Inflammatory Lesions of the Breast and the Role of the Breast Microbiome

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Disclosures

I have no financial disclosures relevant to this talk.

My husband is the Chief Scientific Officer of resTORbio (a biopharmaceutical company).

We own stock in resTORbio and Novartis.

Acknowledgement

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Brigham and Women’s Hospital/Dana Farber Cancer Institute, Breast Pathology Services - cases per year

> 4,000 consultation cases

> 2,000 core needle biopsies

> 1,000 in house surgical specimens

Support for Partners in Health in Rwanda and Haiti

Breast Team:

Jane Brock
Stuart Schnitt
Deborah Dillon
Beth Harrison
Susan Lester
A case from circa 1985 . . . .

A 36 year old woman, who was not pregnant or lactating, developed a painful palpable breast mass.

On examination by her doctor, localized skin erythema was noted. Because the majority of breast infections are due to *Staphylococcus aureus*, she was prescribed an antibiotic for this presumed infection.

Two weeks later, the mass was larger and firmer.

An incision and drainage was performed.

Tissue fragments sent for pathologic evaluation showed a mixed inflammatory infiltrate with neutrophils, lymphocytes, and scattered giant cells ("granulomatous" inflammation). No organisms were seen on AFB and fungal stains.

She was instructed to continue taking the same antibiotic.
Two months later, the mass had increased in size and fistula tracts to the skin had developed.

A swab sample from the lesion was sent for culture.

To the clinician’s surprise, the cultures grew a mixed anaerobic bacterial population.

At the time, the source of anaerobic bacteria causing non-lactational abscesses was a mystery. . .

**Transient bacteremia due to vaginal manipulation?** “In 2 of our patients surgical trauma of the upper vagina and cervical region had occurred, and vaginal examination had been performed in the third.”

**Sexual activity?** “Because the breasts are often involved in sexual activity, manual contamination of the nipple with vaginal and perineal bacteria as well as direct contamination of this area with oral species can occur, and this may, under certain conditions, result in breast infections.”

**Poor hygiene, low socio-economic status?**

**Factitious (Munchhausen’s syndrome)?**


Or . . .

Does the Breast Have Its Own Microbiota?

Learning objectives:

1) Review current knowledge of the breast microbiota

Definitions:

*Microbiota* = all microorganisms at a site

*Microbiome* = the genomes of those organisms

2) Review symptomatic inflammatory lesions of the breast and the possible role of the microbiota in breast infections
There are two main lines of evidence for a normal breast microbiota:

- Breast milk analysis
- Breast tissue analysis
Breast milk was long assumed to be sterile, as the breast was assumed to be sterile.

When an early study found bacteria in the milk of Guatemalan women, the authors concluded:

“The presence of Enterobacteriaceae in human colostrum and milk reflects the low levels of hygiene and environmental sanitation in the population studied.”

However, multiple studies have documented numerous types of bacteria in milk obtained under sterile conditions. The milk microbiome is dominated by *Staphylococcus*, *Streptococcus*, and *Pseudomonas*.

