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M. tuberculosis DNA detection in nasopharyngeal mucosa can precede tuberculosis development in contacts

M. E. Balcells,* M. Huilcamán,*† C. Peña,‡ C. Castillo,§ C. Carvajal,* N. Scioscia,§ P. García§

***Infectious Diseases Department, School of Medicine, Pontificia Universidad Católica de Chile, Santiago, †Hospital Dr Gustavo Fricke, Viña del Mar, ‡Respiratory Department, Hospital San Borja Arriarán, Santiago, §Clinical Laboratory Department, School of Medicine, Pontificia Universidad Católica de Chile, Santiago, Chile**

SUMMARY

BACKGROUND: The nasopharynx is a known gateway for some mycobacterial species such as *Mycobacterium bovis* and *M. leprae*. *M. tuberculosis* can cross lymphoepithelial barriers in vitro, but its ability to colonise the nasopharyngeal mucosa in vivo has not been established.

OBJECTIVE: To determine if *M. tuberculosis* can be transiently detected in nasopharyngeal mucosa of tuberculosis (TB) contacts as a preliminary step in the development of tuberculous infection.

DESIGN: Exploratory study conducted among asymptomatic household contacts of pulmonary TB cases. A chest X-ray, QuantiFERON® TB-Gold or tuberculin skin test and a bilateral nasopharyngeal swab for Xpert® MTB/RIF and mycobacterial culture were performed at baseline and repeated 8–12 weeks later.

RESULTS: Eighty-nine contacts were enrolled a median of 9 days after the diagnosis of the index case. At baseline, 29.9% were positive for latent tuberculous infection and one subject (1.1%) had a positive Xpert in the nasopharyngeal swab with a normal chest X-ray, negative QuantiFERON and negative induced sputum. After 12 weeks' follow-up, this subject developed a new cough and upper lobe infiltrates and *M. tuberculosis* grew in sputum. No other cases of active TB were detected at follow-up.

CONCLUSION: The detection of *M. tuberculosis* DNA in the nasopharyngeal mucosa of contacts is an infrequent event that in this instance preceded the development of pulmonary TB. Its pathogenic role requires further investigation.

KEY WORDS: nasopharyngeal carriage; mucosal colonisation; TB pathogenesis

ALTHOUGH THERE ARE 2 BILLION people worldwide infected with *Mycobacterium tuberculosis* who do not develop active tuberculosis (TB) disease, there is still no certainty as to the key host or pathogen-derived factors that determine whether a recent infection will progress to active TB, clear spontaneously or remain in latency in immunocompetent subjects.[1] Among TB contacts who progress to develop clinical disease, the most vulnerable stages are the first 2 years after initial infection, and active vigilance is recommended for early detection during this period. However, by the time the patient has developed distinctive clinical symptoms or the diagnosis is evident by a positive acid-fast smear in sputum, the disease is well advanced and active transmission is usually already ongoing. The immunological detection of early infection using latent tuberculous infection (LTBI) test conversion requires a window period of at least 8–10 weeks, and even if conversion occurs, this does not necessarily indicate that the subject will develop clinical TB on follow-up.[2]

It has traditionally been accepted that the most common mechanism for TB transmission is the inhalation of droplet nuclei that are small enough to reach the alveolar space and interact directly with alveolar macrophages. Nonetheless, the respiratory tract is anatomically a continuum from the nostrils to the lungs, and the nasopharyngeal mucosa is the site where the first encounter with pathogens occurs. Several pathogens display an upper respiratory tract invasion phase, most commonly seen for respiratory viruses, but also for bacteria such as *Streptococcus pneumoniae* and *Neisseria meningitidis*. For these micro-organisms, a transient phase of asymptomatic nasopharyngeal carriage can precede the development of clinical disease or they may remain as only asymptomatic colonisation of the mucosal niche but with potential for transmission.[3]

We aimed to investigate whether *M. tuberculosis* can be detected in the nasopharyngeal mucosa of

Correspondence to: María Elvira Balcells, Infectious Diseases Department, School of Medicine, Pontificia Universidad Católica de Chile, Lira 63, Santiago, Chile. Fax: (+56) 2 2633 9820. e-mail: balcells@med.puc.cl
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Table Results of latent tuberculous infection tests, Xpert® MTB/RIF polymerase chain reaction and mycobacterial culture in nasopharyngeal swabs of tuberculosis contacts

