotherapy under direct observation [2].

By applying classical epidemiological and molecular strain-typing techniques, population-based studies have recently revealed a high frequency of transmission of MTB, even in countries with a low TB incidence [3–7]. The tuberculin skin test (TST) introduced by Mantoux has been widely used as a screening test to identify individuals with LTBI. In screening for LTBI, the predictive value of a positive TST result (positive predictive value, PPV) is the probability that a contact with a positive TST result is truly infected and, consequently, may profit from chemotherapy [8]. This single parameter combines, on the one hand, the sensitivity and specificity of the method and, on the other, the prevalence of TB infection within the population subset examined. In close contacts, the prevalence of infection plays only a minor role in determining the PPV, because their risk of MTB transmission exceeds by far the risk in the general population. Thus, after this step of contact investigation, the PPV is primarily influenced by the specificity of the test method: the higher the specificity (i.e., low level of false-positive results), the higher the PPV of a positive result.

One problem in the clinical applicability of the Mantoux test is its cross-reactivity with antigens present in other mycobacteria, such as the *Mycobacterium bovis* bacillus Calmette-Guerin (BCG) vaccine strain and environmental mycobacterial species. This cross-reactivity leads to false-positive results and decreases the PPV of the TST. Therefore, alternative diagnostic tools for the detection of LTBI have been explored. One antigen of MTB that induces a strong IFN-γ-secreting CD4 T-cell immune response in experimental models [9, 10], and that is absent in *M. bovis* BCG and most other non-tuberculous mycobacteria, is the early secretory antigenic target 6 (ESAT-6). Counting of ESAT-6-reactive peripheral PBMCs by detection of IFN-γ-spot-forming cells (ELISPOT assay) has been used successfully to identify persons with active and latent MTB infection [11–16].

Whereas the sensitivity of an MTB-specific ELISPOT assay appears comparable to that of the TST [17], the specificity of this test may actually be superior to that of the classical TST. However, studies that addressed this question have so far either been conducted in a country with intermediate MTB incidence [18] (as opposed to the low MTB incidence encountered in most European countries and the USA) or with children [19]. Until now, the ELISPOT assay has not been evaluated for adults contacts directly related to a single source case in a low incidence setting.

From January 2004, extensive contact tracing became necessary in young and middle-aged adults within a police academy in Eutin, a town in the German federal state of Schleswig-Holstein. In mid-September 2003, one of its cadets (a 31-year-old unmarried former military policeman, who had been BCG-vaccinated in 1973) complained of progressive cough and weakness. After a temporary improvement of his condition on broad-spectrum antibiotic therapy, the patient experienced haemoptysis in December 2003. A chest X-ray at that time showed extensive infiltrates and cavitations in the right upper lobe, and he was admitted to a

pulmonary clinic. Sputum samples showed 3+ acid-fast bacilli on Ziehl-Neelsen stain. Isolates were culturally confirmed as MTB, susceptible to first-line drugs. Despite standard quadruple antituberculous therapy AFBs were still present on sputum smears and MTB could still be cultured from the sputum up to 2 months after treatment had been initiated. Thus the patient had to be considered as having been an index patient, highly infectious to his contact persons at the barracks of the police academy, presumably for a period of up to four months.

We participated in contact tracing at the police academy barracks, and we compared the agreement between TST and an ESAT-6-based ELISPOT for the identification of LTBI.

Materials and Methods

Study population

A contact investigation at the police academy yielded a total of 369 persons with identified risk of LTBI due to exposure to the index case since September 2003. Of these, 287 were born in the former West Germany, 74 (20.1%) in the former East Germany and 8 persons were foreign-born and migrated to Germany.

The mean age of the contacts was 28 years (range 15–62). Contacts were predominantly male ($n = 265$, 71.8%). No subject reported any contact with persons with tuberculosis before exposure to the index case. We considered 36 persons (9.8%) to be close contacts (see Figure 1); these comprised 35 police cadets, who were sharing the same classroom and sleeping quarters with the index case (first-year group 5 and 6) and one of the instructors (see below) who had trained the index case in cardio-respiratory resuscitation.

