Russian Journal of Infection and Immunity = Infektsiya i immunitet
2021, vol. 11, no. 3, pp. 491–496

Инфекция и иммунитет
2021, Т. 11, № 3, с. 491–496

MOLECULAR DIAGNOSTICS FOR VERIFICATION OF PLEURAL TUBERCULOSIS IN MOROCCO

I. Chaoui[a], S. Taoudi[b], A. Oudghiri[a], J. Benamor[b], J. Bourkadi[b], M. El Mzibri[a]

[a] *National Center for Energy, Sciences and Nuclear Techniques, Rabat, Morocco*
[b] *Moulay Youssef Hospital, Rabat, Morocco*

Abstract. Pleural tuberculosis (pTB) is a very common form of extrapulmonary tuberculosis (TB). pTB diagnostics represents a major burning challenge worldwide due to the limitations of available conventional diagnostic tools. These latter include microscopic examination of the pleural fluid for acid-fast bacilli, mycobacterial culture of pleural fluid in solid or liquid media, sputum or pleural tissue, and histopathological examination of pleural tissue; these tests have recognized limitations for clinical use. Hence, to overcome these limitations, attention has been devoted to new nucleic acid amplification (NAA) diagnostic tests such as the polymerase chain reaction (PCR) and real-time PCR (RT-PCR), owing to their accuracy, rapidity, high sensitivity and specificity. Within this context, this prospective study was conducted to evaluate the performance of molecular diagnosis methods for differentiation between tuberculosis and non-tuberculosis pleural effusions. Fifty patients with pleural effusion were enrolled in this prospective study in Rabat, Morocco. The efficacy of conventional polymerase chain reaction (PCR) in the diagnostics of tuberculous pleurisy by targeting IS*6110* and mycobacterial internal transcribed spacer (MYITS) was evaluated compared to histopathologic examination and culture data. Our results showed that IS*6110* PCR could "rule in" pTB, the sensitivity and specificity being 41.6% and 85.7%, respectively. Therefore, the findings confirmed that molecular tests exert a relatively high specificity in EPTB but lower sensitivity, thus a positive test is considered as a pTB case whereas negative one cannot exclude the disease. Although the study was limited by a small sample size, it adds to the body of evidence of usefulness of molecular testing as adjuncts to histopathologic examination for accurate diagnosis of pTB, to treat timely and to avoid the emergence and spread of drug resistant pTB. However, further efforts should be made to increase the sensitivity of NAA methods and to identify the best molecular targets to be useful in clinical practice.

Key words: *pleural tuberculosis, Mycobacterium tuberculosis, polymerase chain reaction, IS6110, mycobacterial internal transcribed spacer (MYITS).*

МОЛЕКУЛЯРНАЯ ДИАГНОСТИКА В ВЕРИФИКАЦИИ ТУБЕРКУЛЕЗНОГО ПЛЕВРИТА В МАРОККО

Шауи И.[1], Тауди С.[2], Удгири А.[1], Бенамор Д.[2], Буркади Д.[2], Эль Мзибри М.[1]

[1] *Национальный центр энергетики, ядерных наук и технологий, Рабат, Марокко*
[2] *Больница им. Мулая Юссефа, Рабат, Марокко*

Резюме. Туберкулезный плеврит (pleural tuberculosis — pTB) является очень распространенной формой внелегочного туберкулеза (ТБ). Его диагностика представляет собой серьезную проблему во всем мире из-за недостатков традиционных диагностических методов. К последним относятся микроскопическое исследование плевральной жидкости на наличие кислотоустойчивых микроорганизмов, культивирование микобактерий из плеврального экссудата на твердых или жидких средах и гистологическое исследование биопсии плевры.

