PCR utility over conventional tests for detection of *Mycobacterium* spp. in patients with mastitis and granulomatous lymphadenitis: Case series

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Abstract

Objective: The present study aims to highlight the utility of quantitative polymerase chain reaction (qPCR) as a method for identifying typical and atypical mycobacterial infections in patients with mastitis and granulomatous lymphadenitis.

Materials and methods: A total of five patients initially diagnosed with mastitis or granulomatous lymphadenitis based on their clinical characteristics in whom conventional tests were used as a diagnostic method were selected. **Conclusion:** The present study showed that the routine performance of qPCR for typical and atypical mycobacteria allows for improved diagnosis.

Keywords: Quantitative polymerase chain reaction. *Mycobacterium* spp. Granulomatous mastitis. Granulomatous lymphadenitis.

Introduction

Idiopathic granulomatous mastitis, also known as idiopathic lobular granulomatous mastitis, is a benign chronic inflammatory disease of the breast with an unknown etiology¹. A definitive diagnosis is primarily established through histopathology. Accordingly, biopsies reveal a granuloma formation in combination with localized infiltration of multinucleated giant cells, epithelioid histiocytes, and plasma cells. The diagnosis and treatment of patients with granulomatous mastitis continues to be a challenge for physicians, considering the non-definitive results that can be obtained from ultrasound, mammography, and magnetic resonance imaging. This promotes a prolonged course of the disease with a significant impact on quality of life².

Chronic granulomatous lymphadenitis is a distinct form of inflammatory immune response in which organized collections of epithelial macrophages form granulomas. In general, granulomatous inflammation occurs when the immune system fails to eliminate a foreign body and instead covers it with activated macrophages³. The etiologies of axillary lymph node granulomas are similar to those of breast parenchyma granulomas, and patients generally exhibit tenderness, erythema, swelling, and abscess formation⁴.

Tuberculosis (Tb) is a serious disease that continues to be a public health problem worldwide⁵. *Mycobacterium tuberculosis* remains the primary causative agent of Tb in developing and underdeveloped countries, with atypical mycobacteria rarely being isolated⁶. Although

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the lungs continue to be the primary site of Tb infection, increasing reports of extra-pulmonary Tb have emerged worldwide, suggesting the potential for Tb to spread into various organs throughout the body⁵. Approximately 90% of atypical mycobacterial infections are located in the pulmonary system, followed by the lymph nodes, skin, soft tissues, bones, and less frequently in the central nervous system, ears, and breast⁷.

Mycobacterial identification has traditionally been based on phenotypic characteristics such as growth rate, colony morphology and pigmentation, optimal growth temperature, and reactions as part of a battery of biochemical tests. Many of these tests require growth in solid media that are poorly reproducible and may take several weeks of incubation before species-level identification can be performed. An acid-fast bacilli (AFB) smear should be performed on all samples submitted for mycobacterial testing, except for blood samples, on which an AFB smear cannot be performed. Moreover, a Ziehl-Neelsen or Kinyoun stain is adequate for AFB smears confirming the presence of AFB in a culture. In most cases, cultures using a commercial broth system can return negative at 6 weeks⁸. Because of the above, there is a need for more effective diagnostic methods. One option is the application of the quantitative polymerase chain reaction (qPCR). The qPCR test is considered a high-cost study and, although it is usually performed in other conditions for the detection of *M. tuberculosis*, this is not a daily practice. In Mexico, conventional methods with the lower sensitivity and specificity are still used to detect both pulmonary and extra-pulmonary *Mycobacterium* spp, prolonging diagnosis time and increasing costs. Considering the continued diagnostic difficulties and treatment delays, the current study aims to demonstrate the utility of qPCR for expediting the diagnosis and treatment of granulomatous mastitis and lymphadenitis.

Materials and methods

The present study discusses the cases of five patients diagnosed with idiopathic mastitis and/or granulomatous adenitis who showed no response to treatment based mainly on high doses of steroids. After a multidisciplinary evaluation of the cases, qPCR tests for typical or atypical mycobacteria were performed based on a biopsy or paraffin-embedded tissue section, depending on the patient. qPCR tests were requested despite bacteriological cultures for Tb and Ziehl-Neelsen staining being negative in some cases.

