

6. Eyigor H, Osma U, Eyigor M, Yilmaz MD, Gultekin B, Telli M, et al. Detection of human bocavirus in children with upper respiratory tract infection by polymerase chain reaction. *Clin Lab.* 2013;59:139–42.
7. Günel C, Kırdar S, Ömürlü İK, Ağdaş F. Detection of the Epstein-Barr virus Human Bocavirus and novel KI and KU polyomaviruses in adenotonsillar tissues. *Int J Pediatr Otorhinolaryngol.* 2015;79:423–7.
8. Don M, Söderlund-Venermo M, Valent F, Lahtinen A, Hedman L, Canciani M, et al. Serologically verified human bocavirus pneumonia in children. *Pediatr Pulmonol.* 2010;45:120–6.
9. Kantola K, Hedman L, Allander T, Jartti T, Lehtinen P, Ruuskanen O, et al. Sero-diagnosis of human bocavirus infection. *Clin Infect Dis.* 2008;46:540–6.
10. Söderlund-Venermo M, Lahtinen A, Jartti T, Hedman L, Kempainen K, Lehtinen P, et al. Clinical assessment and improved diagnosis of bocavirus-induced wheezing in children, Finland. *Emerg Infect Dis.* 2009;15:1423–30.

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## Use of adenosine deaminase as a marker for selecting pleural fluids for culture and/or molecular techniques for detection of mycobacteria<sup>☆</sup>



### Uso de adenosin deaminasa como indicador para seleccionar líquidos pleurales para cultivo y/o técnicas moleculares para detección de micobacterias

Dear Editor:

Pleural tuberculosis (PT) is a significant cause of tuberculosis (TB) in our region of Castile and León and, along with genitourinary tuberculosis, is the most common cause of extrapulmonary TB. With respect to all positive cultures for *Mycobacterium tuberculosis* (MT), the proportion of positive cultures in pleural fluid (PF) ranged from 5.18% in 2013 to 6.54% in 2016. The number of cases of PT has remained around 14 annual cases confirmed by culture, while over the same time there was a decrease in the number of cases of pulmonary TB. A previous 17-year study conducted in our Bierzo health board area<sup>1</sup> revealed that only 2.5% of PF samples are positive for MT culture. In view of this situation, we decided to look for a system for selecting PF samples to improve the performance of mycobacterial cultures and optimise the use of molecular techniques on those samples. We assessed a total of 200 PF samples collected from 2015 to 2018. The adenosine deaminase (ADA) value was determined in all of the samples, establishing the cut-off point at 30 U/l, above which PT is suspected in our health board area. All samples were seeded in solid culture media (Coletos and Middlebrook 7H11) and automated liquid media (BacT/ALERT<sup>®</sup> MP) and incubated for at least 6 weeks. When ADA was found to be above the cut-off point, the automated liquid medium was re-seeded at the end of the incubation period and incubation of all the bottles was prolonged for at least three more weeks. Identification of MT was performed by molecular techniques (GenoType<sup>®</sup> MTBC and GenoType<sup>®</sup> MTBDR, Hain) and the PCR on direct sample was performed with GeneXpert<sup>®</sup> MTB-RIF (Izasa<sup>®</sup>). When the ADA value is dispensed with, MT growth/PCR+ is only obtained in 3% of the PF samples. When an ADA value of 30 U/l or above is used (51 cases,

25.5% of PF), the rate of growth/detection by PCR increases to 11.76% (6 cases). As shown in Table 1, there was no doubt that the pleural effusion was predominantly lymphocytic in all the samples, with figures ranging from 54% to 100%. MT growth was obtained in solid media in only two cases, and only two to three colonies grew after the first six weeks of incubation. The growth of MT in liquid media occurred in four cases, with a mean of 22 days; growth was negative in two cases. No MT growth was obtained in any of the 149 PF samples with ADA values below 30 U/l. According to a recent review, the method of determining ADA values and establishing cut-off points adapted to regions with a high TB rate has a high discriminatory power; in contrast, in low-incidence regions, it would have a high negative predictive value.<sup>2</sup>