Адрес для переписки:
Шауи Имане
10001, Марокко, Рабат, п.я. 1382, Национальный центр энергетики, ядерных наук и технологий.
Тел.: +212 537 713 205. Факс: +212 537 711 846.
E-mail: im_chaoui@yahoo.fr, chaoui@cnesten.org.ma

Contacts:
Imane Chaoui
B.P. 1382 R.P. 10001, National Center of Energy, Sciences and Nuclear Techniques, Rabat, Morocco.
Phone: +212 537 713 205. Fax: +212 537 711 846.
E-mail: im_chaoui@yahoo.fr, chaoui@cnesten.org.ma

Для цитирования:
Шауи И., Тауди С., Удгири А., Бенамор Д., Буркади Д., Эль Мзибри М. Молекулярная диагностика в верификации туберкулезного плеврита в Марокко // Инфекция и иммунитет. 2021. Т. 11, № 3. С. 491–496. doi: 10.15789/2220-7619-MDO-1399

Citation:
Chaoui I., Taoudi S., Oudghiri A., Benamor J., Bourkadi J., El Mzibri M. Molecular diagnostics for verification of pleural tuberculosis in Morocco // Russian Journal of Infection and Immunity = Infektsiya i immunitet, 2021, vol. 11, no. 3, pp. 491–496. doi: 10.15789/2220-7619-MDO-1399

DOI: http://dx.doi.org/10.15789/2220-7619-MDO-1399

В связи с этим наше внимание было направлено на разработку новых диагностических тестов, основанных на амплификации нуклеиновых кислот (ПЦР и РТ-ПЦР), как наиболее точных, быстрых, высокочувствительных и специфичных. В этом контексте данное исследование было проведено для оценки эффективности методов молекулярной диагностики при дифференциации микобактерий туберкулеза и нетуберкулезных микобактерий в плевральном экссудате. Пятьдесят пациентов с плевритом были включены в проспективное исследование в г. Рабат, Марокко. Эффективность ПЦР двух ДНК-мишеней — IS*6110* и микобактериального внутреннего транскрибируемого спейсера (MYITS) — для диагностики туберкулезного плеврита оценивали на основании сравнения с результатами гистологического исследования и культивирования. Наши результаты показали, что IS*6110*-ПЦР может выявить рТВ с чувствительностью и специфичностью 41,6 и 85,7% соответственно. Таким образом, полученные результаты подтверждают, что молекулярные тесты имеют относительно высокую специфичность для диагностики внелегочного туберкулеза, но более низкую чувствительность, поэтому положительный результат рассматривается как подтверждение рТВ, тогда как отрицательный тест не может исключать заболевание. Хотя исследование было ограничено небольшим размером выборки, оно добавляет к совокупности доказательств полезности молекулярного тестирования в качестве дополнения к гистологическому исследованию для точной диагностики рТВ, своевременного лечения и предотвращения появления и распространения лекарственно-устойчивого рТВ. Необходимы дальнейшие исследования для повышения чувствительности молекулярных методов и определения оптимальных молекулярных мишеней, которые могут быть полезны в клинической практике.

Ключевые слова: *туберкулезный плеврит, Mycobacterium tuberculosis, полимеразная цепная реакция, IS6110, внутренний транскрибируемый спейсер микобактерий (MYITS).*

Introduction

Tuberculosis (TB) is a major public health concern worldwide. The World Health Organization (WHO) estimated 10.4 million incident cases of TB and 1.67 million TB deaths in 2017. Of the 6.4 million new TB cases recognized by WHO in 2017, 14% were extrapulmonary TB cases (EPTB); incidence rates are ranging from 8% in the Western Pacific Region to 24% in the Eastern Mediterranean Region [23]. It has been reported that pleural tuberculosis (pTB) is the most common extrapulmonary form in adults worldwide. EPTB is referred to as TB involving organs other than lungs. Independent risk factors of EPTB were reported in the literature to be mainly young age, female gender, immunogenetic background and HIV infection [18, 20].