The other 333 contacts had only occasional exposure to the index case during his presumed period of infectiousness, and did not vary substantially in respect of exposure time or proximity. These contacts comprised 187 further cadets, 46 instructors, and 100 individuals

working as members of the vehicle maintenance staff, administration, kitchen personnel, cleaners or uniform store-keepers (see Figure 1).

As required by the current national guidelines for contact tracing in Germany [20] (issued by the DZK, the German Central Committee for the Control of TB) all these contacts were subjected to initial Mantoux TST testing 12 weeks following their last possible exposure to the index case. The TST was performed on the volar aspect of the forearm, and results were read 48–72 hours later. Chest radiographs of the TST-positive contacts were performed, and clinical and radiographic findings were reviewed for evidence of active TB. None of the contacts reported that they were seropositive for HIV, undergoing haemodialysis, currently being treated with corticosteroids or other immunosuppressives, or known to have a malignant disease or diabetes mellitus. None of the non-vaccinated contacts had previously been involved in contact tracing or had given a positive result in an employment-related investigation.

Of all the 369 contacts, 158 (42.8%) had a history of previous BCG vaccination; for this reason, and because the efficacy of LTBI treatment is highly dependent on the proportion of true MTB infections, the skin-test-positive persons were offered the possibility of being tested by ELISPOT in order to provide more information about the PPV of the Mantoux test. For practical reasons, only every tenth TST-negative individual was selected for ELISPOT testing by randomisation, to provide a control group. All individuals agreed to participate in the study.

Cell cultures

PBMC were prepared by Ficoll-Hypaque density-gradient centrifugation from heparinised blood and put into culture in RPMI 1640 medium containing 100 U/ml penicillin, 100 µg/ml streptomycin, 2 mmol/l L-glutamine and 5% FCS (culture medium).

ELISPOT assay

ELISPOT assays for human IFN-γ were performed according to the manufacturer's guidelines (AID, Straßberg, Germany). Briefly, 200,000 PBMC were plated overnight on 96-well plates, which had been pre-coated with a mouse anti-human IFN-γ antibody, in 200 µl culture medium per well. The cells were left unstimulated (negative control), were stimulated with 10 ng/ml anti-CD3 monoclonal antibody (clone X35, Beckman-Coulter, Krefeld, Germany; positive control), with 10 µg/ml of PPD (purified protein derivative of tuberculin; Statens Serum Institut, Copenhagen, Denmark) and with 5 µg/ml of ESAT-6 antigens (kindly provided by AID, Straßberg, Germany). After 20 hours of culture at 37 °C the plates were washed and then incubated at room temperature for 2.5 hours in the presence of a biotinylated mouse-anti-human IFN-γ monoclonal antibody. The plates were again washed repeatedly and then, after a 2-hour incubation with streptavidin-conjugated HRP (horseradish peroxidase), were washed again and the substrate buffer (containing AEC (3-amino-9-ethylcarbazole) and H_2O_2) was added. The reaction was stopped after 20–30 minutes by the addition of water. After repeated washing with water, the plates were allowed to dry. Visualisation and analysis of the spots was performed by using the AID EliSpot Reader System ELR02 (AID, Straßberg, Germany). The response of stimulated cultures was considered positive if the test well contained at least 5 more spots than did the negative control well, and this number was at least twice that in negative control wells. The background number of spots in negative control wells was always less than 5 spots per well.

Statistical analysis

Categorical data were compared by the χ-squared test (or Fisher's exact test, when expected cell sizes were smaller than five). Concordance between TST and ELISPOT assay results was assessed using κ coefficients. Values below 40% indicate weak correlation (if any), values of

0.41–0.60 indicate good agreement and values above 0.6 strong agreement [21].