The main disputable factor is the determination of the cut-off point which enables differentiation of TB from other processes, and varies widely according to the following parameters: (1) the prevalence of TB in the geographical area studied; (2) the age of the patients, as in patients over 55, the cut-off point would drop to 26 IU/l, while in the under-55s it would rise to 72 IU/l,<sup>3</sup> and (3) the number of cases studied. In a study of 2413 cases of pleural effusion duly classified according to aetiology and with a mean age of 65, the mean cut-off point was set at 28.26 IU. Values above 100 IU/l were related to lymphoproliferative processes, carcinomas, para-malignant effusions and empyema.<sup>4</sup> In 2015, the average incidence of TB in Castile and León was 9.93 cases per 100,000 population, with an average incidence of PT of 0.77 cases per 100,000 population.<sup>5</sup> The province of León, and especially the county of Bierzo, continue to be the areas with the highest incidence of TB in the region. The selection of PF samples for TB detection could be established initially through the use of ADA, accompanied by other markers, particularly the percentage of lymphocytes and other biochemical parameters. We would need to start by studying a larger number of samples in order to determine the most appropriate cut-off point in our health area. In our study, if we had used 40 IU/l as the cut-off point, we would have missed two cases. Pleural fluids tend to be paucibacillary samples, and greater sensitivity would raise the limit of microbiological and molecular detection. In view of the fact that in most of the PF samples studied (74.5%) the ADA value was below 30 IU/l, that alone would enable us to select the remaining 25.5% (with ADA >30 IU/l) for application of the microbiological and/or molecular techniques, obtaining both a higher diagnostic yield and cost savings. Based on these preliminary results and in the absence of a more exhaustive study, we conclude that the use of molecular techniques for the detection of MT in PF with ADA values below 30 IU/l does not appear to be reasonable in our area.

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**Table 1**

Cases of pleural tuberculosis in which growth of mycobacteria was obtained, and in which the ADA value, the percentage of lymphocytes in the PF, the result of the culture in solid and liquid media and the detection by PCR in direct sample were specified.

No.	Patient	ADA (U/l)	Lymphocytes in PF (%)	SM cultureNo. of colonies	LM culturedays incubation	PCR	Comments
1	PUF	55	98	NEG	NEG	POS	C. telephone
2	SMM	50	100	NEG	24.3	NEG	
3	SRO	66	56	NEG	25.9	–	
4	MMB	31	54	NEG	24.6	–	
5	LCG	63	96	3 colonies week 6	14.5	POS	Lack of <i>rpoB</i> wild type 3
6	MPP	39	85	2 colonies week 6	NEG	–	

ADA: adenosine deaminase; LM: automated liquid culture medium for mycobacteria (BacT/ALERT<sup>®</sup> MP, bioMérieux); NEG: negative; PCR: detection of *Mycobacterium tuberculosis* complex by genome amplification (GeneXpert<sup>®</sup>, Hain Lifescience); PF: pleural fluid; POS: positive; SM: solid culture media for mycobacteria (Coletos, Middlebrook 7H11).

## References

- López-Medrano R, Fuster Foz C, Raya Fernández C. Rendimiento diagnóstico del cultivo de micobacterias en derrames pleurales de origen tuberculoso. Arch Bronconeumol. 2009;45:307–8.
- Ferreiro L, San José E, Valdés L. Derrame pleural tuberculoso. Arch Bronconeumol. 2014;50:435–43.
- Tay TR, Tee A. Factors affecting pleural fluid adenosine deaminase level and the implication of the diagnosis of tuberculous pleural effusion: a retrospective cohort study. BMC Infect Dis. 2013;13:546.
- Lazo Meneses P, Pérez Rodríguez E, Gotera C, Cámara Fernández J, Barrios Barreto D, Díaz Lobato S, et al. Very high level of ADA in pleural effusions, uncommon in tuberculosis. Chest. 2014;145 Suppl 3:154A.
- Informe Epidemiológico sobre la Tuberculosis en Castilla y León. Año 2015. Servicio de Epidemiología. Dirección General de Salud Pública. Consejería de Sanidad de la Junta de Castilla y León. Available from: <http://www.saludcastillayleon.es/profesionales/en/informacion-epidemiologica/>.

