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ANATOMICAL PATHOLOGY / MICROBIOLOGY

Cystic neutrophilic granulomatous mastitis associated with *Corynebacterium* including *Corynebacterium kroppenstedtii*

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Summary

Granulomatous (lobular) mastitis is a rare inflammatory breast disease affecting parous reproductive-aged women. Once considered idiopathic, there is growing evidence of an association with corynebacteria infection, especially in the setting of a distinct histological pattern termed cystic neutrophilic granulomatous mastitis (CNGM). We describe 15 cases with histological features either confirming (n = 12) or suggesting (n = 3) CNGM, and concurrent microbiological evidence of *Corynebacterium* species. The organism was detected by culture or 16S rRNA gene sequencing of specimens obtained at surgery or fine needle aspiration. In seven cases, Gram-positive organisms were seen within vacuolated spaces. Speciation was performed in nine cases, with *Corynebacterium kroppenstedtii* subsequently identified. These cases provide further evidence in support of this association and in doing so highlight the importance of recognising these histological clues as well as the limitations of Gram stain and microbiological culture in detecting this previously underrecognised disease process.

Key words: Cystic neutrophilic granulomatous mastitis; granulomatous mastitis; granulomatous lobular mastitis; lipogranuloma; breast corynebacteria; lipophilic corynebacteria; *Corynebacterium kroppenstedtii*.

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INTRODUCTION

First described in 1972,[1,2] granulomatous mastitis is a rare inflammatory breast disease of unclear aetiology.[2,3] Granulomatous mastitis most commonly affects parous women of reproductive-age[2,4,5] and presents as a tender, enlarging breast mass, which may be complicated by sinus[5] and abscess formation.[4] Radiographic findings are non-specific,[3,6] necessitating histological examination for diagnosis[6] and to exclude carcinoma.

Granulomatous mastitis is histologically characterised by granulomas in and around lobules, often with suppuration and sometimes associated microabscess formation.[2] Differential diagnoses range from infective, including tuberculous mastitis and fungal infection, to autoimmune, including sarcoidosis and granulomatosis with polyangiitis.[2,7]

There is no consensus as to optimal management for granulomatous mastitis,[2] with treatment options including surgical excision, corticosteroids, antibiotics[2,5] and even radiotherapy.[2] It is often difficult to manage, with many patients undergoing multiple procedures[2] and suffering chronic and recurrent disease.[2,5,7] The average time to recovery exceeds one year.[2]

Granulomatous mastitis is often regarded as idiopathic.[2] After exclusion of mammary tuberculosis, most cases are aseptic[2,3,8] and mechanisms including autoimmune[3,8] and hypersensitivity[2] reactions have been proposed. An infectious aetiology in many cases has been suggested in light of growing evidence of an association with corynebacteria.[2] However, the pathogenic role of the isolated bacteria is not clearly defined as it is usually difficult to distinguish between infection, colonisation and contamination.[9]

An association between corynebacteria infection and granulomatous mastitis was proposed by a clinicopathological review of 34 patients in New Zealand in 2003,[2] and further demonstrated in isolated case reports and small series of four or fewer cases throughout Europe,[1,2,8,10–12] North America,[3,13–15] Asia[16,17] and New Zealand.[18]

There is increasing evidence of an association between corynebacteria infection and a distinct pattern termed cystic neutrophilic granulomatous mastitis (CNGM), characterised by lipogranulomas consisting of clear spaces rimmed by neutrophils and surrounding granulomatous inflammation.[4,7,19,20] A retrospective analysis from the United States of America including 19 cases of CNGM, three with microbiological evidence of *Corynebacterium* species, has recently been published.[7]

We present 15 cases, including 12 cases with demonstrated histological features of CNGM and three cases with suggestive histological features, all with confirmed microbiological evidence of *Corynebacterium* species, presenting to a private pathology laboratory in Australia. These cases provide further evidence in support of this association and emphasise the challenges in making this diagnosis from both a histological and microbiological perspective.

MATERIAL AND METHODS

A systematic search of the electronic histopathology and microbiology records for Sullivan Nicolaides Pathology was conducted to identify all patients with a breast tissue histology specimen collected between 1 January 2010 and 30 June 2015 and with microbiological evidence of *Corynebacterium* species

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(bacterial culture or 16S rRNA gene sequencing) on a specimen taken from the same site at approximately the same time. The cases were reviewed by a breast pathologist (JB) for evidence of a CNGM pattern, specifically clear spaces rimmed by neutrophils and surrounded by granulomatous inflammation.[2,7] Cases lacking characteristic or suggestive histological features of CNGM were not included.

Gram stains were performed on all cases. Stains for mycobacteria (acid-fast bacilli, Wade-Fite and/or Ziehl–Neelsen) and fungi (periodic acid–Schiff and/or Grocott) were negative in all cases.