Since there is no gold standard for the determination of LTBI, we focussed the analysis on estimating the strength of association between the degree of exposure, i.e., the proximity between contact persons and the source case. We used logistical regression procedures to estimate results of odds ratios (ORs) of each test in comparisons between the groups of close and of occasionalcontacts. Beside proximity of exposure (stratification), variables included age, origin at birth (East or West Germany), ELISPOT and TST result and history of BCG vaccination.

All tests were performed as two-sided tests.

Results

Seventy-one (19.2%) of the 369 contacts developed an induration greater than or equal to 5 mm at the TST site: 19 of the 36 close contacts (52.8%) and 52 of the 333 non-close (15.6%; $p < 0.001$). According to the current German guidelines [19], this size was taken as a cut-off for positivity in the test. The patients with positive TST results had a mean age (± SD) of 25.9 (± 11.0) years and were significantly younger than those with negative TST (29.1 ± 11.5, $p < 0.05$), had a similar sex distribution, but differed in respect of origin: 20/74 (27.0%) persons born in the former East Germany had a positive TST result, but only 46/287 (16.0%, $p < 0.001$) of those born in the former West Germany did so. Sixty-six of 74 (89.2%) persons born in East Germany had been previously BCG vaccinated compared to 88/287 (30.7%) born in West Germany ($p < 0.001$).

One of the close contacts, a 39-year-old male instructor, developed tuberculous pleuritis in spring 2004 (diagnosis on 13.4.2005). DNA fingerprint results had become available, and recent transmission due to the index case could be confirmed (see Figure 2). No other contact developed active TB after contact tracing. In an ongoing population based epidemiologic

study in the metropolis of Hamburg, about 60 km away from the town of Eutin, isolates from 1205 patients were investigated by molecular strain typing using IS6110 DNA fingerprint and spoligotyping within the study period from 1997 to 2004. Results of contact tracing and additional patient interviews have been used for further epidemiological analyses. Among all patients, approx. 40% were grouped into clusters based on identical fingerprint patterns of the respective isolates. The size of the RFLP clusters ranged from 2 to 45 isolates, however the majority of cluster comprise just two isolates [22, 23]. The particular strain involved in the contact investigation in Eutin, has caused an outbreak comprising 5 patients in the study period.

The mean induration size (± SD) of the TST was 13.8 (± 6.7) mm in the BCG-vaccinated TST-positives and 16.2 (± 8.0) mm in the unvaccinated contacts, but this difference was not statistically significant.

In total, 83 contacts underwent ELISPOT testing (see Figure 3 and also the crude numbers with respect to the size of induration in Table 1); 56 of the 71 TST-positive contacts (78.9%, and 9.1% (27/298) of the TST-negatives chosen at random. Fifteen TST-positive contacts declined the offer of ELISPOT testing. In total, agreement between TST and ELISPOT was low ($\kappa = 0.16$, 95% CI 0.1–0.25; $p = 0.006$), with concordant results in only 40/83 (48.2%), i.e.13/56 contacts (23.2%) with positive TST results – 8 of the 19 TST-positive close contacts (42.1%) and 5 of the 37 occasional contacts (13.5%) – but 27/27 (100%) students with negative TST results. Among the non-vaccinated individuals, ELISPOT and TST results were in good agreement ($\kappa = 0.5$, 95% CI 0.4–0.6; $p = 0.001$). All of the 18 non-vaccinated contacts who had negative TST results were ELISPOT-negative as well. Nine of the 18 who were TST-positive (50%) were also ELISPOT-positive

There was no correlation between BCG vaccination and ELISPOT result ($\kappa = 0.04$).

Of all contacts, 158 of 369 (42.8%) had ever received BCG vaccination. Of these, 44 (27.8%) gave a positive TST result. However, of the 211 contacts who had not received BCG vaccination, only 27 (12.8%) gave a positive result ($p < 0.0001$).

