Detection of *Corynebacterium kroppenstedtii* in Granulomatous Lobular Mastitis Using Real-Time Polymerase Chain Reaction and Sanger Sequencing on Formalin-Fixed, Paraffin-Embedded Tissues

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• ***Context.***—**Associations between granulomatous lobular mastitis (GLM) and *Corynebacterium kroppenstedtii* have been reported since 2002, but large-scale studies to assess the actual prevalence of this bacterium in GLM have not been performed.**

Objective.—**To assess the prevalence of *C kroppenstedtii* in GLM using real-time polymerase chain reaction and Sanger sequencing.**

Design.—**We analyzed formalin-fixed, paraffin-embedded tissues from 67 cases of GLM by sequential DNA amplification and sequencing to assess the rate of *C kroppenstedtii* detection in GLM. A retrospective analysis including patient demographics, history of pregnancy and lactation, clinical signs and symptoms, radiographic findings, histologic pattern, Gram stain results, and microbial cultures was performed on 67 cases of GLM. In addition, 10 cases of nongranulomatous breast abscess were included as controls.**

Results.—***C kroppenstedtii* 16S rRNA SYBR real-time polymerase chain reaction was positive on formalin-fixed, paraffin-embedded tissues from 46 of 67 (68.7%) GLM cases, while all control cases were negative. Among the positive cases, the majority showed features of cystic neutrophilic granulomatous mastitis.**

Conclusions.—***C kroppenstedtii* was highly prevalent in GLM cases and was not found to be associated with nongranulomatous breast abscess in our study ($P < .001$).**

(*Arch Pathol Lab Med.* 2022;146:749–754; doi: 10.5858/arpa.2021-0061-OA)

Granulomatous lobular mastitis (GLM) is an uncommon benign, inflammatory disease of the breast first described in 1972 by Kessler and Wolloch[1] that characteristically affects women of childbearing age with a recent history of pregnancy and lactation.[2] The most common presenting symptom is a unilateral, firm, and painful breast mass, which may be accompanied by overlying skin changes causing significant concern for malignancy.[3,4] On histopathologic examination GLM is characterized by nonnecrotizing granulomas in and around lobules, often with suppuration and sometimes with microabscess formation.[5] A subset of GLM cases, distinctive for cystic spaces lined by neutrophils in the background of suppurative granulomatous inflammation, was described by Renshaw et al[6] as "cystic neutrophilic granulomatous mastitis (CNGM)." In rare instances, Gram-positive bacilli can be seen within these cystic spaces.[7]

The etiology of GLM is not well established, and the disease is often regarded as idiopathic; however, various mechanisms, including infection, autoimmunity, and hypersensitivity reactions, have been proposed.[8] Interest in a possible infectious etiology has spiked in recent years after a study conducted by Taylor et al[5] in 2003 showed a strong association between *Corynebacteria* and GLM in a review of 34 patients in New Zealand. Since then, several case reports and case series describing an association between *Corynebacteria* species and GLM have been described.[8–16] Among *Corynebacteria*, *Corynebacterium kroppenstedtii* is the most frequently isolated pathogen associated with GLM.[8,17–21] The incidence of *C kroppenstedtii* is reported to be higher in GLM cases with CNGM pattern.[19]

To assess the strength of this association, we performed a retrospective analysis on 67 cases of GLM at our institution over 10 years (2009–2019). In addition to gathering clinical and laboratory information, we performed sequential DNA amplification and sequencing on formalin-fixed, paraffin-embedded (FFPE) tissues from all 67 cases using real-time polymerase chain reaction (PCR) and Sanger sequencing. To

Accepted for publication May 10, 2021.
Published online September 10, 2021.

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The authors have no relevant financial interest in the products or companies described in this article.

The abstract was presented in the poster session at the United States and Canadian Academy of Pathology (USCAP) Annual Meeting; March 2, 2020; Los Angeles, California.