<table>
<thead>
<tr>
<th>Genera</th>
<th>Species</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acinetobacter</td>
<td><em>A. calcoaceticus</em></td>
</tr>
<tr>
<td>Bifidobacterium</td>
<td><em>B. breve, B. adolescentis, B. bifidum, B. longum, B. animalis, B. catenulatum</em></td>
</tr>
<tr>
<td>Enterococcus</td>
<td><em>E. faecalis, E. faecium, E. durans, E. hirae, E. mundtii</em></td>
</tr>
<tr>
<td>Escherichia</td>
<td><em>E. coli</em></td>
</tr>
<tr>
<td>Gemella</td>
<td><em>G. haemolysans</em></td>
</tr>
<tr>
<td>Klebsiella</td>
<td><em>K. oxytoca</em></td>
</tr>
<tr>
<td>Kocunia</td>
<td><em>K. kristinae</em></td>
</tr>
<tr>
<td>Lactobacillus</td>
<td><em>L. rhamnosus, L. crispatus, L. gasseri, L. fermentum, L. plantarum, L. brevis, L. oris, L. animalis</em></td>
</tr>
<tr>
<td>Lactococcus</td>
<td><em>L. lactis ssp. lactis</em></td>
</tr>
<tr>
<td>Leuconostoc</td>
<td><em>L. mesenteroides, L. citreum, L. fallax</em></td>
</tr>
<tr>
<td>Propionibacterium</td>
<td><em>P. acnes</em></td>
</tr>
<tr>
<td>Pseudomonas</td>
<td><em>P. synxantha, P. fluorescens</em></td>
</tr>
<tr>
<td>Rothia</td>
<td><em>R. mucilaginosa</em></td>
</tr>
<tr>
<td>Serratia</td>
<td><em>S. proteomaculans</em></td>
</tr>
<tr>
<td>Staphylococcus</td>
<td><em>S. aureus, S. epidermidis, S. haemolyticus, S. hominis, S. pasteuri, S. warneri</em></td>
</tr>
<tr>
<td>Streptococcus</td>
<td><em>S. salivarius, S. mitis, S. galloyticus, S. australis, S. vestibularis, S. parasanguis, S. pneumoniae</em></td>
</tr>
<tr>
<td>Weisella</td>
<td><em>W. cibaria, W. confusa</em></td>
</tr>
</tbody>
</table>

**Table 1. Bacterial types identified in human breast milk by culture and culture-independent techniques.**

**Other bacterial genera detected but not assigned to species**
- Corynebacterium
- Sphingomonas
- Granulicatella
- Bradyrhizobium
- Prevotella
- Ralstonia
- Actinomyces
- Clostridium
- Veillonella

Data taken from [12–14,29,30,114].

BREAST MILK - not just milk!

Secretory antibodies (sIgA)

Glycans (prevent binding of enteropathogens)

Lactoferrin (bactericidal, antiviral, anti-inflammatory)

Multiple anti-inflammatory mediators

Mediators that enhance the infant’s natural immune system

Stimulation of growth of certain types of colonic bacteria
BREAST MILK - not just milk!

- Secretory antibodies (sIgA)
- Glycans (prevent binding of enteropathogens)
- Lactoferrin (bactericidal, antiviral, anti-inflammatory)
- Multiple anti-inflammatory mediators
- Stimulation of growth of certain types of colonic bacteria

$10^3$-$10^6$ bacteria per ml. in aseptically collected milk.

Breast-fed infants consume 7-8 billion bacteria per day.
Sterile tissue from women without infection have been studied.

Numerous bacterial species are identified by 16S rRNA sequencing and by culture.

The breast has a unique microbiota which differs from the microbiota of the skin, oral cavity, and intestinal tract.

Anaerobic and lipophilic bacteria are common.


The breast microbiota normally exists in the lumens of ducts and lobules – an anaerobic environment filled with secretions and lipids.

Bacteria may be introduced via:

- Woman’s nipple/areolar skin
- Infant’s oral cavity and skin
- “Enteromammary pathway” from gut (?)

Role in normal breast health, infections, and possibly in cancer is not yet understood.
Symptomatic inflammatory breast lesions

Learning objectives:

1) Review current knowledge of the breast microbiota

Definitions:
Microbiota = all microorganisms at a site
Microbiome = the genomes of those organisms

2) Review symptomatic inflammatory lesions of the breast and the possible role of the microbiota in breast infections
## Inflammatory Lesions of the Breast

<table>
<thead>
<tr>
<th>Symptomatic</th>
<th>Usually not symptomatic</th>
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<tbody>
<tr>
<td>Lactational abscesses</td>
<td>Ruptured cysts</td>
</tr>
<tr>
<td>Non-lactational abscesses</td>
<td>Fat necrosis</td>
</tr>
<tr>
<td></td>
<td>Lymphocytic (diabetic) mastopathy</td>
</tr>
<tr>
<td></td>
<td>Duct ectasia</td>
</tr>
<tr>
<td></td>
<td>IgG4 mastitis</td>
</tr>
</tbody>
</table>
Mastitis is common during breastfeeding – ~1-33% of women.

Only 1-3% develop an abscess.

Women present with a tender erythematous mass often accompanied by fever.

