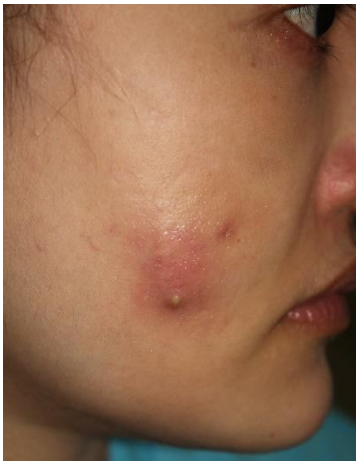
Case 3
A 48-year-old Thai woman from Bangkok
Chief complaint: Painful plaque on right cheek for 6 months



Present illness: A 48-year-old woman received 1 ml injection of hyaluronic acid filler (Restylane®) to correct the deformities in her tear troughs and cheeks at an aesthetic clinic.

Two months later, the patient developed a firm swollen area of approximately 4 cm in diameter on her right cheek around the previous filler injection entry point. After microbial workup, antibiotics were prescribed (clindamycin 600 mg, three times per day plus ciprofloxacin 500 mg, twice daily) for four weeks with improvement resulting in an atrophic scar. Results of pus culture including; aerobe, TB and fungus as well as PCR for TB were negative.

One month later, the skin lesion recurred as an erythematous indurated plaque with pus discharge on the same site. Skin punch biopsy for histopathology and culture was done. The culture was reported *Pseudomonas aeruginosa*. Antibiotic treatment with clindamycin 600 mg, three times per day, ciprofloxacin 500 mg, twice daily and clarithromycin 500 mg, once daily) was started and continued for 16 weeks without improvement. Therefore pus culture from the resistant indurated plaque was repeated and again reported *Pseudomonas aeruginosa*. She denied symptoms of weight loss, anorexia, prolonged fever and chronic cough.

Past history

She was healthy and did not report taking any medications.

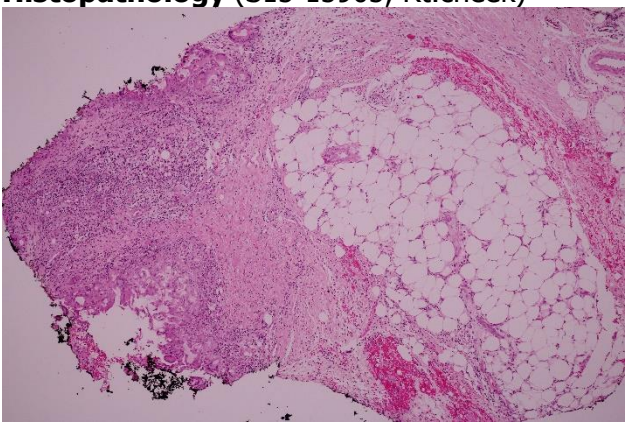
Physical examination

HEENT: not pale, no jaundice
Lymph node: no palpable lymph nodes
Breast: no palpable breast masses
Abdomen: no hepatosplenomegaly

Skin examination

An ill-defined, erythematous to brownish, painful indurated plaque sized 4x4 cm with pustules on right cheek.

Histopathology (S15-15905, Rt.cheek)



- Nodular inflammatory cell infiltrate of lymphocytes, histiocyte, multinucleated giant cell and plasma cells in the deep dermis
- Pale eosinophilic material and multiple small vacuoles within some multinucleated histiocyte
- Special stains failed to indentify the infectious organism

Tissue culture for aerobe:

Pseudomonas aeruginosa

Pus culture for aerobe (after 16 weeks of ATBs treatment):

Pseudomonas aeruginosa

Diagnosis: *Pseudomonas aeruginosa* with biofilm formation as a complication of hyaluronic acid filler

Treatment:

- Ceftazidime 2 g IV every 8 hr plus ciprofloxacin 500 mg PO t.i.d
- Hyaluronidase intralesional injections; total of 10ml hyaluronidase in 3 sessions (2ml, 2ml, 6ml at approximately 7 ,10 and 10 1/4 months after the initial filler injection.)
- 2 ml in 6th and 9th month since hyaluronic acid injection. Four days after that, she also received 6 ml at an aesthetic clinic.

