

Title: *Mycobacterium tuberculosis* bacilli in oral biopsies containing granulomatous inflammation with caseous necrosis.

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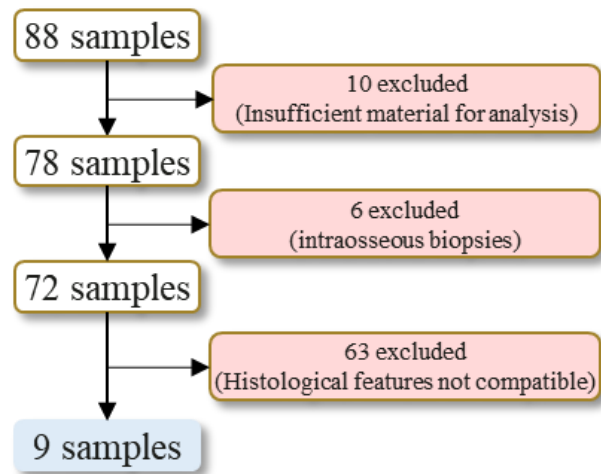
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Supplementary Figures



Supplementary Fig 1 Insufficient material for analysis - paraffin-fixed samples were not found in the FAO-UFMG archive and did not have enough material for all necessary analyses: exclusion of intraosseous biopsies - histological characteristics compatible or not - presence or absence of granuloma with caseous necrosis.

Supplementary Tables

Supplementary Table 1 Clinical and histological diagnosis of selected samples

Sample	Clinical Diagnosis	Histological Diagnosis
1 [†]	-	Tuberculosis
2 ^{†‡}	-	Tuberculosis
3 ^{†‡}	-	Tuberculosis
4	Orofacial granulomatosis	Granulomatous inflammation. Note: Consider orofacial granulomatosis, Crohn's disease, sarcoidosis, tuberculosis, and chronic granulomatous disease.
5	Epidermoid cyst	Suggestive of tuberculosis.
6	SCC	Granulomatous lesion. Note: clinical investigation is suggested to rule out the possibility of tuberculosis.
7 [§]	SCC or Infectious Lesion	Chronic granulomatous inflammatory reaction. Note: although ZN staining did not show bacilli, the possibility of tuberculosis is not ruled out.
8 [§]	SCC or Infectious Lesion	Chronic granulomatous inflammatory reaction. Note: although ZN staining did not show bacilli, the possibility of tuberculosis is not ruled out.
9	SCC	Chronic granulomatous inflammatory process. Note: clinical and laboratory evaluation is required to confirm the diagnosis.

(-): uninformed

[†]Samples where no other type of special staining or clinical characteristics that collaborated for the diagnosis were reported

^{†,§}Samples from the same individual

Abbreviations: SCC: Squamous Cell Carcinoma

Supplementary Table 2 Use of different gene targets for the diagnosis of EPTB and sensitivity and specificity of PCR tests

Reference	TB/Tissue	NAA test	Gene	Primers	Amplicon size	Annealing (°C/min)		Cycle	St (%)	Sp (%)
Barcelos et al., 2008	Spleen (miliary TB)/FFPE	PCR	IS6110	F: CCTGCGAGCGTAGGCGTCGG R: CTCGTCCAGCGCCGCTTCGG	123	65	1	40	N.D.	N.D.
Alli et al., 2011	Smear	PCR	IS6110	F: CCTGCGAGCGTAGGCGTCGG R: CTCGTCCAGCGCCGCTTCGG	123	68	1	30	75,5	94,8
Khosravi et al., 2017	EPTB	nestedPCR	IS6110 Outer	F: ATCGTGGAAGCGACCCGCCAGCC CAGGAT R: CGGGACCACCCGCGGCAAAGCCC GCAGGAC	220	63	0,5	30	N.D.	N.D.
			Inner	F: CCTGCGAGCGTAGGCGTCGG R: CTCGTCCAGCGCCGCTTCGG	123	68	0,5	35	N.D.	N.D.
De Almeida et al., 2013	Culture	PCR	IS6110	F: CCTGCGAGCGTAGGCGTCGG R: CTCGTCCAGCGCCGCTTCGG	123	64	2	40	92,5	100
De Almeida et al., 2015	Smear	PCR	IS6110	F: CCTGCGAGCGTAGGCGTCGG R: CTCGTCCAGCGCCGCTTCGG	123	68	2	40	37.5	100
Antonello et al., 2021	EPTB/FFPE	ddPCR	IS6110	F: ATCTGGACCCGCCAA R: CCTATCCGTATGGTGGATAA	UN	56	1	39	98,5	100
			rpoB	F: GGAGCGCCAAACCG R: AGTCCCGGAACCTCAA	UN	56	1	39	66,5	100
Bandyopadhyay et al., 2008	EPTB/FFPE	GeneXpert® MTB/RIF							79,4	100
		Multiplex PCR	65 kDa	F: CTAGGTCGGGACGGTGAGGCCAG G R: CATTGCGAAGTGATTCTCCGGAT	541	55	2	40	78.9	87.1
			dnaJ	F: AAGAGGAAGGAGAGAGGC R: GTCGTTGAGGTTGAACTC	365					
			IS6110	F: GTGGGCATGGTCGCAGAGAT	165					