In contrast to this, only 4 of the 38 TST-positive contacts with previous BCG vaccination submitted to ELISPOT testing gave a positive ELISPOT result (10.5%), while 9 of 18 non-vaccinated TST-positive contacts (50%) were positive ($p < 0.01$).

Since the numbers of contacts who underwent TST testing and ESAT-6 testing differed markedly, a logistical regression analysis was used to estimate the ODDS between degree of exposure and TST response or ELISPOT response, respectively. The 83 contacts for whom an ESAT-6 result was available were included (see Table 2). Compared with the group of individuals with occasional contact to the index case, the risk of having a positive ELISPOT test was found to be 29.2 times higher (95% CI, 3.5-245.0, $p = 0.002$), and the risk of having a positive TST 19.7 times higher (95% CI, 2.0-190.2, $p= 0.01$) when individuals had close contact to the index case. However, in contrast to ESAT-6 testing (no significant OR) this result was strongly confounded by BCG vaccination [OR 4.8 (95% CI, 1.3-18.0, $p = 0.02$), see Table 3].

Following the current guidelines all individuals with a positive TST (induration > 5 mm) were offered nine months of INH prophylaxis.

Discussion

In this contact study of a tuberculosis outbreak in a police academy in Germany, the degree of exposure to the index case was more closely correlated with the detection of MTB-specific T-cells from PBMCs in an ELISPOT assay than with a positive TST reaction. This finding was independent of age, sex and BCG vaccination status. In a low incidence country for tuberculosis with a substantial proportion of individuals who have been vaccinated with BCG

in the past, the ELISPOT allowed better discrimination between true infection and cross-reactivity, and can thus circumvent the unpredictable influence of BCG on the TST. However, this study has been conducted in a healthy, young population, which does not allow the usefulness of this test to be evaluated in an older population with chronic underlying diseases.

Of the 369 contact persons investigated, 71 (19.2%) were TST-positive. Although the TST is still regarded as the gold standard for the diagnosis of LTBI, TST only offers an indirect diagnosis of LTBI and can produce a substantial number of falsely positive test results [13]. Nevertheless, official guidelines [24] require that a positive test result should be followed by treatment. However, this study has revealed two crucial points that affect a decision to start chemotherapy for presumed LTBI on the basis of a positive TST. First, in absolute numbers, there were more TST-positive persons among the occasional than the close contacts, while relatively speaking there were more than twice as many TST-positive persons among those contacts who had previously been vaccinated (44/158 = 27.9% vs. 27/211 = 12.8%) indicating the possibility of a false positive reaction due to cross-reactivity with *M. bovis* BCG.

Our study demonstrates a poor correlation between the results of TST and those of the ELISPOT-assay among 56 TST-positive contacts when the induration cut-off for the TST was set to 5 mm diameter. This remarkable discrepancy could not be explained by false negative IFN-y-assay results, because the control group showed an excellent concordance [$\kappa = 1.0$]. Moreover, it was surprising that more than 80% of the contacts who had agreed to ELISPOT testing failed to confirm positive TST results. This indicates three possibilities. The first is that ELISPOT is not sensitive enough to confirm a true LTBI. The second is that the cut-off of TST representing a positive result is too low because of an overestimation of the true degree of exposure of a contact and must therefore be raised. The third is that, as in the case of BCG vaccination, there are cross-reactivities with other mycobacteria whose infection prevalence cannot be determined even by laboratory investigations because of the multitude

of species that may be present.

Most of the contact individuals in this study who grew up in former East Germany had received BCG vaccination at birth and again, if a TST control showed re-conversion to negative, during early adolescence. However, BCG vaccination was only performed sporadically in West Germany. The results of both the TST and MTB-specific ELISPOT were sensitive to the degree of contact to the infectious source case. However, owing to the confounding by BCG vaccination, detection of MTB-specific T-cells in the peripheral blood appears to be the better method when a considerable number of contacts have previously been BCG-vaccinated. These findings are in agreement with studies in children [13, 19] where TST was more likely to be positive in BCG-vaccinated than in non-vaccinated children, whereas INF-γ production by MTB-specific T-cells was not associated with BCG vaccination status.

