27th ECCMID Vienna, Austria 22 – 25 April 2017

The congress of ESCMID

Session: P082 Tuberculosis: pathogenesis, diagnostics and drug resistance

Category: 2a. Tuberculosis and other mycobacterial infections

25 April 2017, 12:30 - 13:30
P1667

Diagnosis of Mycobacterium spp. infection by PCR: usefulness of paraffin-embedded tissue sections DNA extracts when fresh biopsy specimens are not available

Berta María Pilar Vela[1], Marta María Gómara[2], Elena Morilla[2], Juan Sahagún[3], Lucía Ferrando[3], Ana Isabel López-Calleja[2], Cruz Villuendas Usón[4], Rebeca Fernández[2], J Viñuelas*[5], Maria Jose Revillo[2]

[1]*Hospital Universitario Miguel Servet; Servicio de Microbiología*

[2]*Hospital Universitario Miguel Servet; Microbiology*

[3]*Hospital Universitario Miguel Servet*

[4]*Hospital Universitario Miguel Servet; Department of Microbiology*

[5]*Hospital Universitario Miguel Servet. Iis Aragon*

Background: Early diagnosis of mycobacteria infection (MI) allows prompt and specific antimycobacterial treatment. Our Microbiology Laboratory often receives paraffin-embedded specimens suspicious of MI from Pathology Department (PD), not submitted for mycobacterial culture. The only option to confirm the diagnosis is to detect mycobacterial DNA by PCR assay. We aimed to analyze the PCR profitability from paraffin-embedded tissue sections DNA extracts *versus* fresh biopsies.

Material/methods: From November 2013 to November 2016, 170 biopsy specimens were collected from patients who were clinically suspected to have MI. 108 of which (63.5%) were sent for mycobacterial culture and 62 (36.5%) were submitted from PD, when histopathologic features of MI were present.

DNA extraction and PCR assay were performed by FluoroType® MTB v.1.0 (Hain Lifescience) according to manufacturer's standards. In case of paraffin-embedded tissue blocks, DNA extraction was performed by using DNA Sample Preparation Kit (Cobas 4800®, Roche).

If high clinical suspicion persisted despite *Mycobacterium tuberculosis complex* (mTB) was not detected or first clinical suspicion was non-tuberculous mycobacteria (NTM) infection, PCR hybridization assay to detect NTM was also performed using GenoType Mycobacterium CM/AS (Hain Lifescience). Clinical, laboratory, epidemiologic data and previous treatment were reviewed.

Results: The study included 170 clinical samples from 106 patients, 60 males (56.6%) and 46 females (43.4%). A total of 171 PCR assays were performed (164 mTB-PCR and 7 NTM-PCR). Of 108 fresh biopsy specimens, 21 (19.4%) obtained a positive result and 87 (80.5%) a negative result by PCR.

Of 62 paraffin-embedded tissue blocks, MI diagnosis was confirmed by PCR in 24 (39%) and excluded in 32 (51,6%) according to mTB/NTM PCR negative results, clinical and histopathologic criteria (Table 1).

Profitability of PCR for detecting *M. tuberculosis* in paraffin-embedded tissue blocks and fresh specimens were 36.7% and 17.3% respectively.

	mTB-PCR (DNA-extracts N=60; fresh-biopsies N=104)					**NTM-PCR** (DNA-extracts N=3; fresh-biopsies N=4)	
Specymen type	**Positive**	**Profitability**	**Specificity**	**Sensitivity**	**Negative**	**Positive**	**Negative**
DNA-extracts(N=62)	**22**	36.7%	100%	84.6%*	**38**	**2**	**1**
Fresh-biopsies (N=108)	**18**	17.3%	100%	75%**	**86**	**3**	**1**
TOTAL (N=170)	**40**				**124**	**5**	**2**

Table 1-Results

*2 false negative results (positive *M. tuberculosis* culture), 2 high clinical suspicion cases with TB treatment, 2 no filiated cases

*3 false negative results (positive *M. tuberculosis* culture), 1 high clinical suspicion case with TB treatment, 5 no filiated cases.

Conclusions: Performance of mTB/NTM PCR from DNA extracts submitted from PD allows a fast and reliable diagnosis, showing high profitability, even for NTM PCR, despite is not validated for direct sample and reduced number of cases reported.

Results showed that 53.3% (24/45) of MI diagnosis were confirmed by mTB/NTM PCR from paraffin-embedded tissue blocks, carrying out an early directed treatment. We suggest this is a suitable method that should be implemented in laboratories.