Laboratory test results	Positive at baseline *n/N* (%)	Repeated at follow-up (8–12 weeks) *n/N* (%)
QFT-G	19/61 (31)	1/25 (4)*
TST	4/16 (25)	ND
Nasopharynx Xpert	1/89 (1.2)	0/33
Nasopharynx *M. tuberculosis* culture	0/89	0/33

* IGRA was repeated at 8–12 weeks only if negative at baseline.
QFT-G = QuantiFERON® TB Gold In Tube; TST = tuberculin skin test; ND = not done; IGRA = interferon-gamma release assay.

asymptomatic TB contacts, and its potential role in the development of LTBI at follow-up.

METHODS

An exploratory, cross-sectional study was conducted in adult (aged $\geqslant$14 years) household contacts of smear-positive pulmonary TB cases in central Santiago, Chile, over an 18-month period. All new pulmonary TB cases diagnosed in that area were directly reported by the local TB programme staff (Hospital San Borja Arriarán, Santiago, Chile) to the research nurse, who immediately initiated a contact investigation. Household contacts were invited to participate and were enrolled at each out-patient clinic where index cases had been identified. A baseline chest X-ray (CXR), blood sample for a QuantiFERON® TB-Gold (QFT; Carnegie, VIC, Australia) or tuberculin skin test (TST) and bilateral nasopharyngeal swab (FLOQSwab™; COPAN, Murrieta, CA, USA) were performed in all contacts. Subjects with any symptoms suggestive of active TB or an abnormal CXR were excluded from the study.

Nasopharyngeal swabs and blood samples were safely stored and transported within 2 h to be processed at a certified quality controlled clinical laboratory at the Red de Salud Universidad Católica-Christus (Laboratorio de Microbiologia y Microbiologia Molecular Universidad Católica). At the laboratory, the sediment (<2.5 ml) from the nasopharyngeal swabs was divided into three aliquots that were processed using Xpert® MTB/RIF (Cepheid, Sunnyvale, CA, USA), and solid (Löwenstein-Jensen) and liquid (MGIT™, BD, Sparks, MD, USA) mycobacterial cultures.

After 8–12 weeks, all subjects were contacted by telephone. Those who agreed were invited to come for a second nasopharyngeal swab for Xpert and mycobacterial culture and, in the case of contacts with a negative result at baseline, a second blood sample for QFT re-testing. If any symptoms suggestive of active TB had developed (fever, night sweats, cough or weight loss), a new CXR and sputum analysis were requested.

Permission to conduct this study was obtained from the Ethical Research Committee of the Pontificia Universidad Católica de Chile, Santiago, Chile; all enrolled subjects signed an informed consent form (CEI-MedUC permission n° 10-221).

RESULTS

Eighty-nine contacts were enrolled (mean age 42 years, 61.8% female) a median of 9 days (range 1–89) after the diagnosis of the index case. In total, 77 contacts had a valid LTBI test result (8 did not return for the TST reading and 4 did not agree to blood sampling). Of these, 29.9% tested positive at baseline (25% on TST >5 mm and 31% on QFT). In the follow-up examination 8–12 weeks later, a further 4% of subjects with a negative QFT at baseline had converted to positive (Table).

In total, 122 bilateral nasopharyngeal swab samples were processed for Xpert testing from the 89 contacts enrolled at baseline and from the 33 contacts who agreed to follow-up tests. One subject had a positive Xpert nasopharyngeal test result at baseline (1.1%) and none at follow-up.

The positive nasopharyngeal Xpert contact was a 19-year-old healthy, asymptomatic woman who had tested QFT-negative (0.13 international unit [IU]/ml) with a normal CXR at day 3 after the index case diagnosis (Figure A). The index case was her elder sister, with whom she shared a small apartment. She had not been in contact with the index case for at least 3 days at the time of sampling, as her sister had been hospitalised. An induced sputum sample was negative for acid-fast smear and *M. tuberculosis* culture at baseline. New nasopharyngeal Xpert testing and mycobacterial cultures performed at 17, 25 and 84 days of follow-up were also negative. After 12 weeks, she developed a new cough, repeat QFT increased to 0.31 IU/ml (still negative), and a second CXR revealed new right upper lobe infiltrates (Figure B). A high-resolution computed tomography chest scan confirmed new bilateral upper lobe nodular infiltrates with tree-in-bud areas highly suggestive of early active TB (Figure C). Two induced sputum samples were acid-fast smear-negative, but a positive mycobacterial culture was obtained, and the contact was started on standard anti-tuberculosis treatment at the local TB clinic. No other cases of active TB were detected among the other enrolled contacts after a median of 7 months of clinical follow-up.