The influence of prior BCG-vaccination on the TST and MTB-specific interferon-γ release assays in the detection of LTBI has been very recently investigated also in adults [19, 18, 25]. In agreement with our findings, only in BCG-unvaccinated individuals positive MTB-specific interferon-γ release assays were highly positively correlated with positive TST results in these studies and BCG-vaccination was a confounder of the TST but not of the interferon-γ release assays [13, 18, 25]. Recently a MTB-specific ELISPOT was compared to the TST in contacts of patients with tuberculosis in the United States. Among 209 non-BCG-vaccinated persons, frequencies of positive TST and ELISPOT results were almost identical. Even in non-BCG-vaccinated persons ELISPOT tended to be closer related to the degree of exposure with the index case than the TST [26].

The present study was not designed to study the effect of BCG-vaccination on MTB-transmission in adults, however, the frequency of a positive MTB-specific ELISPOT was

lower in individuals who received BCG-vaccination compared to BCG-unvaccinated individuals. This difference could be due to a protective effect of BCG-vaccination on MTB-infection as it was recently suggested [27]. The strain involved in this outbreak had a normal level of virulence compared to other strains in this low incidence area. Nevertheless only 13 of 369 contacts could be confirmed by ELISPOT to have LTBI and only one of these 13 (7.7%) developed active tuberculosis within 2 years of follow up. This is a slightly lower rate of secondary cases compared with other outbreaks (e.g. [19], where 9 secondary cases out of 97 ELISPOT confirmed LTBI cases (9.2%) occurred). Secondary cases of tuberculosis did not occur among 32 contacts with LTBI in a study in Denmark [13], where the incidence of tuberculosis is also low. Of note, prior BCG-vaccination did not protect against MTB-infection in that study.

The current German guidelines [20] distinguish between close and occasional contacts, but do not distinguish between different cut off-sizes in the evaluation of the TST reaction. However, when the statistical likelihood that a true MTB infection has occurred is limited by a short exposure time, the possibility that a small TST induration represents a false-positive reaction increases. Thus, we considered the possibility that adopting a higher cut-off point for positivity in the TST might result in higher levels of agreement between the two tests. Following the current guidelines of the American Thoracic Society [28] we raised the cut-off to the next recommend diameter, i.e. ≥ 10 mm. This is the cut-off diameter recommended for persons who are at increased risk, but are not close contacts. This caused an increase in the κ value to 0.29 overall and, for the non-vaccinated contacts, to 0.61, representing strong agreement between both tests. In the logistical regression analysis, the confounding by BCG vanished and the odds ratio for a positive TST finding between the two exposure groups increased to 25.9 (95% CI 2.8–238.1; see Table 4).

The fact that increasing the cut-off to 10 mm for occasional contacts also resulted in a strong agreement between the results from TST and those from ELISPOT in the non-vaccinated population seems to support both the influence of BCG as a confounder and the part played by the actual duration of exposure to the source. This corresponds to the results of other studies, in which a strong agreement between TST and ELISPOT could be seen when a diameter of 10 mm was used as cut-off from the start [29]. Thus, when the TST is performed for tuberculosis contact tracing, a TST with a cut off of 10mm, rather than 5 mm as stated in the ATS/CDC and current German Guidelines should be regarded as positive in BCG-vaccinated individuals and/or occasional contacts. However, even in individuals with no history of BCG-vaccination and a TST > 10 mm, a MTB-specific interferon-γ release assay may be a better indicator for the risk of LTBI than the TST. For the decision to initiate chemotherapy against LTBI a TST > 10 mm should be confirmed by a consecutive MTB-specific interferon-γ release that further minimizes the number of contacts falsely classified to be MTB-infected.