Tissues and aspirates were routinely plated on horse blood agar, chocolate agar and anaerobic agar containing Tween 80, with the latter incubated for 14 days at 35°C. Antibiotic susceptibilities were performed by either disc diffusion or Etest (bioMérieux, France, as per the Etest manufacturer recommendations). Non-species-related breakpoints were utilised as per European Committee on Antimicrobial Susceptibility Testing (EUCAST) guidelines until *Corynebacterium* species clinical breakpoints became available (www.eucast.org) in 2014.

Clinical history was obtained from pathology requests accompanying the specimens and communication with referring practitioners in some cases. Cytology findings were recorded where performed.

Ethical approval was given at an institutional level (Sullivan Nicolaides Pathology) in accordance with the National Health and Medical Research Council guidelines for Negligible Risk Human Research.

RESULTS

Twenty-one cases with microbiological evidence of *Corynebacterium* species were reviewed for possible inclusion. Twelve cases were identified which showed microbiological evidence of *Corynebacterium* species infection in addition to histopathological features of CNGM. Three cases of *Corynebacterium* species infection associated with histological features suggestive of but falling short of a diagnosis of CNGM were also identified. Six cases which did not show characteristic or suggestive histological features of CNGM were excluded. The diagnoses in each of these cases were necrosis, microabscesses and acute on chronic inflammation (n = 1), acute abscess (n = 1), chronic abscess (n = 1), inflamed mammary fistula (n = 1), granulomatous and foreign body giant cell reaction to retained surgical material (n = 1) and infected breast implant capsule (n = 1). No cases with the histological features described in idiopathic granulomatous mastitis were identified. The key clinical, histology, microbiology and cytology findings of each case are summarised in Table 1.

Demographics

Women were aged between 33 and 58 years, with a mean age of 38.7 years. Among the five cases where ethnicity was known, four were of Asian origin born outside Australia and the fifth was of Maori descent.

Clinical history

While an abscess and/or infectious process were clinically suspected in six patients, the remaining majority presented with a mass, often with a clinical concern of malignancy. Disease was bilateral in three cases. All except for two patients underwent multiple procedures over a period of up to 32 months. Management included antibiotics and surgical intervention. In Case 3, the patient continued to deteriorate with flucloxacillin and ciprofloxacin. In Case 4, the patient had successive courses of antibiotics including combinations of β-lactams, rifampicin, clarithromycin, trimethoprim-sulfamethoxazole and isoniazid, with suspicion for mycobacterial disease due to negative culture results. The patient had a clinical response when doxycycline was instituted. Improvement with doxycycline was also seen in Case 11. In Case 6, the patient had an initial response to doxycycline but later relapsed. Clinical deterioration was observed with substitution for amoxicillin-clavulanate whilst surgical intervention and drainage with adjuvant clindamycin brought resolution of symptoms. In Case 7, a combination of surgery and clindamycin also resolved symptoms.

Histology

Cases 1–12 showed features consistent with a diagnosis of granulomatous mastitis, and in particular, the distinct pattern of CNGM[4,7,19,20] (Fig. 1). All cases showed granulomatous inflammation centred on vacuolated spaces surrounded by neutrophils in a background of acute on chronic inflammation including neutrophils, plasma cells, lymphocytes and eosinophils. The vacuolated spaces were mostly microcystic; however, large cleared spaces were also seen. The distribution varied from lobulocentric to being florid and widespread with obliteration of normal architecture. Several cases also showed evidence of suppuration and fibrosis. In four cases CNGM was seen in association with abscess formation. In one case the initial core biopsy was characteristic whilst the subsequent lumpectomies showed cavitating granulomatous abscess formation with variable sized vacuolated spaces suggestive of but falling short of a definite diagnosis of CNGM.

Cases 13–15 showed features suggestive of but falling short of a diagnosis of CNGM in the setting of *Corynebacterium* species infection. In all three cases, histology demonstrated tissue consistent with material from the wall of an abscess along with vacuolated cystic spaces of presumed lipid surrounded by histiocytes and associated with focal acute inflammation. Lipogranulomas were identified in Case 14, were poorly formed in Case 13 and were absent in Case 15. In Cases 13 and 14, samples were core biopsies with only limited available tissue.

Gram stain

Seven of the 15 cases showed evidence of Gram-positive organisms within vacuolated spaces (Fig. 1), one with distinct coryneform features. Four were identified at the time of initial reporting and three were stained and identified as positive retrospectively. No stains that were initially reported as negative were subsequently identified as positive. On histology, the staining of bacterial organisms was variable with the appearances varying from definite bacilli in four cases to coccobacilli in three cases. Gram stains were negative in the remaining eight cases.

Microbiology

All cases were associated with the detection of corynebacteria in invasive breast specimens. *Corynebacterium* species were grown in 14 cases, one also with confirmed 16S rRNA gene sequencing. In the remaining case, cultures were negative, however *C. kroppenstedtii* was detected using 16S rRNA gene sequencing of two separate aspirates. Nine cases were specifically identified as *C. kroppenstedtii* and six were not further speciated. In four cases of CNGM, corynebacteria were grown in the presence of other organisms including *Propionibacterium acnes*, Group B *Streptococcus*, and