Of breast abscesses requiring surgery, only 14% are related to lactation.
In rare cases in which I&D is performed, the specimen shows neutrophils and necrosis. Bacteria may be present associated with neutrophils.
The causative organism is typically *Staphylococcal* spp. (*S. aureus* ~50%).

Standard teaching has been that the infections arise from cracks and fissures in the nipple skin providing a portal of entry of bacteria from skin and infant oral microbiome.

However –

Abscesses are not located at the nipple.

Portal of entry is likely deeper in the breast.

Source of bacteria is more likely the milk/breast microbiota escaping from the duct/lobular system.
Lactational Mastitis – Clinical Features

Readily treated with antibiotics directed against *Staphylococcal* spp.

Probiotics containing lactobacilli are under investigation as an alternative treatment.

Continued breast feeding is generally possible.

*Because lactational mastitis is more common than non-lactational mastitis, the two are often confused clinically.*

Non-Lactational Abscesses

Etiology:

Infection

Exogenous organisms

Endogenous (breast microbiota) organisms?

Other causes of inflammation
Breast Infections: Exogenous organisms

Vanishingly rare.

Tuberculosis

Fungi

Parasites
TB involving the breast is very rare – including locations with endemic TB.

Most common in young women but also reported in males and older women.

Appearance is the same as TB seen elsewhere –

Granulomas – location scattered throughout tissue (i.e. not associated with ducts and lobules - location unlike Granulomatous Lobular Mastitis).

Central caseating necrosis.

Caseating granulomas are scattered throughout breast tissue and not specifically associated with ducts and lobules.
Extremely rare.

**Fungi**

Histoplasmosis – necrotizing granulomatous inflammation associated with yeast forms

Blastomycosis – necrotizing granulomas associated with yeast forms

Cryptococcus – yeast forms associated with histiocytes
31 year old woman from Rwanda, HIV positive, with fever, a palpable breast mass, and lymphadenopathy.

The fungal forms were positive for mucicarmine and Fontana-Masson stains.
Parasites

Rarely involve the breast in locations where parasite is found.

Filariasis – roundworms (Africa, Southeast Asia, South America, Caribbean, Australia) – serpiginous calcifications (dead worms in lymphatics)

Schistosomiasis – parasitic fluke (South East Asia, South America, Caribbean) – calcified ova seen in lobules and ducts

Cysticercosis – larval form of *Taenia solium* (Africa, Asia, Eastern Europe, Latin America) – cysts containing viable larva or calcified dead organisms

Sparginosis – tapeworm larva of the *Spirometra* genus (Southeast Asia)

Sparginosis

Infection may occur due to drinking contaminated water or eating raw snakes and frogs.

The parasite penetrates the intestinal wall and migrates throughout the body.

Patients present with a palpable mass – usually in subcutaneous tissue rather than in the breast.

Diagnosis can sometimes be made by identifying movement of a living larval worm.
An eosinophilic tegument surrounds a myxoid matrix containing smooth muscle fibers and calcareous bodies.
Non-Lactational Abscesses

Etiology:

Infection

Exogenous organisms

Endogenous (breast microbiota) organisms?

Other causes of inflammation
Two candidate diseases:

Cystic neutrophilic granulomatous mastitis (CNGM) and granulomatous lobular mastitis (GLM)

Squamous metaplasia of lactiferous ducts (SMOLD)
Histologic features and association of CNGM with *Corynebacteria* first well described in series of patients from New Zealand in 2003.

Only women with a history of pregnancy or prolactinemia (drug induced, pituitary adenoma) have been affected.

Patients present with a painful palpable breast mass.


Cystic neutrophilic granulomatous mastitis

A mixed inflammatory infiltrate is present including neutrophils, lymphocytes, and giant cells.

The hallmark of the disease is the lipid vacuoles ("cysts" – also termed "suppurative lipogranulomas").
Cystic (lipid vacuoles)

Neutrophilic (polymorphonuclear leukocytes)

Granulomatous (giant cells)

Mastitis
Cystic neutrophilic granulomatous mastitis

Gram positive bacteria may be present within the lipid vacuoles.

Bacteria are typically absent from areas of the inflammatory infiltrate.
Recurrence and/or persistence of symptoms is common.

Fistula tracts can open onto the skin – usually away from the areola.