Blood testing for a interferon-γ production by MTB-specific T-cells, either by ELISA or by ELISPOT, have been showing very promising results for the diagnosis of active and latent TB in recent years [11, 16, 18, 30–38]. Tests using MTB-specific antigens such as ESAT-6 and the cultured filtrated protein (CFP)-10 out of the region of differences (RD)-1 in the MTB genome have a better specificity than the test with PPD antigen [28, 35, 36, 39]. The RD-1 is absent in most non-tuberculous mycobacteria (with the exception of *M. marinum, M. szulgai and M. kansasii*), and thus false positive results are less likely to be obtained than with the PPD-based TST. The sensitivity of these assays can be increased by using a combination of two antigens, a limitation in the present study. However, specificity of these assays is close to 100%, even when only ESAT-6 antigen is used [11, 15, 36]. At present it is unclear whether

test results with ELISA and ELISPOT are equivalent, and studies comparing the two tests concurrently with the TST are needed.

In conclusion, the data presented here suggest that an MTB-specific ELISPOT is superior to the TST in detecting LTBI and is the test of choice when considering the possibility of administering chemotherapy to persons who have earlier received BCG vaccination and/or whose contact status is unclear. For occasional and BCG vaccinated contacts the TST cut off should be raised from 5 mm to 10 mm, in order to minimize the number of false positive results. This study showed that the knowledge of the BCG-vaccination status is important in the diagnosis of LTBI.

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Competing interests

The author(s) declare that they have no competing interests.

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Tables and figures

Figure 1: Pattern of exposure among 369 contacts of a police cadet with 3+ AFB smear positive pulmonary tuberculosis

