



DOI: 10.32768/abc.202293SI249-253



The Emerging Role of *Corynebacteria* in the Pathogenesis of Granulomatous Lobular Mastitis

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Granulomatous Lobular Mastitis (GLM) is a non-neoplastic, inflammatory disease of the breast that most commonly affects women of childbearing age.¹ The most common clinical manifestation of GLM is a unilateral and painful breast lump that raises concern for a malignant breast neoplasm and causes significant distress to the patient. The distress is further augmented by the chronic and relapsing nature of this disease which often results in the formation of sinuses, fistulae, and fluid collections within the breast as well as ulceration of the overlying skin causing serious breast disfigurement.² Despite being a benign condition, GLM is a locally aggressive disease that causes long-term pain and discomfort; hence, many patients require incision and drainage of fluid collections, debridement of inflamed tissue, and even mastectomy to alleviate the symptoms. Even though it is a well-recognized clinical entity, there is no consensus in the field regarding optimal patient management. This obscurity concerning optimal treatment guidelines largely stems from the uncertainty in the etiology of GLM. Various mechanisms, such as autoimmunity, hypersensitivity, lactation, trauma, chemical irritation, and hormonal imbalance have been proposed,³⁻⁷ but the etiology is unclear which explains why the disease is commonly referred to as “idiopathic” granulomatous mastitis.

In recent years, there has been increasing evidence pointing towards an infectious etiology, particularly the role of *Corynebacteria*.⁸⁻¹¹ This has resulted largely from increased awareness and better understanding of

the histopathologic features of GLM among breast pathologists. In histologic sections, GLM shows characteristic noncaseating granulomas centered in and around the lobules often accompanied by suppurative inflammation and microabscess formation,⁸ (Figure 1A&B). Additionally, a subset of cases show distinctive cystic spaces lined by neutrophils and surrounded by an outer cuff of epithelioid histiocytes (Figure 1C), a highly characteristic histologic picture commonly referred to as cystic neutrophilic granulomatous mastitis (CNGM).⁹ It was within these cystic spaces that the presence of Gram-positive *Corynebacteria* was first described by Taylor *et al.*,⁸ in 2003 (Figure 1D), paving the way for further exploration. Initially, CNGM was thought to represent a distinct subtype of GLM; however, recent evidence suggests that GLM and CNGM are different stages of the same disease process. In the early stages, the granulomatous inflammation is lobulocentric with mild suppurative inflammation. This results in an overall preserved lobular architecture in histologic sections that is more defining of classic GLM. In more advanced stages, the suppurative inflammation predominates and effaces the underlying lobular architecture resulting in microabscess and cystic space formation, giving the distinctive appearance of CNGM. Ever since the initial description of Gram-positive *Corynebacteria* within these cystic spaces by Paviour *et al.*, several studies describing an association between *Corynebacteria* species and GLM have been reported. *Corynebacteria*, also known as ‘Diphtheroids’ are gram-positive, catalase-positive, aerobic bacilli that have a characteristic club-shaped appearance on Gram stain. These bacteria are a part of endogenous skin flora and, hence, are frequently regarded as contaminants in tissue biopsies. However, in recent years they have been increasingly implicated in human diseases, particularly GLM.¹¹

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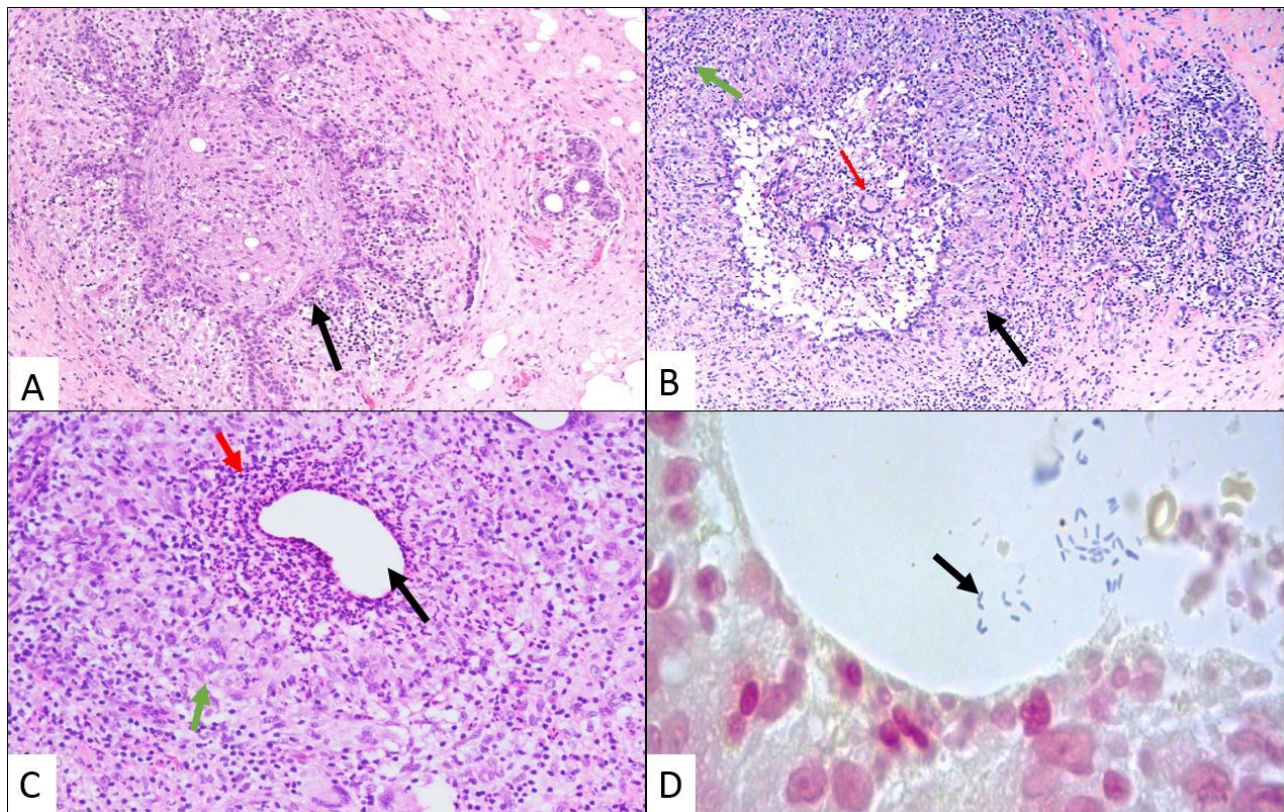