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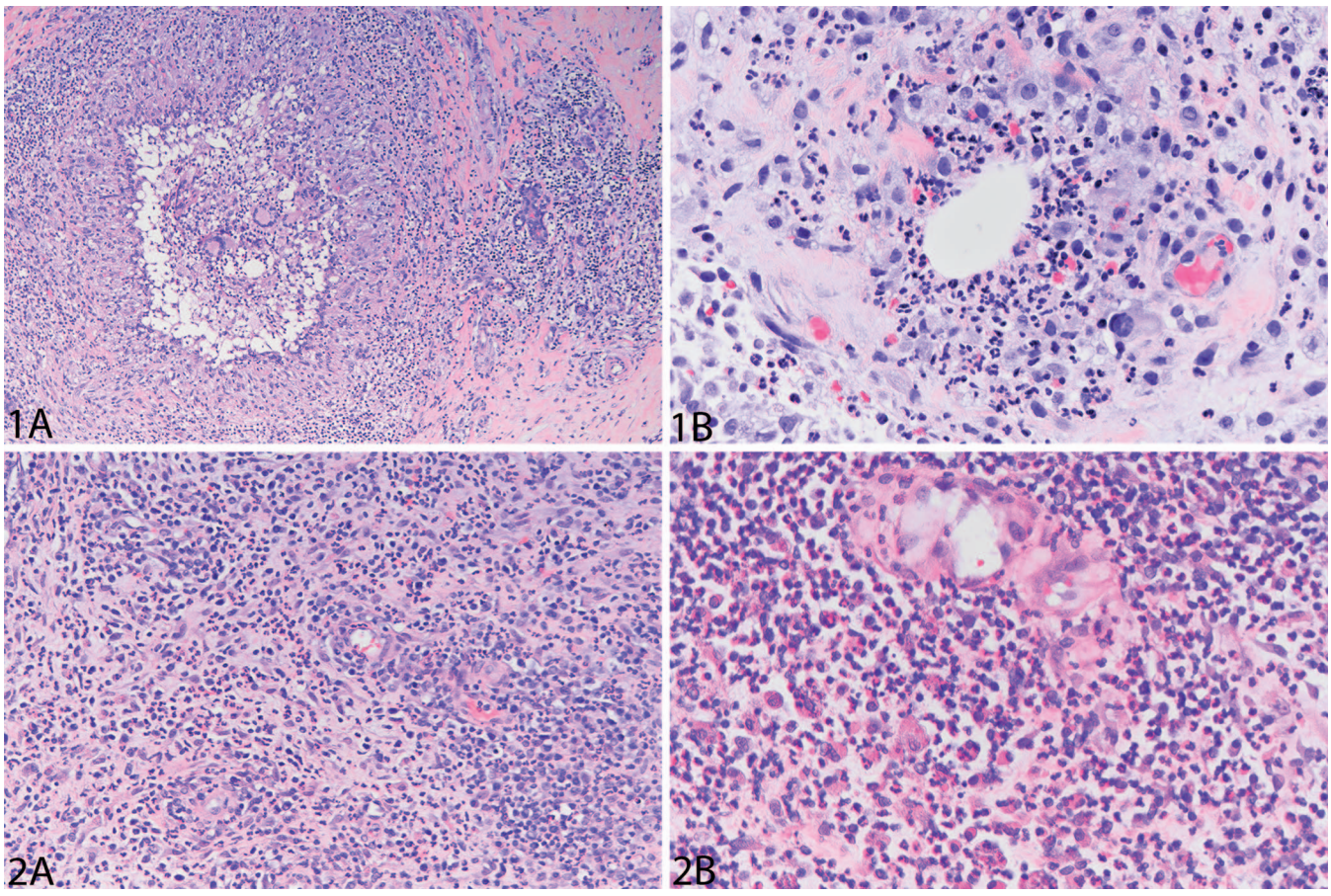

Figure 1. *A, Granulomatous lobular mastitis (GLM) with lobulocentric granulomatous inflammation. B, GLM with cystic neutrophilic granulomatous mastitis pattern characterized by cystic spaces surrounded by neutrophils (hematoxylin-eosin, original magnifications ×10 [A] and ×40 [B]).*

Figure 2. *A, Nongranulomatous breast abscess (control group). B, Nongranulomatous breast abscess (control group) showing suppurative acute inflammation (hematoxylin-eosin, original magnifications ×20 [A] and ×40 [B]).*

our knowledge, this is the largest study to date using molecular techniques to detect *C kroppenstedtii* in GLM cases.

MATERIALS AND METHODS

We retrospectively identified cases of GLM during a 10-year period, using keyword searches within the anatomic pathology information system, in addition to 10 cases of nongranulomatous breast abscess included as controls. Patient demographics including age, ethnicity, body mass index, gravidity and parity, history of lactation and hyperprolactinemia, clinical presentation, radiographic findings, histologic features, Gram stain, and microbial culture results were reviewed. Hematoxylin and eosin–stained slides from all cases were reviewed by a breast pathologist to assess for features of GLM. Only cases with defining histologic features of GLM (ie, nonnecrotizing granulomatous inflammation in and around the lobules) were included in this study (Figure 1, A). All other causes of granulomatous inflammation (infectious and autoimmune) were ruled out clinically and histologically in each case. The GLM cases were further assessed for histologic features of CNGM, defined as clear spaces (microcysts) surrounded by a rim of neutrophils and further surrounded by histiocyte-rich granulomatous inflammation (Figure 1, B), and based on this assessment were subdivided into CNGM and non-CNGM categories. Similarly, 10 nongranulomatous breast abscess cases were reviewed by a breast pathologist to ensure that features of GLM were absent (Figure 2, A and B). These cases were included in the control group. Gram, acid-fast bacillus, and Gömöri methenamine silver histochemical stains were performed on all 67 GLM cases as well as the 10 control cases.

Molecular Methodology

FFPE tissue blocks from all 67 GLM and 10 control cases were retrieved. A representative block was selected from each case for molecular study (1 block per case). Five-µm to 10-µm-thick FFPE scrolls were collected from each FFPE block into an Eppendorf tube using PCR precaution protocol to avoid contamination. FFPE sections were deparaffinized by CitriSolv, and DNA was extracted from FFPE sections using the EZ1 DNA Tissue Kit (Qiagen) on the BioRobot EZ1 System (Qiagen). DNA concentration was measured by the NanoDrop spectrophotometer (Thermo Fisher Scientific).