Figure 1. **A:** low lymph node section with a fragment of fatty tissue (3.2 × 3.0 cm) and multiple light yellow semi-firm nodules measuring 1.0 to 2.5 cm. **B:** tissue sections showed lymph nodes with an architecture altered by the presence of chronic inflammatory infiltration of lymphocytes, plasma cells, macrophages, and a few multinucleated Langhans-type giant cells, all surrounding areas of caseous necrosis. Ziehl-Neelsen staining did not show bacilli, while findings of malignancy were not observed.

Case 1

A 31-year-old male patient with no prior history of Tb or other important background sought treatment for asthenia, adynamia, night fever for 2 weeks, and weight loss of 4 kg in 3 weeks. He also reported intense pain in his right armpit. A physical examination revealed palpable, slightly painful, 3-cm adenopathies at the axillary level. An ultrasound of the armpit revealed a 4-cm mass of purulent content, while a subsequent puncture culture did not reveal bacterial growth with a gram stain. Contrast-enhanced computed tomography of the chest and neck showed lymphadenopathies with an inflammatory appearance at cervical levels IA, IB, IIA, IIB, II, IV, VA, and BV.

Moreover, images showed smooth, well-defined edges, and a density similar to a muscle, with the largest lymphadenopathy (7.5 cm) located on the left IIA. A Quantiferon-TB Gold test returned positive for what was suspected to be nodal TB. Low dissection of the conglomerate lymph node on the right armpit was then performed, after which a diagnosis of granulomatous lymphadenitis negative for malignancy and Ziehl-Neelsen staining was established (Fig. 1). qPCR for typical and atypical mycobacteria revealed positive genotyping for *Mycobacterium kansasii*. After completing a treatment course comprising rifampicin, isoniazid, pyrazinamide, and ethambutol, the patient showed adequate resolution and remission of symptoms.

Case 2

A 69-year-old female patient with a history of Tb 32 years prior and infiltrating ductal adenocarcinoma of

the right breast, for which she underwent a radical mastectomy, adjuvant chemotherapy, and hormone therapy, sought treatment for a palpable lymphadenopathy in the right armpit that was present for 4 weeks, which was associated with a probable recurrence of breast cancer. Physical examination revealed a 4.0 × 3.5 cm soft, non-tender mass at the right axillary tail of the inner arm. Ultrasound revealed post-surgical-type changes consistent with BI-RADS 5. An ultrasound-guided Tru-cut biopsy revealed chronic granulomatous lymphadenitis with caseous necrosis and a Ziehl-Neelsen stain negative for bacilli. qPCR was performed for *M. tuberculosis* which was negative. However, due to the progression of the disease, another qPCR was requested a month later for atypical mycobacterium, which was positive for *Mycobacterium abscessus*. After completing a 6-month treatment course comprising ertapenem, levofloxacin, and clarithromycin, the patient exhibited complete response throughout follow-up.

Case 3

A 28-year-old female patient with no history of Tb or other important background sought treatment for pain in her left breast with a palpable mass present for 8 months beginning in April 2019. AFB revealed ductal ectasia, while an ultrasound of the left breast showed mastitis and abscess (BI-RADS 3). Abscess drainage was performed; however, recurrence was noted and managed with debridement and surgical cleaning on multiple occasions. Tissue biopsy confirmed granulomatous mastitis with negative Ziehl-Neelsen staining for bacilli. For this reason, the disease was established as idiopathic, and treatment with high doses of prednisone was initiated without success. qPCR for typical and atypical mycobacteria revealed positive genotyping for *M. tuberculosis*. The patient subsequently started on rifampicin, pyrazinamide, ethambutol, and isoniazid for 9 weeks, followed by supportive treatment for 4 months, with adequate results and resolution of injuries and symptoms.