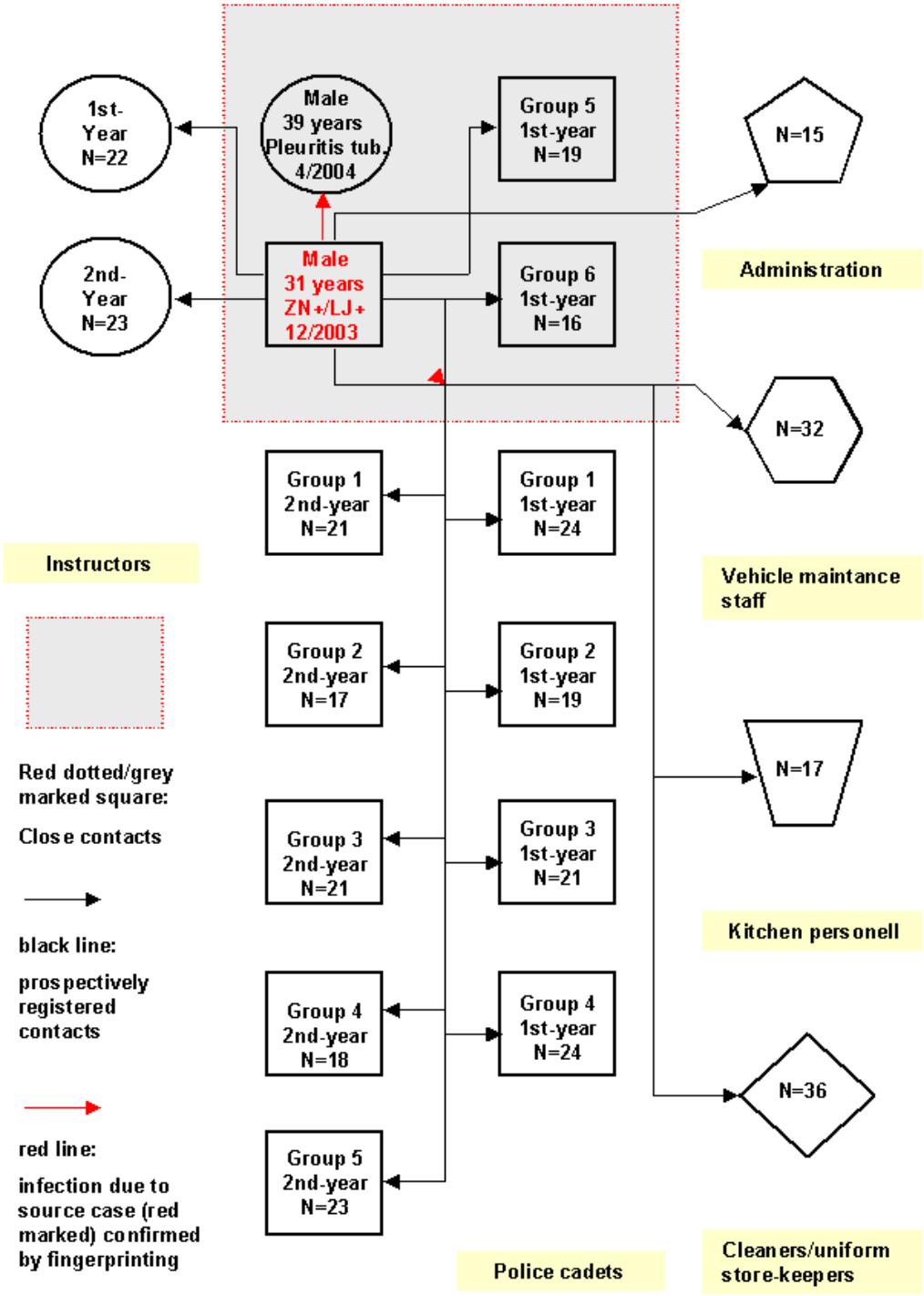

Figure 2: Spoligotyping patterns of MTB-isolates of the index case and a contact who developed pleural tuberculosis