DISCUSSION

It is widely known that TB is an airborne disease; it is largely assumed that *M. tuberculosis* accesses and infects the human body after the bacilli undergoes

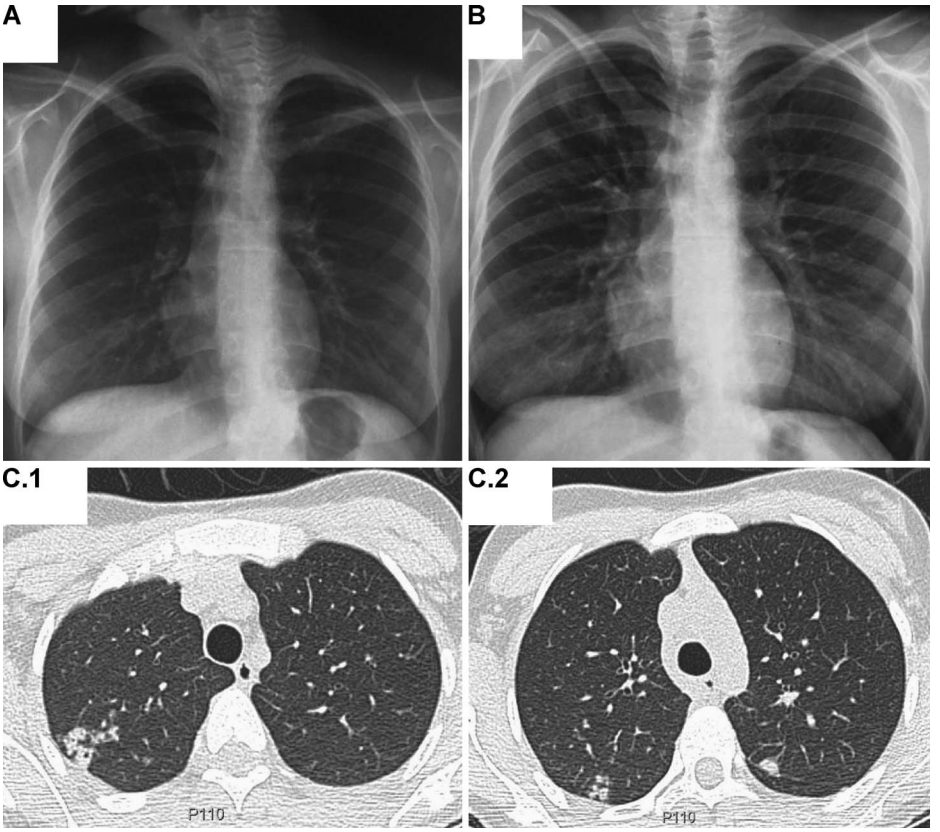

Figure **A)** Baseline (21 April 2015) CXR in nasopharyngeal Xpert-positive contact. **B)** CXR at 12 weeks (30 July 2015) showing new right upper lobe infiltrates. **C** 1 and 2) high-resolution chest computed tomography scan at 12 weeks confirming bilateral upper lobe nodular infiltrates with tree-in-bud areas. CXR = chest X-ray.

phagocytosis by alveolar macrophages. However, definitive data for this model are lacking, and a significant number of lymphatic or disseminated cases of TB occur in the absence of obvious primary lung infection. Many other pathogenic mycobacteria are commonly able to cross mucosal barriers, where dendritic cells and macrophages allow their replication due to the permissive immunological environment in lymphoepithelial tissues; the most classic example is *M. bovis* invasion of gut mucosa after ingestion of unpasteurised dairy products.[4,5] Nasopharyngeal mucosa-associated lymphoid tissue can also be a port of entry for some non-tuberculous mycobacteria, such as *M. leprae*, which shows affinity for the nasal mucosa in both mouse models and humans, where it can have an important role as a primary infection site and focus for local bacillary spread.[6,7] In vitro, *M. leprae* can invade both human alveolar and nasal epithelial cells, and both cell types are capable of sustaining bacterial survival. Furthermore, the introduction of *M. leprae* to the nasal septum of mice also results in macrophage and epithelial cell infection in lung tissue, supporting the idea that the upper airways constitute an important entry route for *M. leprae* into the body.[8] Similarly, *M. bovis* has been recovered from nasal secretions of experimentally infected cattle that do not show signs of active pulmonary disease.[9]