In Morocco, the proportion of EPTB was 53% among all TB reported cases, and the diagnosis is based on clinical signs and/or histology. Of note, pTB and ganglionnary TB (tuberculous lymphadenitis) are the most common forms of EPTB in Morocco [8]. Although tissue biopsy is the most effective method for EPTB diagnosis, it is invasive and sometimes inaccessible. In contrast, pleural, peritoneal, and pericardial fluids are easily accessible and provide valuable diagnostic clues in EPTB patients [12]. However, a body fluid biopsy may not exclude EPTB as atypical features may be present. Indeed, tuberculous pleural fluid is invariably an exudate, with lymphocytic predominance in about 90% of cases [3]. Usually, conventional diagnostic tests such as microscopic examination of pleural fluid, biochemical assays, mycobacterial culture from pleural liquid and histopathological examination of pleural fluid are widely used but still have limitations. Indeed, it has been reported that microscopy of the pleural fluid for acid-fast bacilli (AFB) is positive in less than 5% of tuberculous pleurisy cases, except for HIV-infected patients and tuberculous empyema. Moreover, a mycobacterial culture of pleural fluid has also low sensitivity ranging from 24 to 58%; it is also limited by lengthy delays of up to 8 weeks to get results if solid culture media are used [1, 21]. To overcome these limitations, attention has been paid to new nucleic acid amplification (NAA) diagnostic technologies such as the polymerase chain reaction (PCR) and real-time PCR, owing to their rapidity, high sensitivity, and specificity. Hence, the present study aimed to evaluate the performance of *MYITS* (mycobacterial internal transcribed spacer) and IS*6110* PCR based assays for the detection of *Mycobacterium tuberculosis* complex (MTBC) strains in pleural liquid compared to results generated by culture and histologic examination of pleural fluid as well as culture and to assess efficiency and practicality of the PCR based methods for MTBC detection and better management of EPTB in Morocco.

Materials and methods

Patients. A total of 50 patients suspected of having pleural effusion (PE) or had evidence of PE according to chest X-ray results and medical records, referred to Moulay Youssef university hospital in Rabat, from June 2015 to July 2016 were included in this prospective study. The study was approved by the Ethical Committee for Biomedical Research, School of Medicine and Pharmacy, Mohammed V University of Rabat. Patients were informed on the objectives of the study and gave written informed consent for their participation in the present study.

Study design and sample collection. For each subject, the pleural fluid collected in a sterile tube was used for diagnosis including (i) Histology of pleural fluid; (ii) Ziehl–Neelsen (ZN) microscopy of pleural

fluid; (iii) Culture of pleural fluid for *M. tuberculosis* and (iv) PCR amplification to detect mycobacteria belonging to *Mycobacterium* genus and species belonging to *M. tuberculosis* complex. To fulfill those purposes, an aliquot of 5–10 ml of pleural fluid was submitted for cytology, biochemical testing, and histopathologic examination. An aliquot of 2 ml of the pleural fluid was centrifuged at 3,000g for 15 min, the supernatant was decanted out, and a 0.5 ml of sediments was subjected to molecular analysis. Another 2 ml of the concentrated pleural fluid was simultaneously sent to mycobacteriological laboratory for ZN staining followed by smear microscopy and mycobacterial culture on Lowenstein-Jensen medium for 4 to 8 weeks.

DNA extraction. Sediments from 0.5 ml of PE were subjected to microcentrifugation at 11,000g for 10 min and resuspension in 400 µL of distilled water. DNA was then extracted by simple boiling method for 10 minutes to release DNA [6].

Amplification of mycobacterial DNA. DNA was subjected to conventional PCR amplification by two sets of specific primers, *MYITS* and IS*6110*, to detect DNA of the members of *Mycobacterium* genus and *M. tuberculosis* complex species, respectively. The sequences and characteristics of used primers are given in Table 1.