DNA was amplified by SYBR real-time PCR (PowerUp SYBR Green Master Mix, Thermo Fisher Scientific) with primers specifically targeting the *C kroppenstedtii* 16S rRNA gene region (*C kroppenstedtii*–specific primer set)[22] on the ABI 7900HT real-time PCR System (Thermo Fisher Scientific). For each sample, PCR was performed in duplicate. *C kroppenstedtii*–negative DNA control and non-DNA template controls were included in each run to monitor PCR contamination issues. In addition, a human control gene cyclophilin (Cy FW and RV primer set)[23] was amplified along with the *C kroppenstedtii* PCR to monitor DNA quality and PCR efficiency.

C kroppenstedtii PCR–positive samples were bidirectional Sanger sequenced using BigDye Terminator Cycle Sequencing Kit (Thermo Fisher Scientific) on the ABI 3130xl Genetic Analyzer. Obtained

Table 1. Comparison of Demographic and Clinical Features in Patients With Granulomatous Lobular Mastitis (GLM) Versus Controls

	GLM (n = 67)	Non-GLM (n = 10)
Age, range (mean age in y)	17–61 (36.4)	17–58 (34.4)
Ethnicity	Hispanic: 54/67 (80.5%) White: 8/67 (11.9%) Other: 5/67 (7.4%)	Hispanic: 5/10 (50%) White: 5/10 (50%)
BMI, range (mean BMI in kg/m^2)	19.39–51.1 (28.6)	18.3–49.5 (31.2)
Patients with at least 1 prior pregnancy	63/67 (94.0%)	7/10 (70%)
Multigravida patients	50/63 (79.4%)	3/10 (30%)
Nulligravida patients	4/67 (5.9%)	3/10 (30%)
Mean gravidity	G 3.13	G 1.3
History of lactation in past 5 y	36/67 (53.7%)	1/10 (10%)
Patients with pituitary hyperprolactinemia[a]	4/67 (5.9%)	0.0%
History of diabetes	8/67 (11.9%)	6/10 (60%)
Most common presenting symptoms	Palpable painful mass, erythema of skin overlying breast, nipple inversion, and discharge	Acute onset breast pain, swelling, erythema, and fever
Duration of symptoms	2 wk to 1 yr	1–7 d

Abbreviation: BMI, body mass index.

[a] Four (5.9%) of 67 patients in the GLM group had a pituitary source of hyperprolactinemia and presented with galactorrhea. Out of 4, 3 had prolactin-secreting pituitary adenomas, while 1 had hyperprolactinemia due to partial empty sella syndrome.

sequences were analyzed using DNASTAR Lasergene 10 software (DNASTAR, Inc.). Resulting sequences were queried in the GenBank database using BLASTn (accessed on September 2019), and results with the highest alignment scores were analyzed. In addition, sequences from all *C kroppenstedtii*–positive cases were aligned against *C kroppenstedtii* partial 16S rRNA sequence (strain DSM 44385, GenBank# NR_074408) using the AliView program (version 1.26, Larsson, A., 2014).

RESULTS

Sixty-seven cases fulfilling histologic criteria for GLM were identified within the 10-year period. In addition, 10 non-GLM cases with histologic features of breast abscess were included in the control group. The clinical characteristics including patient age, ethnicity, body mass index, obstetric history, history of lactation and hyperprolactinemia, and history of diabetes, and the presenting symptoms in GLM and non-GLM patients are summarized in Table 1.

Of 67 GLM cases, 38 (56.7%) showed definitive histologic features of CNGM. Gram stain performed on the histologic sections showed rare Gram-positive bacilli in only 12 of 67 (17.9%) GLM cases. All 12 Gram-positive cases were in the CNGM category and showed rare bacteria exclusively inside the cystic spaces lined by neutrophils (Figure 3). All 10 cases in the control group were negative for bacteria on Gram stain and did not show features of CNGM. Acid-fast bacillus and Gömöri methenamine silver were negative for microorganisms in all 67 GLM cases as well as the 10 control cases. Breast aspirates for microbial cultures were obtained in only 38 of 67 (56.7%) of the GLM cases at the time of the biopsy procedure, of which 22 of 38 (57.8%) were negative for bacterial growth, 14 of 38 (36.8%) were positive for *Diphtheroids*, 1 grew coagulase-negative *Staphylococci*, and 1 was positive for *Streptococcus angionosus*. In the control group, breast aspirates for microbial cultures were obtained in 7 of 10 (70%) cases. Three of 7 were positive for *Staphylococcus aureus*, 2 of 7 were positive for *Actinomyces israelii*, and 1 each was positive for *Staphylococcus lugdunensis* and *Streptococcus angionosus*.