Clinical TB of the nasopharyngeal mucosa is a rare clinical presentation for TB, which suggests that normal upper airway mucosa are relatively resistant to natural infection or to local progression. Nonetheless, there are several reports of cases of primary nasal, tonsil or pharyngeal TB without pulmonary involvement.[10] Within mucosa-associated lymphoid tissue, there are highly specialised epithelial cells, microfold (M) cells, that carry out the transepithelial transport of macromolecules and micro-organisms for presentation to mononuclear phagocytes and lymphocytes within and below the epithelium.[11]

M-cells exist in overlying nasal-associated lymphatic tissue (NALT), the tonsils of Waldeyer's ring and bronchus-associated lymphatic tissue. Researchers have recently shown, using in vitro assays, that rapid *M. tuberculosis* translocation can occur across M-cells in NALT through a Type VII secretion system, and that M-cell depletion immediately prior to *M. tuberculosis* aerosol infection significantly delays mortality in mice.[12] Similarly, in an in vitro model of tissue culture, human nasal and bronchial mucosa

were successfully infected with *M. tuberculosis*, resulting in effective active bacterial replication and tissue invasion for a short period (14 days), as revealed by electronic microscopy and culture. Both experimental studies suggest that in vitro NALT mucosal colonisation and invasion is possible, and if they occur in vivo this happens for only brief periods of time, with shorter periods for *M. tuberculosis* than for other mycobacteria.[13] Only one earlier clinical report also explored the presence of *M. tuberculosis* in nasal swabs from pulmonary TB patients and household contacts, and found one positive swab among 13 contacts tested, although no follow-up was reported.[14]

The present clinical study supports the view that *M. tuberculosis* nasal colonisation may occur in the early stages after exposure and is only transient, given that repeated testing in the following weeks in this contact resulted negative.

As a limitation of the present study, we cannot exclude with absolute certainty that our findings may be due to a false-positive Xpert result. However, this is unlikely for two reasons: 1) the very high specificity of the Xpert test (99%, 95% credibility interval [95%CI] 98–99) as reported by the largest meta-analysis;[15] and 2) this finding was expected to be a rare event, and early TB progression, which is also an uncommon event in immunocompetent adult contacts (<1.5% during the first 4 years of follow-up), also coincided at follow-up with the only positive patient, strengthening the likelihood of a possible association of events.[16] The discordance between the Xpert result and *M. tuberculosis* culture may be related to the fact that a very small volume from the swab (<0.5 ml) was left for culture in this particular case, in accordance with having obtained an Xpert result in the very low positive range of amplification.

Finally, Xpert testing in nasopharyngeal aspirate has been shown to be effective in detecting active pulmonary TB in children, with low sensitivity (39.3%, 95%CI 23.6–57.6) but very high specificity (99.3%, 95%CI 97.4–99.8) at this sampling site.[17] At the time of sampling, our positive contact was completely asymptomatic and had negative induced sputum and normal CXR, making it unlikely that active pulmonary TB was then present.

It is to be noted that QFT testing remained negative and was not able to accurately predict the development of clinical TB in the exposed contact. Discordance between the TST and interferon-gamma release assays (IGRA) has already been described, with only 48% of tuberculin TST converters among recently exposed household contacts of pulmonary TB cases in Brazil having a positive IGRA. A lower cut-off value for IGRA should therefore be considered to allow closer monitoring for potential conversion.[18] Although contact screening with a nasopharyngeal swab for DNA detection could represent an attractive strategy for subjects with recent TB exposure at risk of developing TB, the transience of the event as reported here is a strong barrier for negative test result interpretation. We propose that future research should instead explore longer lasting biomarkers indicative of *M. tuberculosis* penetration of the nasopharyngeal mucosa, such as local NALT innate immunity activation markers. Any new approach requires validation with a large prospective cohort of contacts.