PCR reactions were performed in a final volume of 25 µL containing 5 µl of 5X MyTaq reaction buffer (Bioline); 0.5 µl of each of 10 µM oligonucleotide primers; 1U of MyTaq DNA polymerase (Mytaq, Bioline); 16.8 µl of Nuclease free water and 2 µL of crude DNA. The reaction was subjected to a PCR protocol as follows: an initial denaturation at 95°C for 2 min followed by 30 cycles each containing denaturation at 95°C for 15 sec, annealing at corresponding Tm for 15 sec and extension of 72°C for 30 sec followed by a final extension at 72°C for 7 min at the end of the last cycle. For each set of analyses, a positive control (DNA of *M. tuberculosis* H37Rv) and a negative control (2 µl of PCR grade water) were included. The amplified DNA products were visualized by UV illumination after agarose gel electrophoresis and green viewer staining.

Results

Patients and pleural fluid characteristics. 50 patients enrolled in this study originated mainly from Rabat, Morocco. The demographic and clinical data showed that the mean age of patients was 34.3 years ranging from 17 to 81 years; the sex-ratio being 1.2 (28 males vs. 22 females). For pleurisy localization, 48% of patients had right side pleurisy, 42% had left side pleurisy, whereas 10% had pleurisy on both sides. The effusion was an exudate with high protein content in 90% of cases. The cytological examination revealed lymphocyte predominance in 92% of pleural effusions. Thoracentesis revealed that pleural fluid was yellow-colored in 88% of cases whereas turbid and sero-sanginous aspects were reported each in 6% of cases.

The ZN staining smear microscopy and LJ medium culture were negative for all cases. Histological examination showed that 72% of patients had pTB (36/50), whereas 20% had inconclusive results (10/50). The histology examination was not performed for 8% of patients (4/50) because of insufficient volume of pleural fluid.

Molecular analysis by PCR using specific primers targeting *MYITS* and IS*6110* regions showed in positive cases the PCR products of 350–500 bp and 125 bp respectively. Out of 50 suspected patients with pleurisy, 34% (17/50) and 38% (19/50) were IS*6110* PCR and *MYITS* PCR-positive, respectively (Table 2). For instance, 30% of specimens were positive for both *MYITS* and IS*6110* (15/50); 4% of specimens were determined to be positive by IS*6110* but negative by *MYITS* (2/50). Of particular interest, all IS*6110* positive cases (17/50) had conventional characteristics in favor of EPTB, including clinical signs, histological results. All these patients had clinical follow-up, and consequently received anti-TB treatment. In contrast, 8% of samples were rather positive for *MYITS* and negative for IS*6110* (4/50); this result is likely to rule out pTB. For these cases, the histopathologic examination of the pleural fluid was not conclusive.

Table 1. Primers for PCR amplification

Target	Primer	Sequence	Tm	Fragment length	Reference
MYITS	***MYITS* F**	5'-GATTGGGACGAAGTCGTAACAAG-3'	60°C	350–500 bp	[16]
	***MYITS* R**	5'-AGCCTCCCACGTCCTTCATCGGCT-3'			
IS*6110*	**IS F**	5'-CCTGCGAGCGTAGGCGTCGG-3'	65°C	123 bp	[14]
	IS R	5'-CTCGTCCAGCGCCGCTTCGG-3'			

Table 2. AFB smear microscopy, LJ culture, PCR IS*6110*, and PCR *MYITS* results in suspected patients with pleural tuberculosis (n = 50)

No. of specimens	AFB smear		L/J culture		*MYITS* PCR		IS6110 PCR		*MYITS* PCR and IS*6110* PCR	
	Pos	Neg	Pos	Neg	Pos (%)	Neg	Pos (%)	Neg	Pos (%)	Neg
50	0	50	0	50	19 (38%)	31	17 (34%)	33	15 (30%)	35

Table 3. Comparison of PCR IS*6110* results with histopathologic data

N = 50	Confirmed pTB by histologic examination (n = 36)	No conclusive exam for pTB by histologic examination (n = 14)	Total
PCR IS*6110*+	15	2	17
PCR IS*6110*–	21	12	33
Total	36	14	50
Sensitivity (%)	41.6%		
Specificity	85.7%		

Correlation between molecular and histopathological results showed that out the 36 pTB cases confirmed by histopathological examination, 15 cases were MTBC positive by PCR method targeting IS*6110* in contrast to ZN staining smear microscopy and LJ culture that failed to detect positive MTBC cases (Table 2).