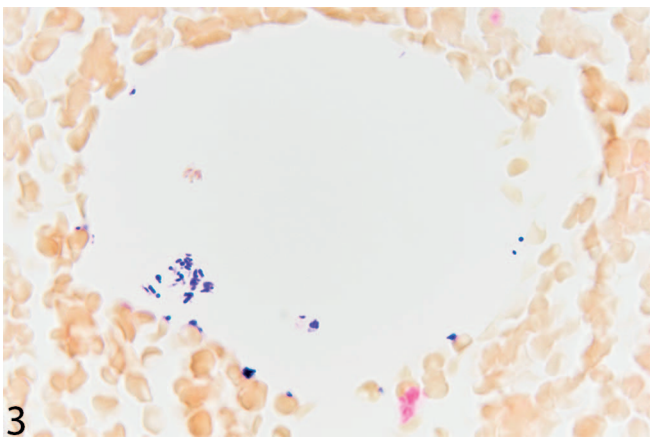

Figure 3. *Gram stain showing Gram-positive bacilli inside a cystic space lined by neutrophils (original magnification ×100).*

For molecular testing, human control gene cyclophilin (Cy) was used to determine the suitability of the FFPE samples for PCR-based assay, and all 77 samples showed Cy amplification by PCR. In the GLM category, 46 of 67 (68.7%) cases were positive for *C kroppenstedtii* by 16S rRNA SYBR real-time PCR. All PCR-positive cases were confirmed by Sanger sequencing. Clean sequences were obtained from all 46 cases with sequence lengths ranging from 104 bp to 136 bp. BLASTn search results indicated that sequences from these 46 cases were a 100% match with the *C kroppenstedtii* partial 16S rRNA sequence (strain DSM 44385, GenBank# NR 074408). In addition, the alignment study by the AliView program also confirmed that all 46 sequences were aligned to the *C kroppenstedtii* partial 16S rRNA sequence (see Figure 4). The remaining 21 of 67 (31.3%) of GLM cases in our study were either negative (n = 17) for *C kroppenstedtii* real-time PCR or weak positive but the sequence failed to confirm the *C kroppenstedtii* strain (n = 4). All 10 control cases were negative for *C kroppenstedtii* by PCR. Our findings suggest that *C kroppenstedtii* is specific to GLM and is not seen in association with nongranulomatous breast abscess ($P < .001$, Fisher exact test).

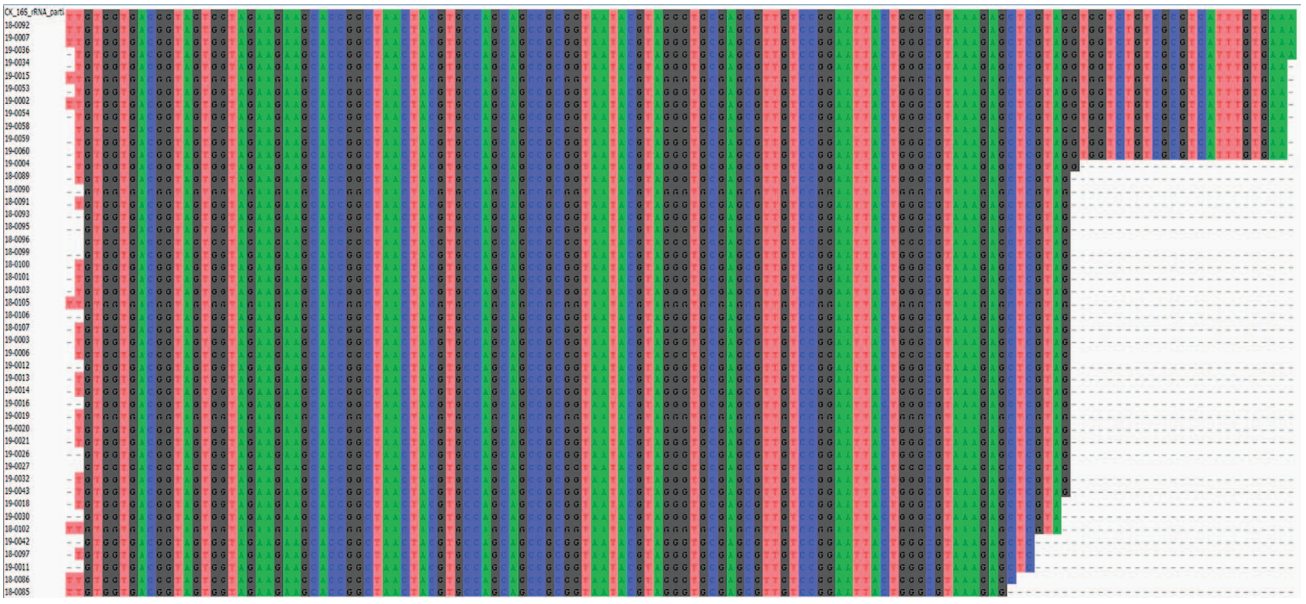

Figure 4. *Sequences obtained from 46* Corynebacterium kroppenstedtii *(Ck) positive cases were aligned to the Ck partial 16S rRNA sequence by the Aliview program. Perfect alignment was observed on all 46 sequences from granulomatous lobular mastitis cases. Case numbers were labeled on the left, and sequences were color coded for different nucleotides.*

Histologically, 28 of 46 (60.9%) of *C kroppenstedtii* PCR–positive cases were in the CNGM category. Gram stain was positive for Gram-positive bacilli in only 8 of 46 (17.4%) of the *C kroppenstedtii*–positive cases by SYBR real-time PCR. Of note, 4 cases negative for *C kroppenstedtii* by PCR were positive for Gram-positive bacilli by Gram stain on tissue sections. Microbial cultures were obtained in only 25 of 46 of *C kroppenstedtii*–positive cases. Of the 25, 10 (40%) were positive for *Diphtheroids* and 1 (4.0%) was positive for coagulase-negative *Staphylococci*, while 14 (56%) were negative for microbial growth. The clinical, histologic, Gram stain, and microbial culture findings in *C kroppenstedtii* PCR–positive versus negative cases are summarized in Table 2.