Case 4

A 35-year-old female patient with no history of Tb sought treatment for asthenia, adynamia, diaphoresis (thrice), and lymphadenopathy on the left side of the neck that was present for 1 month. Excisional biopsy of the neck lymphadenopathy revealed mixed cellular-type Hodgkin lymphoma (Fig. 2). The patient also

Figure 2. A: ganglion 1. Irregular, yellowish-brown tissue with a dimension of 1.8 × 1.5 × 1.4 cm from which two white and semi-firm nodules, measuring 1.0 and 1.5 cm at their greatest diameter, were dissected.

B: ganglion 2. White nodule previously sectioned during trans operative study, with a 2.5 × 1.6 cm dimension and a smooth capsular surface. When cut, the nodule was light brown with a semi-firm consistency. Tissue sections of the lymph node showed a proliferation of large cells with clear cytoplasm, large nuclei, vesicular with prominent nucleoli, and some binucleated (Reed-Stenberg cells) and mononucleated (Hodgkin's cells) cells with a scattered distribution and surrounded by an inflammatory infiltrate comprising lymphocytes, plasma cells, eosinophils, and histiocytes.

underwent computed tomography, which suggested probable pneumonia. qPCR for typical and atypical mycobacteria revealed positive genotyping for *M. tuberculosis*. After completing treatment, the patient showed adequate results and resolution of lesions and symptoms.

Case 5

A 47-year-old female patient with no history of Tb sought treatment for asthenia, adynamia, and nodulation in her right breast that was present for 6 months and nocturnal hyperthermia without diaphoresis. A nodular resection was performed; however, recurrence was noted. Physical examination of her right breast revealed a yellowish milky exudate and blood at the lesion site and two ulceration and induration sites with hyperthermia and pain on palpation. A bilateral mammary ultrasound revealed a heterogeneous fibroglandular-like echogenicity in the right breast and increased vascularity on Doppler ultrasound associated with ectatic peripheral ducts. Moreover, bilateral contrasted-enhanced magnetic resonance imaging showed data suggestive of breast abscesses with a fistula at the

Figure 3. **A:** fresh trans operative histopathological study; the product of right mammary gland lesion resection (3.4 × 2.5 × 2.3 cm). **B-C:** histological sections stained with hematoxylin and eosin showed a mammary tissue with altered architecture due to various cysts and ducts with eosinophilic granular luminal secretion, few macrophages, double-row coating of polygonal cells, uniform without atypia with some luminal projections, and intact basement membrane, as well as dense fibroconnective stroma with periductal lymph histiocytic infiltrate, various mature acini, and congestive adipose tissue. Altogether, no data suggesting malignancy were noted.

ipsilateral areolar border (BI-RADS 4a). Accordingly, a diagnosis of granulomatous mastitis was established. qPCR for typical and atypical mycobacteria revealed positive genotyping for *M. tuberculosis* and *Mycobacterium kansasii*. The patient started and finished treatment with adequate results and resolution of injuries and symptoms (Fig. 3).

Table 1 shows additional information regarding the characteristics of the samples (biopsies) from which the qPCR test was performed and their results.

Discussion

Given the lack of defined clinical features, breast Tb has generally been misdiagnosed as another breast disease⁹. On clinical presentation, up to a third of all patients with granulomatous mastitis show abscess-like symptoms, such as pain, erythema, massive fluid accumulation on ultrasound, and reactive lymphadenopathy. Such patients may undergo puncture, drainage, or incision of the abscess depending on the size of the lesion. Aspiration can fail given that the abscess-like mass often has necrotic tissue at the center that makes the aspirate thick and difficult to remove. Typically, microbiological cultures return negative².

From the clinical cases currently presented, in cases 1 and 5, qPCR was requested for typical and atypical mycobacteria, obtaining positivity for an atypical mycobacterium in both cases; however, case 5 was also positive for *M. tuberculosis* (typical mycobacterium). In contrast, in case 2, in which qPCR was requested for

typical mycobacteria, the result became negative. A month later, qPCR was requested again for atypical mycobacteria, resulting in a positive for atypical mycobacteria (*M. abscessus*). Therefore, we believe that with nonspecific clinical data, suggestive of mastitis and granulomatous lymphadenitis, qPCR should be requested for typical and atypical mycobacteria.