The efficiency of the IS*6110* PCR analysis compared with the histopathological examination was estimated by the calculation of sensitivity and specificity (Table 3).

Discussion

The diagnosis of extrapulmonary infection with the MTBC is challenging because of the absence of a uniform reference standard [22]. As such, definitive and accurate diagnosis is often problematic, since the number of bacteria in extrapulmonary specimens is by far lower than the one in pulmonary specimens [10]. Furthermore, the collection of extrapulmonary material often requires invasive procedures. Moreover, it may be difficult to obtain additional samples for patient follow-up. Consequently, monitoring of treatment outcomes is difficult to establish. Recently, Pang et al. reported that the diagnosis of most of extrapulmonary TB cases relies on clinical symptoms, thus generating diagnostic delays as well as misdiagnosis of extrapulmonary TB cases [18].

The pTB is the most common etiology of pleural effusion [13]. Its diagnosis relies on ZN smear microscopy, which is albeit its low cost and speed to be performed, is not an efficient tool (sensitivity ranging from from 0 to 40%) in pTB patients with the low mycobacterial load. A concentration of 10,000 AFB/ml is required to be seen on microscopy, thus, ZN smear microscopy is not an efficient tool in pTB patients with the low mycobacterial load. Moreover, this conventional technique is not able to differentiate between MTBC species and nontuberculous mycobacteria. Likewise, culture identification, although recognized as the gold standard method, has a sensitivity ranging from 0 to 80% in EPTB as 10 to 100 viable bacilli are mandatory for positivity. Additionally, mycobacterial culture takes 6 to 8 weeks to get results which causes diagnosis delays and affects clinical and therapeutic management and clinical and therapeutic decisions [15].

In the present investigation, ZN staining smear and culture did not detect any positive pTB case. These results are in agreement with those reported by Makeshkumar et al. (2014), who found 0% sensitivity of both smear microscopy and culture identification [14]. However, other studies reported the sensitivity of culture ranging from 12 to 56% [7, 10]. Therefore, more advanced techniques for diagnosis are highly sought by clinicians and worldwide TB national control programs.

During the last decade, considerable efforts have been made towards the development of NAA tests for rapid and accurate diagnosis of pTB using different gene targets such as IS*6110*, GCRS and MPB-64 [15]. Of note, the IS*6110* PCR is a widely used test to detect MTBC strains because of the presence of multiple copies within the MTBC genome. In the present investigation, out of 19 patients positive for *MYITS*, 78.9% had IS*6110* in their genome. Likewise, Diraa et al. (2005) reported that 92% of MTB strains from Morocco harbored 6–14 copies of IS*6110* [4]. However, in some MTBC strains, especially from Asia, there are few or zero copies of IS*6110* in the genome [19], which underlines the importance of the combination of two or more gene targets either by mono or multiplex PCR to conclude for pTB.

The results from this study reported that 41.6% of confirmed pTB are due to species belonging to MTBC (15/36); this rate could increase by applying more accurate techniques for DNA extraction and by testing other target genes to reach better sensitivity. Of note, four pleural fluid specimens were positive for *MYITS* but negative for IS*6110* meaning that those patients were infected by NTM species.

These results confirmed that molecular tests have a relatively high specificity in EPTB but lower sensitivity; thus, a positive test is treated as a pTB case whereas negative one cannot exclude the disease. This low sensitivity can be attributed to some technical constraints in nucleic acid extraction, PCR failure due to the presence of inhibitors in the pleural fluid such as host proteins, and to the paucibacillary nature of the disease.