DISCUSSION

The etiology of GLM is not well established, and the disease is often regarded as idiopathic; however, recent research indicates that human breast tissue is not sterile but contains a diverse microbiome that includes *Corynebacteria* species.[24–26] Dysbiosis involving this microbiome may contribute to the development of several disease states.[25] The association between GLM and lipophilic *Corynebacteria* in cultures was communicated for the first time in 1996 by Binelli et al.[27] Subsequently, Taylor et al[5] demonstrated the presence of lipophilic bacteria with Coryneform morphology inside the neutrophil-lined cystic spaces in histologic sections of GLM. This prompted them to perform 16S rRNA gene sequencing on selected culture specimens that yielded *C kroppenstedtii* as the most common pathogen. This was followed by a study by Yu et al[20] in which the microbiota of GLM was studied using 16S rDNA metagenomic sequencing on fresh breast aspirates. They found *Corynebacteria* in all 19 of their patients with *C kroppenstedtii* being the most common species. A similar study using 16S rRNA gene sequencing on fresh breast samples from 15 CNGM patients by Johnstone at al[8] showed *C kroppenstedtii* to be the most prevalent pathogen in these patients.

Data on the yield of *C kroppenstedtii* using 16S rRNA on FFPE tissues from GLM patients are limited. In 2018, Gautham et al[28] described *Corynebacteria* within neutrophil invested microcysts in 4 of 5 patients with CNGM using Gram stain on histologic sections; however, their attempt at

Table 2. Comparison of Age, Histologic Pattern, Gram Stain, and Microbial Cultures in *C kroppenstedtii* Polymerase Chain Reaction (PCR)–Positive Versus *C kroppenstedtii*–Negative Cases

	***C kroppenstedtii* 16S rRNA SYBR Real-Time PCR–Positive Cases, 46/67 (68.7%)**	***C kroppenstedtii* 16S rRNA SYBR Real-Time PCR–Negative and Inconclusive Cases, 21/67 (31.3%)**
Mean age, y	35.2	36.4
CNGM pattern	28/46 (60.9%)	10/21 (47.6%)
Gram stain positive cases	8/46 (17.4%)	4/21 (19%)
Microbial cultures not obtained	21/46 (45.7%)	8/21 (38.1%)
Microbial cultures obtained and negative for bacterial growth	14/25 (56.0%)	8/21 (38.1%)
Microbial cultures obtained and positive for *Diphtheroids*	10/25 (40%)	4/21 (19.0%)
Microbial cultures obtained and positive for bacteria other than *Diphtheroids*	1/25 (4.0%) (Coagulase-negative *Staphylococcus*)	1/21 (4.8%) (*Streptococcus angionosus*)

Abbreviation: CNGM, cystic neutrophilic granulomatous mastitis.

performing 16S rRNA PCR on FFPE tissues on these cases was unsuccessful. In 2018, Fuji et al[22] for the first time demonstrated *C kroppenstedtii* genome encoding 16S rRNA in DNA extracted from FFPE sections of GLM cases using a *C kroppenstedtii*–specific primer set yielding 7 positive cases of 18. Most recently, Naik et al[21] identified *Corynebacteria* by 16S rRNA sequencing on DNA extracted from FFPE tissues in 12 of 23 GLM cases. We performed this study to test the strength of this reported association of *C kroppenstedtii* with GLM via SYBR real-time PCR on FFPE tissues at a larger scale and to compare the rate of *C kroppenstedtii* detection in GLM cases with nongranulomatous breast abscess cases. Our study showed that 46 of 67 (68.7%) GLM cases were positive for *C kroppenstedtii* 16S rRNA by SYBR real-time PCR. All 46 cases had the highest alignment score with *C kroppenstedtii* partial 16S rRNA sequence. Our yield of detecting *C kroppenstedtii* by SYBR real-time PCR on FFPE tissues of 68.7% is higher than reported by Fuji et al[22] and Naik et al[21] in their studies (28.8% and 52.1%, respectively). In addition, in our study, all 10 cases of nongranulomatous abscess in the control group were negative for *C kroppenstedtii* DNA suggesting that *C kroppenstedtii* is highly prevalent and specific to GLM and does not appear to be associated with nongranulomatous breast abscess ($P < .001$). Among the *C kroppenstedtii*–positive cases, the majority showed CNGM histology (60.9%).