In conclusion, the detection of *M. tuberculosis* DNA in the nasopharyngeal mucosa of contacts was a rare (<2%) occurrence that preceded the development of culture-proven pulmonary TB by 3 months in one case. NALT transient colonisation as a port of entry for *M. tuberculosis* in humans thus has in vitro as well as in vivo support and requires further research, indicating that strict, close follow-up of any contact with positive detection of *M. tuberculosis* nasal carriage is required. Future implications may include targeted treatment for LTBI and earlier screening for patients at risk of developing active TB.

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Conflicts of interest: none declared.

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RESUME

CONTEXTE : Le nasopharynx est une porte d'entrée connue pour certaines espèces de mycobactéries comme *Mycobacterium bovis* et *M. leprae*. *M. tuberculosis* peut traverser les barrières lymphoépithéliales in vitro, mais sa capacité à coloniser la muqueuse nasopharyngée in vivo n'a pas été établie.

OBJECTIF : Déterminer si *M. tuberculosis* peut être transitoirement détecté dans la muqueuse nasopharyngée de contacts avec la tuberculose (TB) comme une étape préliminaire du développement de l'infection tuberculeuse.

SCHÉMA : Etude exploratoire réalisée chez des contacts domiciliaires asymptomatiques de cas de TB pulmonaire. Une radiographie pulmonaire, un test QuantiFERON® TB-Gold ou un test cutané à la tuberculine et un écouvillonnage nasopharyngé bilatéral pour Xpert® MTB/RIF et culture de mycobactéries ont été faits au départ et répétés 8 à 12 semaines plus tard.

RÉSULTATS : Ont été enrôlés 89 contacts après le diagnostic du cas index (délai médian de 9 jours). Au départ, 29,9% avaient un test de TB latente positif et un sujet (1,1%) a eu un Xpert positif sur l'écouvillonnage nasopharyngé, avec une radiographie pulmonaire normale, un QuantiFERON négatif et une expectoration induite négative. Après 12 semaines de suivi, ce sujet a développé une nouvelle toux et des infiltrats du lobe supérieur et *M. tuberculosis* a poussé sur les crachats. Aucun autre cas de TB active n'a été détecté lors du suivi.

CONCLUSION : La détection de l'ADN de *M. tuberculosis* dans la muqueuse nasopharyngée des contacts est une éventualité rare qui, dans ce cas, a précédé le développement de la TB pulmonaire. Son rôle pathogène mérite d'autres investigations.

RESUMEN

MARCO DE REFERENCIA: La nasofaringe es una puerta de entrada conocida para algunas especies de micobacterias como *Mycobacterium bovis* y *M. leprae*. *M. tuberculosis* es capaz de cruzar barreras linfoepiteliales in vitro, pero su habilidad para colonizar la mucosa nasofaríngea in vivo no ha sido aún establecida.

OBJETIVOS: Determinar si *M. tuberculosis* puede ser detectado transitoriamente en la mucosa nasofaríngea de contactos de casos de tuberculosis (TB), como paso preliminar en el desarrollo de esta infección.

DISEÑO: Estudio exploratorio realizado en contactos intradomiciliarios asintomáticos de casos de TB pulmonar. En ellos, se realizó una radiografía de tórax, un test de QuantiFERON® TB-Gold o test de tuberculina cutánea, y un hisopado nasofaríngeo bilateral para Xpert® MTB/RIF y cultivo de micobacterias, al ingreso y tras 8–12 semanas.

RESULTADOS: Se enrolaron 89 contactos, a una mediana de 9 días posterior al diagnóstico del caso índice. Bante, 29,9% presentaba un test de TB latente positivo y solo un sujeto (1,1%) resultó con un Xpert positivo en hisopado nasofaringeo, con una radiografia de tórax normal y un test de QuantiFERON negativo. Tras 12 semanas, este sujeto desarrolló tos, infiltrados nuevos en lóbulos superiores y un cultivo positivo para *M. tuberculosis* en esputo inducido. No hubo otros casos de TB activa al seguimiento.

CONCLUSIÓN: La detección de DNA de *M. tuberculosis* en la mucosa nasofaríngea de contactos es un evento infrecuente, que en esta oportunidad precedió el desarrollo de TB pulmonar clínica. Su rol patogénico merita mayor investigación.