Rapid diagnosis of Tb has been widely regarded as essential for treatment and transmission control. Although extra-pulmonary forms of Tb have low communicability, they tend to be more aggressive or generate sequelae that can significantly limit the patient's quality of life¹⁰. Early control of mycobacterial infections relies on rapid identification of complex nontuberculous and tuberculous mycobacterial infections from a clinical sample, which can reduce both morbidity and mortality by allowing the implementation of the most appropriate pharmacological treatment¹¹.

Although rare, mastitis and granulomatous lymphadenitis are often diagnosed and treated as idiopathic diseases, given the negative results for Ziehl-Neelsen staining or bacteriological cultures. They usually present a torpid evolution, disease progression, and important cosmetic sequelae. Although a reliable and timely diagnosis is vital for disease detection, smear microscopy (developed more than 100 years ago), which detects disease through AFB observation, has remained the most commonly used diagnostic method. While smear microscopy does allow for a relatively quick diagnosis, it requires between 5000 and 10,000 bacilli per mL of sample. Moreover, given that smear microscopy

Table 1. Sample's characteristics and mycobacteria detected

Case	Sample type	Method	Genotyping of typical and atypical mycobacteria	Mycobacteria detected
Case 1	Fresh from Tissue. Low lymph node dissection	qPCR	<i>Mycobacterium szulgai</i> , <i>Mycobacterium abscessus</i> , <i>Mycobacterium kansasii</i> , <i>Mycobacterium scrofulaceum</i> , <i>Mycobacterium simiae</i> , <i>Mycobacterium peregrinum</i> , <i>Mycobacterium lentiflavium</i> , <i>Mycobacterium intracellulare</i> , <i>Mycobacterium parascrofulaceum</i> , <i>Mycobacterium malmoense</i> , <i>Mycobacterium fortuitum</i> , <i>Mycobacterium phlei</i> , <i>Mycobacterium smegmatis</i> , <i>Mycobacterium genavense</i> , <i>Mycobacterium avium</i> , <i>Mycobacterium bovis</i> , <i>Mycobacterium heckeshornense</i> , <i>Mycobacterium marinum</i> , <i>Mycobacterium xenopi</i> , <i>Mycobacterium bohemium</i> , <i>Mycobacterium haemophilum</i> , <i>Mycobacterium celatum</i> , <i>Mycobacterium gordonae</i> , <i>Mycobacterium chelonae</i>	<i>Mycobacterium kansasii</i>
Case 2	Paraffin-embedded tissue. Axillary node biopsy	qPCR	<i>Mycobacterium szulgai</i> , <i>Mycobacterium abscessus</i> , <i>Mycobacterium kansasii</i> , <i>Mycobacterium scrofulaceum</i> , <i>Mycobacterium simiae</i> , <i>Mycobacterium peregrinum</i> , <i>Mycobacterium lentiflavium</i> , <i>Mycobacterium intracellulare</i> , <i>Mycobacterium parascrofulaceum</i> , <i>Mycobacterium malmoense</i> , <i>Mycobacterium fortuitum</i> , <i>Mycobacterium phlei</i> , <i>Mycobacterium smegmatis</i> , <i>Mycobacterium genavense</i> , <i>Mycobacterium avium</i> , <i>Mycobacterium bovis</i> , <i>Mycobacterium heckeshornense</i> , <i>Mycobacterium marinum</i> , <i>Mycobacterium xenopi</i> , <i>Mycobacterium bohemium</i> , <i>Mycobacterium haemophilum</i> , <i>Mycobacterium celatum</i> , <i>Mycobacterium gordonae</i> , <i>Mycobacterium chelonae</i>	<i>Mycobacterium abscessus</i>
Case 3	Biopsy tissue	qPCR	<i>Mycobacterium avium</i> , <i>Mycobacterium intracellulare</i> , <i>Mycobacterium kansasii</i> , <i>Mycobacterium malmoense</i> , <i>Mycobacterium xenopi</i> , <i>Mycobacterium szulgai</i> , <i>Mycobacterium mucogenicum</i> , <i>Mycobacterium scrofulaceum</i> , <i>Mycobacterium haemophilum</i> , <i>Mycobacterium smegmatis</i> , <i>Mycobacterium simiae</i> , <i>Mycobacterium celatum</i> , <i>Mycobacterium bovis</i> , <i>Mycobacterium africanum</i>	<i>Mycobacterium tuberculosis</i>
Case 4	Neck lymph node	qPCR	<i>Mycobacterium tuberculosis</i>	<i>Mycobacterium tuberculosis</i>
Case 5	Paraffin-embedded tissue	qPCR and hybridization to microarrays	<i>Mycobacterium szulgai</i> , <i>Mycobacterium abscessus</i> , <i>Mycobacterium kansasii</i> , <i>Mycobacterium scrofulaceum</i> , <i>Mycobacterium simiae</i> , <i>Mycobacterium peregrinum</i> , <i>Mycobacterium lentiflavium</i> , <i>Mycobacterium intracellulare</i> , <i>Mycobacterium parascrofulaceum</i> , <i>Mycobacterium malmoense</i> , <i>Mycobacterium fortuitum</i> , <i>Mycobacterium phlei</i> , <i>Mycobacterium smegmatis</i> , <i>Mycobacterium genavense</i> , <i>Mycobacterium avium</i> , <i>Mycobacterium bovis</i> , <i>Mycobacterium heckeshornense</i> , <i>Mycobacterium marinum</i> , <i>Mycobacterium xenopi</i> , <i>Mycobacterium bohemium</i> , <i>Mycobacterium haemophilum</i> , <i>Mycobacterium celatum</i> , <i>Mycobacterium gordonae</i> , <i>Mycobacterium chelonae</i>	<i>Mycobacterium tuberculosis</i> and <i>Mycobacterium kansasii</i>