The yield of Gram stain for *Corynebacteria* in FFPE tissue sections in our study was significantly lower than by PCR (17.9% versus 68.7%). *Corynebacteria* are notorious for staining poorly in clinical samples,[29] and the sensitivity is even lower in Gram-stained FFPE tissue sections. This is also explained by the fact that the bacteria in GLM cases, as shown in our study, are scant and found exclusively inside the neutrophil-rimmed cystic spaces. Microbial culture data were only available in 38 of 67 (56.7%) GLM cases. This is because many of our patients presented with a palpable breast mass that was clinically concerning for malignancy and samples for cultures were not procured at the time of the biopsy procedure due to low clinical suspicion for an infectious or inflammatory process. Of the 38 GLM samples that were cultured, 16 were positive for bacterial growth (42.1%). Overall, 14 of 38 (36.8%) cases with available culture data were positive for "*Diphtheroids*." Species-level identification was not pursued in any of these 14 cases likely because many C*orynebacteria* are part of the normal microflora of human skin, mucous membranes, and body fluids and are usually regarded as commensals/contaminants in the lab. Nevertheless, in our study *Diphtheroids* were the most common isolates among the positive cultures. Our study shows that the yield of detecting *C kroppenstedtii* in GLM cases is significantly higher by SYBR real-time PCR on FFPE tissues as compared with Gram stain on histologic sections (68.7% versus 17.9%). The yield of detecting *C kroppenstedtii* by microbial cultures versus SYBR real-time PCR on FFPE tissues is difficult to analyze in our study as the cases positive for *Diphtheroids* were not pursued further for speciation; however, it is likely lower than PCR given that only 14 of 38 (36.8%) cases with available culture data were positive for *Diphtheroids* (versus 68.7% by PCR). If we take into consideration the limitations of performing PCR on FFPE tissues, the yield is likely higher than 68.7% and can be further improved by the use of fresh samples.

We recommend that fresh samples be obtained in all cases of suspected GLM for microbial studies. This will not only improve the detection rate of *C kroppenstedtii* by PCR in these cases but also allow antimicrobial susceptibility testing in culture-positive cases that may open doors to targeted antibiotic therapies and hopefully improved clinical outcomes. At present, there is no consensus in the literature regarding the treatment for GLM. The proposed treatment regimens include steroids, immunosuppressants, antibiotic therapy, surgical debridement, and watchful waiting.[30–33] Irrespective of the treatment strategy employed GLM has a high rate of relapse.[34,35] An infectious etiology has long been a leading hypothesis with regard to GLM, but the frequent negative microbial cultures[13] and the lack of response to antibiotic therapy[19] have led to some skepticism. The negative cultures are likely attributable to the lipophilic nature of *Corynebacteria*, which typically require specific media and prolonged incubation. Furthermore, "*Diphtheroids*" in smears and colonies are frequently regarded as commensals or contaminants in the lab and are not pursued further, as seen in our study. In addition, *Corynebacteria* are poorly susceptible to the beta-lactam antibiotics that are traditionally prescribed for breast infections and many are multidrug resistant.[36] In recent years several case reports and case series claiming successful treatment of GLM solely with an extended course of lipophilic antibiotics targeting *C kroppenstedtii* have been described.[17,37–40] Large-scale studies focused on improved detection of *C kroppenstedtii* in GLM and treatment with targeted lipophilic antibiotics are needed to ensure a convincing response to therapy.

Of note, 21 of 67 (31.3%) of GLM cases in our study were negative for *C kroppenstedtii* by PCR. These 21 cases include four Gram-stain positive and 9 cases positive for *Diphtheroids* in microbial samples. This discrepancy may be in part due to formalin-related nucleic acid degradation.[41] Second, only 1 block per case was selected for PCR, hence, a false-negative result due to sampling error is a very likely possibility. Third, many of our FFPE specimens were small core biopsies, hence, the lesional tissue in the block may have been exhausted during processing. Last, the contents of the cystic spaces may be lost with deparaffinization and DNA extraction leading to false low yield. The use of fresh biopsy samples obtained for microbial studies might improve the detection sensitivity by PCR-based methods.

Certain important limitations of our study should also be noted. First, several factors may influence PCR results, such as small tissue size, variability in tissue processing, contamination of tissue or blocks, and the effects of formalin fixation upon nucleic acid quality and quantity. We have attempted to mitigate these factors through the use of analytic controls as well as case controls to minimize false-negative or false-positive results. Also, since we expected relatively small nucleic acid fragments in FFPE tissue, only a *C kroppenstedtii*–specific primer set (with PCR product size of 134 bp) was selected for this study. The use of this species-specific primer precludes the detection of other bacteria in our study. Larger studies using multiplex PCR techniques are needed to exclude the presence of other bacterial species in GLM.