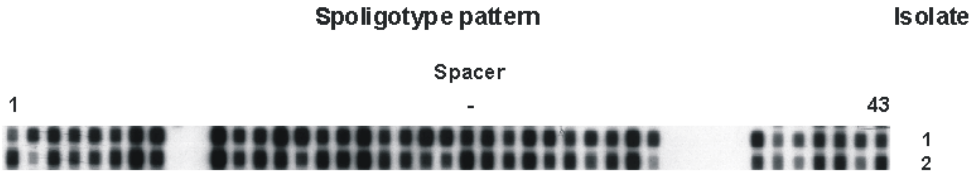

Figure 3: Distribution of test results according to history of BCG vaccination

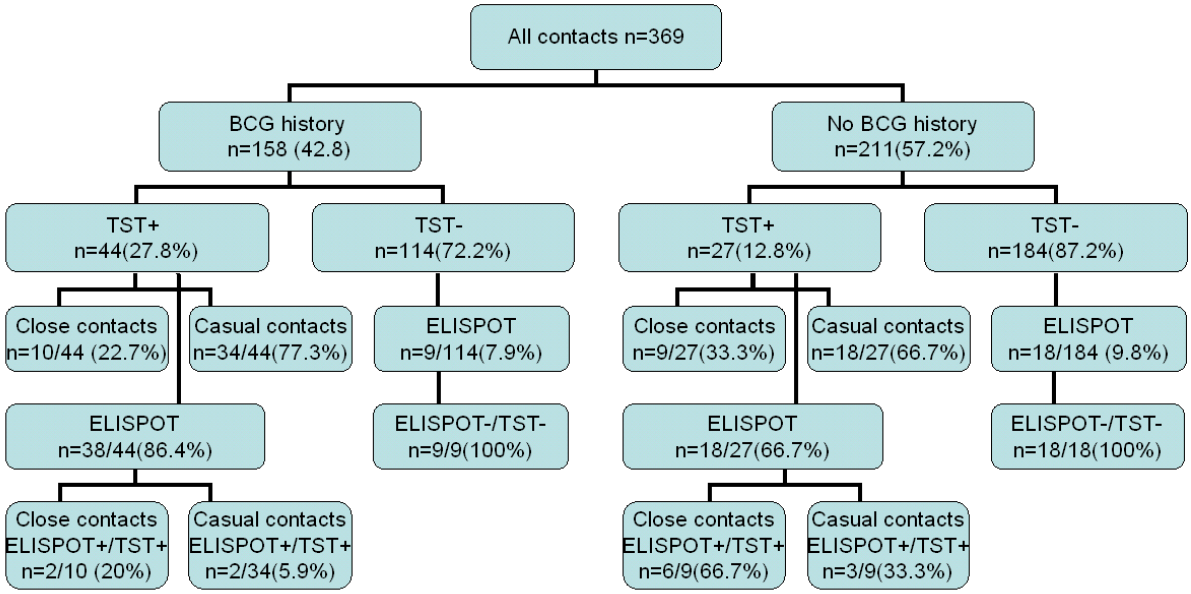

Table 1. Results of MTB-specific interferon-γ release assay (ELISPOT) among TST-positive contacts

BCG	TST Induration size [mm]	Degree of exposure	No. of positive TST results	No. of ELISPOT results	
				negative	positive
no	6	occasional	1	1	
	13	occasional	2	2	
	14	occasional	1	1	
	16	occasional	1		1
	17	occasional	1	1	
	21	occasional	1	1	
	27	occasional	1		1
	30	occasional	3		3
	total		11	6	5
	10	close	3	3	
	15	close	1		1
	22	close	3		3
	total		7	3	4
yes	7	occasional	1	1	
	8	occasional	3	3	
	9	occasional	1	1	
	10	occasional	5	5	
	11	occasional	4	4	
	12	occasional	1	1	
	13	occasional	1	1	
	14	occasional	1		1
	15	occasional	3	3	
	16	occasional	1	1	
	17	occasional	1	1	
	18	occasional	2	2	
	19	occasional	1	1	
	22	occasional	1	1	
	42	occasional	1		1
	total		27	25	2
	10	close	1	1	
	13	close	2	2	
	14	close	1	1	
	17	close	1	1	
	22	close	1	1	
	24	close	1	1	
	25	close	1		1
	27	close	1		1
	total		9	7	2

Table 2. Results of multiple logistical regression: Odds ratio for a positive ESAT-6

result in a comparison between the groups of close and occasional contacts.

Risk factor	Odds ratio	95% Confidence interval	*p*
Stratification (Close versus occasional contacts)	29.2	3.5 – 245.0	0.002
Age	1.1	0.99 – 1.2	0.6 (n.s.)
Origin (East/West Germany)	2.2	0.56 – 8.5	0.3 (n.s.)
BCG vaccination	0.31	0.05 – 1.9	0.2 (n.s.)

Table 3. Odds ratio for a positive TST result with a cut-off of 5 mm.

Risk factor	Odds ratio	95% Confidence interval	*p*
Stratification (Close versus occasional contacts)	19.7	2.0 – 190.2	0.01
Age	0.98	0.95 – 1.0	0.9 (n.s.)
Origin (East/West Germany)	1.8	0.64 – 5.3	0.3 (n.s.)
BCG vaccination	4.8	1.3 – 18.0	0.02

Table 4. Odds ratio for a positive TST result with a cut-off of 5 mm in close and of 10 mm in occasional contacts.

Risk factor	Odds ratio	95% Confidence interval	*p*
Stratification (Close versus occasional contacts)	25.9	2.8 – 238.1	0.004
Age	0.99	0.95 – 1.1	0.89 (n.s.)
Origin (East/West Germany)	0.98	0.4 – 2.4	0.96 (n.s.)
BCG vaccination	3.5	0.98 –12.8	0.054 (n.s.)