Although NAA tests are of great value to shorten the turnaround time, they present major drawbacks and may generate either a high risk of false-positive results due to either laboratory contamination or the presence of killed or dormant bacilli in the patient specimens. Also, there is high risk of false-negative outcomes due to the paucibacillary nature of the disease, unequal distribution of acid-fast bacilli in large volume of fluids, lack of adequate clinical sample volume [5].

To enhance the sensitivity of molecular tests, real-time PCR to quantify bacterial load, reducing hands-on time and decreasing the risk of cross contamination was developed [17]. In this field, the PCR based commercially available Xpert MTB/RIF assay, allowing the identification of both *M. tuberculosis* and rifampicin resistance, was developed [2, 9].

EPTB diagnosis is crucial to timely treat patients. Like for TB, the recommended therapy for pTB is the standard 6-month regimen consisting of a two-month intensive phase of Rifampicin, Isoniazid, Pyrazinamide, Ethambutol (RHZE), followed by a four-month continuation phase with RH [24]. Along with the drug regimen, a therapeutic thoracentesis in patients with large, symptomatic effusions should be performed. Given that pleural effusions have low microbial load, shorter regimens have been administered and had a low relapse rate [11]. Of note, drug susceptibility testing of pleural biopsy must be considered in settings where the prevalence of drug-resistant TB is high. Treatment regimens are identical to those administered for pulmonary TB.

This study highlights the need for implementing NAA techniques in EPTB management. However, it has some limitations related to the technical constraints: (i) PCR results from this study were evaluated against histopathological examination and comprehensive clinical follow-up since smear microscopy and culture were negative for all cases, (ii) the study was performed on crude DNA. To definitely conclude about the sensitivity and specificity of molecular tests, NAA techniques should be performed preferably on purified DNA, and other targets genes should be tested in combination.

Conclusion

To the best of our knowledge, this is the first study aiming to diagnose TB pleural effusion in Morocco by NAA tests. In spite of its limitations, it adds to the body of evidence on the usefulness of molecular testing as adjunct approach for accurate diagnosis and timely treatment of patients with pTB, thus allowing to avoid the emergence and spread of drug-resistant pTB. However, further efforts should be made to find the accurate method for DNA extraction from pleural fluid to increase the sensitivity of IS*6110* PCR methods and to identify the best molecular targets to be helpful in clinical practice.

Acknowledgements

We would like to thank all patients for their participation. We are sincerely grateful to the clinicians and nurses of the Moulay Youssef University Hospital for their fruitful collaboration in this study.

Conflict of interest

The authors have no conflict of interest.

Ethics approval and consent to participate

The study protocol was approved by the Ethical Committee for Biomedical Research, School of Medicine and Pharmacy, Mohammed V University of Rabat, and a written informed consent was obtained from each study subject.

Funding

The authors declare that no funding was received from any agency.