CONCLUSIONS

We conclude that *C kroppenstedtii* is highly prevalent in GLM, supported by the detection of *C kroppenstedtii* DNA in 68.7% of GLM FFPE tissues by real-time PCR. In contrast, *C kroppenstedtii* was not found in nongranulomatous breast abscesses in our study. The prevalence of *C kroppenstedtii* is

higher in GLM with histologic features of CNGM as compared with non-CNGM. Gram stain has low sensitivity for detecting *Corynebacteria* in GLM tissue sections and when positive shows bacteria exclusively inside the neutrophil-rimmed cystic spaces. Microbial cultures positive for "*Diphtheroids*" in smears and colonies should not be dismissed as commensals/contaminants in the setting of GLM, and species-level identification should be pursued in all cases. We strongly recommend obtaining fresh biopsy samples for microbiologic studies in all cases of suspected GLM. This will not only improve the detection rate of *C kroppenstedtii* by PCR in these cases but also allow antimicrobial susceptibility testing in culture-positive cases that may open doors to targeted antibiotic therapies and hopefully improved clinical outcomes.

References

1. Kessler E, Wolloch Y. Granulomatous mastitis: a lesion clinically simulating carcinoma. *Am J Clin Pathol*. 1972;58(6):642–646.
2. Imoto S, Kitaya T, Kodama T, et al. Idiopathic granulomatous mastitis: case report and review of the literature. *Jpn J Clin Oncol*. 1997;27(4):27–277.
3. Baslaim M, Khayat H, Al-Amoudi S. Idiopathic granulomatous mastitis: a heterogeneous disease with variable clinical presentation. *World J Surg*. 2007; 31(8):1677–1681.
4. Heer R, Shrimankar J, Griffith C. Granulomatous mastitis can mimic breast cancer on clinical, radiological or cytological examination: a cautionary tale. *Breast*. 2003;12(4):283–286.
5. Taylor G, Paviour S, Musaad S, Jones W, et al. A clinicopathological review of 34 cases of inflammatory breast disease showing an association between corynebacteria infection and granulomatous mastitis. *Pathology*. 2003;35(2):109–119.
6. Renshaw AA, Derhagopian RP, Gould EW. Cystic neutrophilic granulomatous mastitis: an underappreciated pattern strongly associated with gram-positive bacilli. *Am J Clin Pathol*. 2011;136(3):424–427.
7. D'Alfonso TM, Moo T-A, Arleo EK, Cheng E, Antonio LB, Hoda SA. Cystic neutrophilic granulomatous mastitis: further clinical and pathological characterization of an under-recognized entity based on eleven cases. *Am J Surg Pathol*. 2015;39(10):1440–1447.
8. Johnstone K, Robson J, Cherian S, et al. Cystic neutrophilic granulomatous mastitis associated with *Corynebacterium* including *Corynebacterium kroppenstedtii*. *Pathology*. 2017;49(4):405–412.
9. Riegel P, Liégeois P, Chenard MP, Mathelin C, Monteil H. Isolations of *Corynebacterium kroppenstedtii* from a breast abscess. *Int J Med Microbiol*. 2004;294(6):413–416.
10. Ang LM, Brown H. Corynebacterium accolens isolated from breast abscess: possible association with granulomatous mastitis. *J Clin Microbiol*. 2007;45(5): 1666–1668.
11. Bercot B, Kannengiesser C, Oudin C, et al. First description of NOD2 variant associated with defective neutrophil responses in a woman with granulomatous mastitis related to corynebacteria. *J Clin Microbiol*. 2009;47(9): 3034–3037.
12. Kieffer P, Dukic R, Hueber M, Kieffer C, Bouhala M, Riegel P, Wilhelm JM. A young woman with granulomatous mastitis: a corynebacteria may be involved in the pathogenesis of these disease [in French]. *Rev Med Interne*. 2006;27(7): 550–554.
13. Stary CM, Lee YS, Balfour J. Idiopathic granulomatous mastitis associated with *Corynebacterium* sp. infection. *Hawaii Med J*. 2011;70(5):99–101.
14. Gautier N, Lalonde L, Tran-Thanh D, El Khoury M, David J, Labelle M, Patocskai E, Trop I. Chronic granulomatous mastitis: imaging, pathology and management. *Eur J Radiol*. 2013;82(4):165–175.
15. Tauch A, Fernández-Natal I, Soriano F. A microbiological and clinical review on *Corynebacterium kroppenstedtii*. *Int J Infect Dis*. 2016;48:33–39.
16. Dobinson HC, Anderson TP, Chambers ST, Doogue MP, Seaward L, Werno AM. Antimicrobial treatment options for granulomatous mastitis caused by *Corynebacterium* species. *J Clin Microbiol*. 2015;53(9):2895–2899.
17. Kutsuna S, Mezaki K, Nagamatsu M, et al. Two cases of granulomatous mastitis caused by *Corynebacterium kroppenstedtii* infection in nulliparous young women with hyperprolactinemia. *Intern Med*. 2015;54(14):1815–1818.