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## Osteoarticular infections in pediatric patients: The aetiological importance of *Kingella kingae*



### Infecciones osteoarticulares en pediatría: importancia etiológica de *Kingella kingae*

Osteoarticular infection frequently remains microbiologically unconfirmed in the pediatric age.<sup>1</sup> Since the early 1990s *Kingella kingae* has emerged as a major etiological agent of osteomyelitis and septic arthritis in children aged less than 4 years.<sup>2</sup> Recently, the implementation of molecular detection assays (MDA) has established the real role of this microorganism in osteoarticular infections.<sup>2–5</sup>

We conducted a retrospective study in a cohort of pediatric patients (<18 years at inclusion) diagnosed with septic arthritis and osteomyelitis between January 2012 and May 2016 at Hospital Sant Joan de Déu (Barcelona). Samples were obtained through arthrocentesis or arthrotomy in arthritis and bone debridement in osteomyelitis. Depending on the volume of the drained material, samples were partly inoculated in situ in aerobic blood culture bottles (SBCB) (BacT/ALERT, BioMérieux; USA) by the orthopedic surgeon. In febrile patients, blood cultures (BC) from peripheral veins were also obtained. Samples were processed by means of routine microbiological procedures (gram staining; blood agar, blood anaerobic agar, chocolate agar and tioglicolate medium cultures), inoculated in BCB, when it was not previously done, and to be tested by specific real-time PCR.

PCR targeting the *rtxA* toxin gene of *K. kingae* (accession number EF067866) slightly adapted from a previously PCR design.<sup>5</sup> The primers and probe used in our site were KK-forward (5'-GCGACAAGCAGGTGTACAA-3'), KK-reverse (5'-ACCTGCTGCTACTGTACCTGTTTAG-3') and the TaqMan probe (6FAM-5'-CTTTGAACAAAGCTGGACACGCAGC-3'-BBQ). Real-time

for *LytA* gene of *Streptococcus pneumoniae* and *CtxrA* gene for *Neisseria meningitidis* were also performed.<sup>6,7</sup>

During the study period, samples from 88 patients (42.0% females, median [IQR] age: 2.1 [1.2–6.8] years) were processed. In 51 (58.0%) patients, blood cultures were also performed. Diagnosis included 67 septic arthritis and 21 osteomyelitis.

Overall, 49 cases (55.7%) were microbiologically confirmed, including 38 (56.7%) cases of septic arthritis and 11 (52.4%) cases of osteomyelitis. *K. kingae* (25 cases, 51%) and *Staphylococcus aureus* (18 cases, 36.7%) were the most frequent pathogens. Hip (13), knee (11), ankle (8), elbow (4), shoulder (2) tibia (5), femur (2) ulna (1), fibula (1), astragalus (1) and sternum (1) were the joints and bones affected.

Table 1 shows the distribution of culture and real-time PCR results in patients with microbiologically confirmed osteoarticular infection according to the clinical diagnosis, the age and the species detected.

*Streptococcus agalactiae* was isolated in 2 out of the 3 cases of septic arthritis diagnosed in patients younger than 6 months.

All 25 cases (92% with septic arthritis) of osteoarticular infections caused by *K. kingae* were observed in patients aged between 6 months and 4 years. Only 10 (40%) strains of *K. kingae* were identified by one of the culture methods used (10 in SBCB and 3 also in routine cultures), but 100% of them tested positive by real-time PCR. In patients aged over 4 years (54.8% with septic arthritis) *S. aureus* was the most frequent pathogen and was found in 17 (54.8%) out of 31 cases. Most *S. aureus* isolates (15 out of 18) grew both in BCB and routine culture, while the other 3 were only isolated in BC.

Etiological diagnosis was exclusively attributable to real-time PCR in 17 out of 49 cases (34.7%).

Overall, patients with arthritis were younger (1.5 vs 6.5 years) and more often diagnosed with *K. kingae* infections (34.3% vs 9.5%) than those affected with osteomyelitis.