References

1. Aggarwal A.N., Gupta D., Jindal S.K. Diagnosis of tubercular pleural effusion. *Indian. J. Chest., 1999, vol. 41, no. 2, pp. 89–100.*
2. Asghar M.U., Mehta S.S., Cheema H.A., Patti R., Pascal W. Sputum smear and culture-negative tuberculosis with associated pleural effusion: a diagnostic challenge. *Cureus, 2018, vol. 10, no. 10: e3513. doi: 10.7759/cureus.3513*
3. Cohen L.A., Light R.W. Tuberculous pleural effusion. *Turk. Thorac. J., 2015, vol. 16, no. 1, pp. 1–9. doi: 10.5152/ttd.2014.001*
4. Diraa O., Elmdaghri N., Laaboudi L., Boudouma M., Gutiérrez M.C., Benbachir M. IS6110 restriction fragment length polymorphism of Mycobacterium tuberculosis isolates from an area of Casablanca, Morocco. *Int. J. Tuberc. Lung. Dis., 2005, vol. 9, no. 11, pp. 1294–1296.*
5. Galimi R. Extrapulmonary tuberculosis: tuberculous meningitis new developments. *Eur. Rev. Med. Pharmacol. Sci., 2011, vol. 15, no. 4, pp. 365–386.*
6. Gholoobi A., Masoudi-Kazemabad A., Meshkat M., Meshkat Z. Comparison of culture and PCR methods for diagnosis of Mycobacterium tuberculosis in different clinical specimens. *Jundishapur. J. Microbiol., 2014, vol. 7, no. 2: e8939. doi: 10.5812/jjm.8939*
7. Greco S., Girardi E., Masciangelo R., Capoccetta G.B., Saltini C. Adenosine desaminase and interferon gamma measurements for the diagnosis of tuberculous pleurisy: a meta-analysis. *Int. J. Tuberc. Lung. Dis., 2003, vol. 7, no. 8, pp. 777–786.*
8. Haddaoui H., Mrabet F.Z., Aharmin M., Bourkadi J.E. Multidrug-resistant extrapulmonary tuberculosis: about 7 cases. *Pan. Afr. Med. J., 2019, vol. 32: 196. doi: 10.11604/pamj.2019.32.196*
9. Hillemann D., Rüsch-Gerdes S., Boehme C., Richter E. Rapid molecular detection of extrapulmonary tuberculosis by the automated GeneXpert MTB/RIF system. *J. Clin. Microbiol., 2011, vol. 49, no. 4, pp. 1202–1205. doi: 10.1128/JCM.02268-10*
10. Jeon D. Tuberculous pleurisy: an update. *Tuberc. Respir. Dis., 2014, vol. 76, no. 4, pp. 153–159. doi: 10.4046/trd.2014.76.4.153*
11. Jørstad M.D., Aßmus J., Marijani M., Sviland L., Mustafa T. Diagnostic delay in extrapulmonary tuberculosis and impact on patient morbidity: a study from Zanzibar. *PLoS One, 2018, vol. 13, no. 9: e0203593. doi: 10.1371/journal.pone.0203593*
12. Lee J.Y. Diagnosis and treatment of extrapulmonary tuberculosis. *Tuberc. Respir. Dis., 2015, vol. 78, no. 2, pp. 47–55. doi: 10.4046/trd.2015.78.2.47*
13. Light R.W. Pleural effusion in pulmonary embolism. *Semin. Respir. Crit. Care. Med., 2010, vol. 31, no. 6, pp. 716–722. doi: 10.1055/s-0030-1269832*