18. Wong SCY, Poon RWS, Chen JHK, et al. *Corynebacterium kroppenstedtii* is an emerging cause of mastitis especially in patients with psychiatric illness on antipsychotic medication. *Open Forum Infect Dis*. 2017;4(2):ofx096.
19. Troxell ML, Gordon NT, Doggett JS, Ballard M, Vetto JT, Pommier RF, Naik AM. Cystic neutrophilic granulomatous mastitis: association with gram-positive bacilli and *Corynebacterium*. *Am J Clin Pathol*. 2016;145(5):635–645.
20. Yu HJ, Deng H, Ma J, et al. Clinical metagenomic analysis of bacterial communities in breast abscesses of granulomatous mastitis. *Int J Infect Dis*. 2016; 53:30–33.
21. Naik MA, Korlimarla A, Shetty ST, Fernandes AM, Pai SA. Cystic neutrophilic granulomatous mastitis: a clinicopathological study with 16s rRNA sequencing for the detection of *Corynebacteria* in formalin-fixed paraffin-embedded tissue. *Int J Surg Pathol*. 2020;28(4):371–381.
22. Fujii M, Mizutani Y, Sakuma T, et al. *Corynebacterium kroppenstedtii* in granulomatous mastitis: analysis of formalin-fixed, paraffin-embedded biopsy specimens by immunostaining using low-specificity bacterial antisera and real-time polymerase chain reaction. *Pathol Int*. 2018;68(7):409–441.
23. Sanchez-Vega B, Vega F, Medeiros LJ, Lee MS, Luthra R. Quantification of bcl-2/JH fusion sequences and a control gene by multiplex real-time PCR coupled with automated amplicon sizing by capillary electrophoresis. *J Mol Diagn*. 2002; 4(4):223–229.
24. Urbaniak C, Cummins J, Brackstone M, et al. Microbiota of human breast tissue. *Appl Environ Microbiol*. 2014;80(10):3007–3014.
25. Xuan C, Shamonki JM, Chung A, et al. Microbial dysbiosis is associated with human breast cancer. *PLoS One*. 2014;9(1):e83744.
26. Hieken, T., Chen, J., Hoskin, T, et al. The microbiome of aseptically collected human breast tissue in benign and malignant disease. *Sci Rep*. 2016;6: 30751.
27. Binelli C, Lorimier G, Bertrand G, et al. Granulomatous mastitis and corynebacteria infection. Two case reports. *J Gynecol Obstet Biol Reprod*. 1996; 25(1):27–32.
28. Gautham I, Radford D, Kovacs C, et al. Cystic neutrophilic granulomatous mastitis: the Cleveland Clinic experience with diagnosis and management. *Breast J*. 2018;25(1):80–85.
29. Winn W, Allen S, Janda W, et al. *Koneman's Color Atlas and Textbook of Diagnostic Microbiology*. Philadelphia, PA: Lippincott Williams & Wilkins; 2006.
30. Lai EC, Chan WC, Ma TK, et al. The role of conservative treatment in idiopathic granulomatous mastitis. *Breast J*. 2005;11(6):454–456.
31. Taghizadeh R, Shelley OP, Chew BK, et al. Idiopathic granulomatous mastitis: surgery, treatment, and reconstruction. *Breast J*. 2007;13(5):509–513.
32. Maffini F, Baldini F, Bassi F, et al. Systemic therapy as a first choice treatment for idiopathic granulomatous mastitis. *J Cutan Pathol*. 2009;36(6):689–691.
33. Kehribar DY, Duran TI, Polat AK, Ozgen M. Effectiveness of methotrexate in idiopathic granulomatous mastitis treatment. *Am J Med Sci*. 2002;360(5):560–565.
34. Neel A, Hello M, Cottereau A, et al. Long-term outcome in idiopathic granulomatous mastitis: a western multicentre study. *QJM*. 2013;106(5):433–441.
35. Akbulut S, Yilmaz D, Bakir S. Methotrexate in the management of idiopathic granulomatous mastitis: review of 108 published cases and report of four cases. *Breast J*. 2011;17(6):661–668.
36. Soriano F, Zapardiel J, Nieto E. Antimicrobial susceptibilities of *Corynebacterium* species and other non-spore-forming gram-positive bacilli to 18 antimicrobial agents. *Antimicrob Agents Chemother*. 1995;39(1):208–214.
37. Waldron R, Mohamed A, Boyle B, Al-Azawi D, O'Connell B. Successful treatment of granulomatous mastitis associated with *Corynebacterium kroppenstedtii* with prolonged antimicrobial therapy. *Infect Dis Clin Pract*. 2019;27(2): 107–109.
38. Brownson KE, Bertoni DM, Lannin DR, et al. Granulomatous lobular mastitis-another paradigm shift in treatment. *Breast J*. 2019;25(4):790–791
39. Johnson MG, Leal S, Plongla R, Leone PA, Gilligan PH. the brief case: recurrent granulomatous mastitis due to *Corynebacterium kroppenstedtii*. *J Clin Microbiol*. 2016;54(8):1938–1941.
40. Farouk O, Abdelkhalek M, Abdallah A, et al. Rifampicin for idiopathic granulomatous lobular mastitis: a promising alternative for treatment. *World J Surg*. 2017;41(5):1313–1321.
41. Imrit K, Goldfischer M, Wang, J et al. Identification of bacteria in formalin-fixed, paraffin-embedded heart valve tissue via 16S rRNA gene nucleotide sequencing. *J Clin Microbiol*. 2006;44(7):2609–2611.