					R: CTCGATGCCCTCACGGTTCA							
Chakravorty et al., 2005	EPTB/ and Culture	Smear	PCR	IS6110	N.D.	UN	52	1	45	60	75	
				devR	F: ATCTGTTGTCCCGCATGCC R: GTCCAGCGCCCACATCTTT	UN				47	67	
				Combined (devR + IS6110)						67	75	
Da Cruz et al., 2010	Blood urine	and	nestedPCR	IS6110	F: CCGCAAAGTGTGGCTAAC R: ATCCCCTATCCGTATGGTG	409	68	1	30	53.4	89.8	
				Outer	F: AACGGCTGATGACCAAAC R: GTCGAGTACGCCTTCTTGTT	316	57	0,5	30			
Qing Jun, et al., 2023	EPTB/FFPE		GeneXpert®	MTB/RIF						57,8	100	
Allahyartorkaman et al., 2019	PTB/FFPE		GeneXpert®	MTB/RIF						95,5	96,7	
	EPTB/FFPE									76,5	95,9	
Mustafa et al., 2006	EPTB		nestedPCR	IS6110	F: CCTGCGAGCGTAGGCGTCGG R: CTCGTCCAGCGCCGCTTCGG F: TTCGGACCACCAGCACCTAA R: TCGGTGACAAAGGCCACGTA	123	68	1	45	N.D.	N.D.	
						92	58	1	35	N.D.	N.D.	

Abbreviations: ddPCR: Droplet Digital PCR; EPTB: Extrapulmonary Tuberculosis; PTB: Pulmonary Tuberculosis; N.D.: Not Done; St: Sensitivity; Sp: Specificity; UN: uninformed

Supplementary Table 3 Primers used and reaction parameters

Primers	Product	Annealing Temperature	Cycles
1 st PCR			
5'CCT GCG AGC GTA GGC GTC GG3'	123 bp	55°C	45
5'CTC GTC CAG CGC TTC GG3'			
2 nd PCR			
5'TTC GGA CCA GCA CCT AA3'	92 bp	55°C	45
5'TCG GTG ACA AAG GCC ACG TA3'			

Supplementary Table 4 Means and standard deviation of ages, time of evolution, and size of lesions

Variable	Mean (SD)	Min - Max	Included n %	
Age (years)	50 (23.08)	19 - 89	7/7	(100.0%)
Duration of Symptoms (months) [†]	2.81 (2.40)	0.26 - 6	4/7	(57.14%)
Size (mm) [‡]	22 (22.528)	5 - 60	5/9	(71.4%)

Abbreviations: Max: Maximum; Min: Minimum; mm: millimeter; SD: Standard Deviation.

[†]missing data for 3 cases of this variable.

[‡]missing data for 2 samples of this variable and 2 was indeterminate.

Supplementary Table 5 Frequency data of selected samples

Variable	n	(%)
Age	07	(100)
Less than 20 years	01	(14.2)
From 21 to 60 years	04	(57.1)
Greater than 60 years	02	(28.5)
Sex	07	(100)
Female	02	(28.5)
Male	05	(71.4)
Lesion Type	09	(100)
Ulcer	06	(66.7)
Cystic	01	(11.1)
Tumor	02	(22.2)
Lesion Color	09	(100)
Erythematous	03	(33.3)
Similar to Oral Mucosa	01	(11.1)
Missing Data	05	(55.6)
Symptomatology	07	(100)
Symptomatic	02	(28.5)
Asymptomatic	01	(14.2)
Missing Data	04	(57.1)
Biopsy type	09	(100)
Incisional Biopsy	06	(66.7)
Excisional Biopsy	01	(11.1)
Missing Data	02	(22.2)
HIV Status	07	(100)
Positive	01	(14.2)
Not Reported	06	(85.7)
Location	09	(100)
Tongue	04	(44.4)
Lower Alveolar Ridge	02	(22.2)
Lip	01	(11.1)
Buccal floor	01	(11.1)
Buccal Mucosa	01	(11.1)
Evolution Time (months)	07	(100)
Up to 1 month	01	(14.2)
From 1 to 2 months	01	(14.2)
From 2 to 3 months	01	(14.2)
More than 3 months	01	(14.2)
Indeterminate	02	(28.5)
Missing Data	01	(14.2)
Size (mm)	09	(100)
Up to 10mm	03	(33.3)
From 10 to 59mm	01	(11.1)
Equal to or greater than 60mm	01	(11.1)
Missing Data	04	(44.4)