qPCR: quantitative polymerase chain reaction.

has a sensitivity between 5 and 20% for extra-pulmonary samples, the possibility of obtaining a negative smear when no pulmonary symptoms are present should be considered¹².

At present, nucleic acid-based technologies have been used to detect tuberculous mycobacteria with very high sensitivity and specificity, overcoming some of the problems associated with classical standard laboratory methods. Although rapid and cost-effective, microscopic examination of AFB has low sensitivity and specificity, particularly in paucibacillary specimens and cultures. Despite being considered the gold standard

due to its high sensitivity, the microscopic examination requires several weeks to produce a result. It has a lower sensitivity for the detection of extra-pulmonary Tb⁵. Notably, the use of qPCR for the identification of *M. tuberculosis* has shown excellent results, with a sensitivity varying from 65 to 100% and a specificity of 98%, as well as a decrease in the number of days before treatment initiation (approximately 3-5 days)¹³.

Current traditional methods lack sensitivity and specificity, and a rapid diagnosis of Tb is essential to initiate adequate treatment. However, in many cases, the performance of qPCR is postponed or not considered due

to the costs that it may present for patients. Still, with the cases presented, we can realize the usefulness of performing qPCR for both typical and atypical mycobacteria to get an accurate diagnosis and start treatment early.

Conclusions

The present study shows that the routine performance of qPCR for typical and atypical mycobacteria allows for improved diagnosis, increased diagnosis of infectious cases according to the different types of mycobacteria, increased treatment specificity, and adequate progression and treatment of patients.

Conflicts of interest

The authors declare no conflicts of interest.

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Ethical disclosures

Protection of human and animal subjects. The authors declare that the procedures followed were in accordance with the regulations of the relevant clinical research ethics committee and with those of the Code of Ethics of the World Medical Association (Declaration of Helsinki).

Confidentiality of data. The authors declare that they have followed the protocols of their work center on the publication of patient data.

Right to privacy and informed consent. The authors have obtained the written informed consent of the patients or subjects mentioned in the article. The corresponding author is in possession of this document.

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