Contralateral disease can develop.

The course of the disease is often over months to years – resulting in significant morbidity to affected women.

Dixon JM, Khan LR. Treatment of breast infection. BMJ 2011;342:bmj.d396
Granulomatous lobular mastitis (GLM)

Described in the 1970’s, prior to the description of CNGM.

Like CNGM, all affected women have given birth (1 month to 15 years prior to presentation) and most are about 30 years of age (range 17 to 43 years).

Presents as a firm, sometimes tender, breast mass.

Bilateral in about 25% of cases.

In rare long-standing cases, sinus tracts to the skin may be present.
Granulomatous lobular mastitis (GLM)

Unlike tuberculosis, the granulomas of GLM are centered on ducts and lobules.
Granulomatous lobular mastitis

The granulomas push and distort the epithelium.
“Microabscesses” (lipid vacuoles) are sometimes present within the granulomatous inflammation – very similar to CNGM
Granulomatous lobular mastitis (GLM)

GLM is likely the same disease as CNGM as the clinical settings and histologic features overlap.

GLM was usually seen in surgical excisions of persistent masses, whereas CNGM is currently typically seen in core needle biopsies of masses when patients first present with symptoms.

GLM may be a more advanced or chronic form of CNGM.
Origin from bacteria in breast microbiome?

Bacteria are often seen in lipid vacuoles on Gram stain and are surrounded by an inflammatory response.

Multiple series and case reports have identified bacteria from these lesions using cultures.

More recent studies have used 16S rRNA assays.
Non-Lactational: Corynebacteria (red) were present in 13 of 19 (11 were C. kroppenstedii) and dominated in 7 cases. All had mixed bacterial populations.

Lactational: Staph (black) and Strep (yellow) were almost pure populations.

Cystic neutrophilic granulomatous mastitis

*Corynebacteria kroppenstedtii* – most common bacteria reported

Natural habitat unknown
Lipophilic

Also –

*C. tuberculostearicum*
*C. amycolatum*

Less common:

*Enterobacteriaceae*
*Staphylococcus aureus*
*Propionibacterium acnes*
Corynebacteria are fastidious and difficult to culture. May require special media and long incubation times.

Specific culture for anaerobes and Corynebacteria need to be specified when the specimen is submitted.

In the past, these organisms may have been considered contaminants and disregarded as clinically important.

The types of organisms isolated, and the presence of mixed populations, would be consistent with origin from the breast microbiota.
Cystic neutrophilic granulomatous mastitis

Theory of pathogenesis:

Prior pregnancy (or galactorrhea) predisposes to “leaky” lobular epithelium prone to releasing secretions into stroma.

Bacteria are also released. Anaerobic lipophilic bacteria can survive in pools of lipids.

The lipid pool protects the bacteria from the immune response, resulting in a chronic inflammatory response that is difficult to eradicate.
In general, inflammation with giant cells is associated with mycobacterial or fungal infection.

However, in the breast, this type of inflammatory response is most commonly associated with bacterial infections.

Don’t forget the Gram stain when granulomatous inflammation is seen!
Breast Infections: Breast Microbiota

Two candidate diseases:

Cystic neutrophilic granulomatous mastitis (CNGM) and granulomatous lobular mastitis (GLM)

Squamous metaplasia of lactiferous ducts (SMOLD)
Males and females are affected.

Strongly associated with smoking history.

Presents as a painful erythematous subareolar breast mass.

Typically thought clinically to be an infection and treated with *Staphylococcal* spp. directed antibiotics.

Recurrence is common – another name for this disease is “Recurrent Subareolar Abscess.” Also sometimes referred to as Zuska’s disease after the author of an early study and his wife who suffered from the disease.
Squamous Metaplasia of Lactiferous Ducts (SMOLD)

Normal – keratin plug can be extruded

Keratin extends deep into a duct orifice

Keratin fills a dilated lactiferous sinus
If the duct ruptures, an intense inflammatory reaction with giant cells ensues in response to keratin.
This 50 year old woman has developed a very painful subcutaneous mass on her areola.