14. Makeshkumar V., Madhavan R., Narayanan S. Polymerase chain reaction targeting insertion sequence for the diagnosis of extrapulmonary tuberculosis. *Indian J. Med. Res., 2014, vol. 139, no. 1, pp. 161–166.*
15. Mehta P.K., Raj A., Singh N., Khuller G.K. Diagnosis of extrapulmonary tuberculosis by PCR. *FEMS Immunol. Med. Microbiol., 2012, vol. 66, pp. 20–36. doi: 10.1111/j.1574-695x.2012.00987.x*
16. Mokaddas E., Ahmad S. Development and evaluation of a multiplex PCR for rapid detection and differentiation of Mycobacterium tuberculosis complex members from non-tuberculous mycobacteria. *Jpn. J. Infect. Dis., 2007, vol. 60, no. 2–3, pp. 140–144.*
17. Molaudzi M., Molepo J. The use of real-time polymerase chain reaction and an adenosine deaminase assay for diagnosing pleural tuberculosis. *Afr. J. Lab. Med., 2019, vol. 8, no. 1: 731.*
18. Pang Y., An J., Shu W., Huo F., Chu N., Gao M., Qin S., Huang H., Chen X., Xu S. Epidemiology of extrapulmonary tuberculosis among inpatients, China, 2008–2017. *Emerg. Infect. Dis., 2019, vol. 25, no. 3, pp. 457–464. doi: 10.3201/eid2503.180572*
19. Roychowdhury T., Mandal S., Bhattacharya A. Analysis of IS6110 insertion sites provides a glimpse into genome evolution of Mycobacterium tuberculosis. *Sci. Rep., 2015, vol. 5: 12567. doi: 10.1038/srep12567*
20. Sankar M.M., Singh J., Diana S.C., Singh S. Molecular characterization of *Mycobacterium tuberculosis* isolates from North Indian patients with extrapulmonary tuberculosis. *Tuberculosis (Edinb.), 2013, vol. 93, no. 1, pp. 75–83. doi: 10.1016/j.tube.2012.10.005*
21. Seibert A.F., Haynes J. Jr., Middleton R., Bass J.B. Jr. Tuberculous pleural effusion. Twenty-year experience. *Chest, 1991, vol. 99, no. 4, pp. 883–886.*
22. Tyagi S., Sharma N., Tyagi J.S., Haldar S. Challenges in pleural tuberculosis diagnosis: existing reference standards and nucleic acid tests. *Future Microbiol., 2017, vol. 12, pp: 1201–1218. doi: 10.2217/fmb-2017-0028*
23. WHO. Global tuberculosis Report 2018. *Geneva: WHO, 2018. URL: https://apps.who.int/medicinedocs/documents/s23553en/s23553en.pdf (11.11.2019)*
24. Zhai K., Lu Y., Shi H.Z. Tuberculous pleural effusion. *J. Thorac. Dis., 2016, vol. 8, no. 7, pp. E486–E494. doi: 10.21037/jtd.2016.05.87*

Авторы:

Шауи И., кандидат наук, научный сотрудник отдела медико-биологических исследований департамента наук о жизни Национального центра энергетики, ядерных наук и технологий, Рабат, Марокко
Тауди С., кандидат наук, научный сотрудник отделения фтизиопульмонологии больницы им. Мулая Юссефа, Рабат, Марокко
Удгири А., кандидат наук, научный сотрудник отдела медико-биологических исследований департамента наук о жизни Национального центра энергетики, ядерных наук и технологий, Рабат, Марокко
Бенамор Д., профессор, врач-фтизиопульмонолог отделения фтизиопульмонологии Больницы им. Мулая Юссефа, Рабат, Марокко
Буркади Д., профессор, врач-фтизиопульмонолог отделения фтизиопульмонологии Больницы им. Мулая Юссефа, Рабат, Марокко
Эль Мзибри М., кандидат наук, руководитель Департамента наук о жизни Национального центра энергетики, ядерных наук и технологий, Рабат, Марокко.

Authors:

Chaoui I., PhD, Researcher, Biology and Medical Research Unit, Department of Life Sciences, National Center of Energy, Sciences and Nuclear Techniques, Rabat, Morocco;
Taoudi S., PhD, Researcher, Department of Pneumo-Phtisiology, Moulay Youssef Hospital, Rabat, Morocco;
Oudghiri A., PhD, Researcher, Biology and Medical Research Unit, Department of Life Sciences, National Center of Energy, Sciences and Nuclear Techniques, Rabat, Morocco;
Benamor J., Professor, Pneumo-Phytisiologist, Department of Pneumo-Phtisiology, Moulay Youssef Hospital, Rabat, Morocco;
Bourkadi J., Professor, Pneumo-Phytisiologist, Department of Pneumo-Phtisiology, Moulay Youssef Hospital, Rabat, Morocco;
El Mzibri M., PhD, Research Director, Head of the Life Sciences Department, National Center of Energy, Sciences and Nuclear Techniques, Rabat, Morocco.

Поступила в редакцию 03.03.2020
Принята к печати 03.06.2020

Received 03.03.2020
Accepted 03.06.2020