Figure 1. Histologic features of Granulomatous Lobular Mastitis (GLM)

- Early-stage GLM shows non necrotizing granulomatous inflammation involving the terminal duct lobular unit of the breast (black arrow). The lobular architecture is preserved (hematoxylin and eosin stain, x100)
- In the intermediate stage, the granulomatous inflammation is more pronounced resulting in the disruption of the terminal duct lobular unit (black arrow). The accompanying acute and chronic inflammatory cells are increased (green arrow) and a giant cell reaction can be seen (red arrow) (hematoxylin and eosin stain, x100)
- In the advanced stage, the lobular architecture is totally effaced and replaced by cystic spaces (black arrow) lined by neutrophils (red arrow) and an outer rim of granulomatous inflammation (green arrow) (hematoxylin and eosin stain, x200)
- Gram-positive *Corynebacteria* (black arrow) are typically seen inside the neutrophil rimmed cystic spaces (Gram stain, x1000)

Corynebacteria were first detected in microbial cultures from fresh breast aspirates from GLM patients in 2002.¹⁰ In 2003 Taylor *et al.*,⁸ described their presence in histologic sections and performed 16S rRNA gene sequencing on fresh breast aspirates for species identification. *Corynebacterium kroppenstedtii* was the most commonly isolated species in their study. In 2011, Renshaw *et al.*,⁹ coined the term “cystic neutrophilic granulomatous mastitis” (CNGM) to draw attention to this distinct histologic appearance and the presence of *Corynebacteria* within neutrophil rimmed cystic spaces. In 2016, Yu *et al.*¹² studied the microbiota of GLM using 16S rDNA sequencing on fresh breast aspirates. They found *Corynebacteria* in all 19 of their patients, with *Corynebacterium kroppenstedtii* being the most common species. This was shortly followed by a similar study by Johnstone *et al.*,¹³ which also demonstrated *Corynebacterium kroppenstedtii* to be the most prevalent organism in fresh breast aspirates from GLM patients. These studies demonstrating

Corynebacteria in fresh breast aspirates spiked an interest in assessing their prevalence in Formalin-Fixed, Paraffin-Embedded (FFPE) tissues of breast biopsies from GLM patients that are more readily available and do not require fresh tissue. In a study by Fujii *et al.*,¹⁴ in 2018, the presence of *Corynebacterium kroppenstedtii* genome in DNA extracted from FFPE sections of GLM cases was demonstrated for the first time using a *Corynebacterium kroppenstedtii* specific primer set; however, their cohort was small (18 cases) and did not include a control group of non-granulomatous breast abscesses.

We recently reported our study assessing the prevalence of *Corynebacterium kroppenstedtii* in FFPE tissues by real-time PCR in a large cohort of GLM cases, the largest study of this type to date.¹⁵ In this study, we included FFPE tissues from 67 cases fulfilling the histologic criteria of GLM as well as 10 cases of non-granulomatous breast abscess as controls. DNA was extracted from FFPE tissues of all