A giant cell response to keratin is seen.
Squamous metaplasia of lactiferous ducts

From Lester S. “The Breast”, Robbins and Cotran Pathologic Basis of Disease, Chapter 23, 10th edition
Squamous metaplasia of lactiferous ducts

A single fistula tract opening at the edge of the areola is typical with recurrences.

CNGM – multiple fistula tracts to skin away from nipple.
Patients are encouraged to cease smoking.

Appropriate surgical treatment requires excision of the duct and the fistula tract (if present) via a nipple wedge resection.

With this approach, recurrences are infrequent.

However, because the disease is rare, many patients may not receive appropriate treatment.

There is a case in the literature of a woman undergoing a subcutaneous mastectomy due to the morbidity of recurrent abscesses. This removes all the breast tissue, except for the tissue causing the disease – the involved nipple duct.

Unfortunately, she recurred in the nipple.
The initial specimen from an I&D rarely reveals squamous metaplasia. More typically, a mixed infiltrate of chronic and acute inflammatory cells with occasional giant cells is present.
In some cases, keratin debris associated with giant cells can be seen in the inflammatory infiltrate.
Squamous metaplasia of lactiferous ducts

Squamous metaplasia in ducts and lobules deeper in the breast is usually only seen in larger excisions after one or more recurrences.
At presentation, the lesions may be sterile as the inflammation is due to the keratin debris in stroma.

After recurrence, and sometimes after surgical intervention (e.g. I&D), secondary infections can develop.

Often due to mixed anaerobes – which would be consistent with origin from the breast microbiota.

However, no recent studies using DNA techniques have evaluated the associated bacterial populations.
<table>
<thead>
<tr>
<th></th>
<th>CNGM/GLM</th>
<th>SMOLD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gender</td>
<td>Female</td>
<td>Female and male</td>
</tr>
<tr>
<td>Age</td>
<td>~30 to 40’s</td>
<td>~20’s to 60’s</td>
</tr>
<tr>
<td>Risk factors</td>
<td>Prior pregnancy and/or prolactinemia</td>
<td>Smoking</td>
</tr>
<tr>
<td>Location</td>
<td>Anywhere in breast</td>
<td>Subareolar</td>
</tr>
<tr>
<td>Fistula tract</td>
<td>May be multiple, open to skin</td>
<td>Solitary, open at edge of areola</td>
</tr>
<tr>
<td>Histology</td>
<td>Mixed inflammatory infiltrate with giant cells</td>
<td>Mixed inflammatory infiltrate with giant cells</td>
</tr>
<tr>
<td></td>
<td>Lipid vacuoles, bacteria in vacuoles</td>
<td>+/- squamous metaplasia, keratin</td>
</tr>
<tr>
<td>Infection</td>
<td>Primary Usually Corynebacteria</td>
<td>Secondary Mixed anaerobes</td>
</tr>
<tr>
<td>Treatment</td>
<td>Long term antibiotics (often doxycycline)</td>
<td>Smoking cessation</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Surgery to remove involved duct and fistula</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Antibiotics if there is secondary infection</td>
</tr>
</tbody>
</table>
Non-lactational breast abscesses are the second most common type of symptomatic breast disease undergoing biopsy.

The typical clinical course is a long duration of symptoms with multiple recurrences, causing considerable morbidity to affected women.

There continues to be an associated social stigma – although awareness of a normal breast microbiota should mitigate this.

Both patients and care providers become frustrated with the chronic nature of these abscesses and the poor response to treatment.
The etiology and best treatment for non-lactational abscesses are difficult to study for many reasons:

- Affected women are rare.
- Care is often dispersed among primary care providers, gynecologists, surgeons, infectious disease specialists, and others.
- Bacteria are difficult to culture.
- Literature is confusing (e.g. “granulomatous mastitis” is often discussed as being a specific disease).
Pathologists can play a key role in helping these women.

Although a specific diagnosis is often not possible, including cystic granulomatous mastitis (CNGM) or squamous metaplasia of lactiferous ducts (SMOLD) in the differential diagnosis of appropriate cases can educate clinicians about these disease possibilities.

If new specimens become available, this awareness can lead to better allocation of tissue for culture.

Awareness of these disease can aid in providing patients with appropriate treatment options (e.g. correct antibiotics, surgery, steroids).
Thank you for your attention!