67 GLM cases as well as the 10 control cases and amplified via SYBR real-time PCR with primers specifically targeting the *Corynebacterium kroppenstedtii* 16S rRNA gene region. Samples that were positive for *Corynebacterium kroppenstedtii* genome were bidirectional Sanger sequenced, analyzed, and queried in the GenBank database using BLASTn. Our results showed that 46/67 (68.7%) GLM cases were positive for *Corynebacterium kroppenstedtii* by 16S rRNA SYBR real-time PCR. The remaining 21 GLM cases were either negative for *Corynebacterium kroppenstedtii* real-time PCR, or weak positive but the sequence failed to confirm the strain. In contrast, all 10 control cases of non-granulomatous breast abscess were negative for *Corynebacterium kroppenstedtii* by 16S rRNA SYBR real-time PCR. Taking into consideration the limitations of performing PCR on FFPE tissues and the scarcity of the organisms, the prevalence of *Corynebacterium kroppenstedtii* in GLM FFPE tissues is likely to be higher than 68.7%. Nonetheless, our findings reinforced the observation that *Corynebacterium kroppenstedtii* is highly prevalent in GLM. In addition, all of our non-granulomatous breast abscess control cases were negative for *Corynebacterium kroppenstedtii* genome suggesting that this bacterium is specific to GLM.

Even though we established a strong association between GLM and *Corynebacterium kroppenstedtii* in our study, the use of species-specific primer set precluded the detection of other bacteria. This raises the question of whether *Corynebacterium kroppenstedtii* is the only pathogen in GLM or is it a polymicrobial process. In a more recent study, Jiaxin Bi *et al.*,¹⁶ studied the infectious landscape of GLM using metagenomic next-generation sequencing (mNGS) - a new and powerful platform capable of identifying nucleic acids from multiple different species, unlike targeted PCRs that are species-specific. Their study detected pathogens in 17 of 25 GLM cases out of which 14 (82.3%) were *Corynebacterium kroppenstedtii*. Other organisms such as *Pseudomonas oleovorans*, Human gammaherpesvirus 4, *Acinetobacter baumannii*, and *Tepidiphilus thermophilus* were detected in a much smaller proportion of cases often in combination with *Corynebacterium kroppenstedtii*. Another recent study by Li *et al.*,¹⁷ also showed high prevalence of *Corynebacterium kroppenstedtii* in GLM specimens by nanopore sequencing. These studies unequivocally establish that *Corynebacterium kroppenstedtii* is highly prevalent in GLM but the question arises whether this bacterium is truly pathogenic in GLM or it represents normal skin flora secondarily colonizing inflamed breast tissue. We argue that the identification of the *Corynebacterium kroppenstedtii*

early in the clinical course of GLM, its presence in the deep breast tissue, the evocation of surrounding granulomatous and suppurative inflammatory reaction, a high prevalence in GLM tissues, and its absence in non-granulomatous inflammatory breast diseases are all strong arguments in favor of its true pathogenic role in GLM.

Historically, the idea of a microbial etiology for GLM has met with skepticism largely due to the frequently negative microbial cultures, non-responsiveness to antibiotics, and symptomatic improvement with corticosteroids. Firstly, *Corynebacterium kroppenstedtii* is a lipophilic and fastidious organism that is difficult to culture and requires specialized media as well as prolonged incubation. Secondly, “Diphtheroids” in smears and colonies are frequently regarded as commensals or contaminants in the laboratory and not pursued further resulting in underreporting. Thirdly, the amount of bacteria is scant as evidenced by the histologic features which results in under-sampling. Lastly, *Corynebacteria* are often multidrug-resistant and poorly susceptible to beta-lactam antibiotics that are traditionally prescribed for breast infections. The symptomatic relief seen with corticosteroids is attributable to the reduction in the inflammatory response associated with the infection and explains why steroids alone have a very low efficacy in attaining complete remission in these patients. In recent years, several studies describing successful treatment of GLM with lipophilic antibiotics with a high volume of distribution such as rifampicin, doxycycline, trimethoprim-sulfamethoxazole, and clarithromycin have been described.^{16,18-22} Additionally, being a lipophilic bacterium, *Corynebacterium kroppenstedtii* thrives in lipid-rich states such as hyperprolactinemia and lactation. Supplementing lipophilic antibiotics with prolactin inhibitors and cessation of lactation may result in a more effective response.¹⁶

In conclusion, there is substantial scientific evidence pointing towards the pathogenicity of *Corynebacterium kroppenstedtii* in GLM. We recommend obtaining fresh breast aspirates and/or tissues for microbial studies in all patients with suspected GLM. Microbial specimens positive for “Diphtheroids” in smears and colonies must not be dismissed as contaminants and species-level identification should be pursued in all cases. Even though *Corynebacteria* are difficult to culture, modern methods such as matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS), 16S rRNA, and *rpoB* gene sequence amplification with PCR can accurately identify *Corynebacterium* species in the vast majority of cases.²³ Collecting fresh breast



specimens will not only improve the detection rate of *Corynebacterium kroppenstedtii* in GLM patients, but also allow antimicrobial susceptibility testing. This

will allow more targeted antibiotic therapy that will hopefully result in more effective management of this chronic, distressing, and disfiguring disease.

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How to Cite This Article

Tariq H, Nazarullah AN. The Emerging Role of Corynebacteria in the Pathogenesis of Granulomatous Lobular Mastitis. Arch Breast Cancer. 2022; 9(3): 249-53.

Available from: <https://www.archbreastcancer.com/index.php/abc/article/view/579>