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Discussion:

Hyaluronic acid is the most commonly used injectable filler. [1] Although it is considered safe, complications may develop**.** Infection with biofilm formation is one of the rare complications previously reported.[2, 3] Bacterial biofilm is difficult to distinguish from a low-grade hypersensitivity reaction. As they are usually culture negative, these nodules were previously thought to be due to an allergic or a foreign body reaction to the filler.

A microbial biofilm is 'a structured consortium of microbial cells surrounded by a self-produced polymer matrix'. The concept of biofilm infections in medicine was initiated by Høiby's observations of *Pseudomonas aeruginosa* in sputum and lung tissue from chronically infected cystic fibrosis patients. The term biofilm was

introduced into medicine in 1985 by Costerton. During the following decades it became obvious, that biofilm infections are widespread in medicine and odontology, and their importance is now generally accepted.[4]

There are many risks for biofilm formation in humans such as attachment on an artificial surface in the host, or exposure to sublethal doses of antibiotics.[5-7]

Biofilms usually cause chronic infections. Many bacterial species form biofilms, and as biofilms progress they become more antibiotic resistant and difficult to culture.[8] Persistent infectious conditions not showing improvement with adequate antibiotics therapy and inflammatory nodules that recur after resolution may also indicate a biofilm.[8]

Biofilms are small size in vivo.[9, 10] Therefore, the search for biofilms in clinical samples may be difficult, and may result in false-negative results if the samples are not representative of the focus of the biofilm infection.

Biopsy tissues are considered the most reliable samples to reveal biofilm in wounds. The use of swabs to collect biofilm samples from the wound surface is considered an inadequate method.[11]

Confocal laser scanning microscopy and scanning electron microscopy are the most appropriate to reveal biofilms structures but they are not available for routine. Specific microscopic identification of the biofilm microorganisms in tissue biopsies or swabs can be done by means of species-specific fluorescence in situ hybridization probes and fluorescence microscopy.[12]

In 2014, the European Society for Clinical Microbiology and Infectious Diseases (ESCMID)

developed general features of clinical and laboratory indications for diagnosis of biofilm infections[13] as below

- Clinical signs of infection e.g. the classical but frequently low-grade inflammatory reactions tumor, rubor, odor, loss of function and sometimes low-grade fever
- Medical history of biofilm-predisposing condition (e.g. implanted medical device, cystic fibrosis)
- Persisting infection lasting >7 days (this is unspecific, and other reasons are frequent such as resistance to the antibiotics used)
- Failure of antibiotic treatment and recurrence of the infection (particularly if evidence is provided that the same organism is responsible on multiple time points)
- Documented evidence/history of antibiotic failure
- Evidence of systemic signs and symptoms of infection that resolve with antibiotic therapy, only to recur after therapy has ceased.

Microbiological diagnostics:

- Microscopic evidence from fluid/tissue samples obtained from the focus of the suspected infection
    - Microscopy revealing the presence of microbial aggregates and biofilm structure (smear or fluid sample, but ideally from tissue sample if possible)
    - Microscopy revealing evidence of microbial aggregates co-localized with inflammatory cells
    - Microbiological evidence of aggregated microorganisms consistent with infectious etiology

- Positive culture/non-culture-based techniques (PCR) of fluid or tissue sample
    - Culture-based identification of microbial pathogen (MALDI-TOF)
    - Presence of mucoid colonies or small colony variants of P. aeruginosa in culture positive samples—which may indicate antibiotic recalcitrance)
    - PCR, quantitative PCR or multiplex PCR positive results for pathogen associated with infection (e.g. Staphylococcus aureus with implant, Pseudomonas aeruginosa with cystic fibrosis)
    - fluorescence in situ hybridization positive results for known pathogen showing aggregated microorganisms
    - Non-culture-based identification of microbial pathogen (pyrosequencing, next-generation sequencing).

This patient presented with recurrent inflammatory plaque on the right cheek after previous hyaluronic acid filler injection not responding to antibiotic treatment. The biopsy revealed suppurative granuloma. The tissue culture reported *Pseudomonas aeruginosa* and repeated pus culture four months after receiving ATBs reported the same organism. Therefore according to the ESCMID 2014 guideline[13], the diagnosis in this patient should be biofilm due to *Pseudomonas aeruginosa* infection as a complication of hyaluronic acid filler injection.

Biofilm infections are difficult to treat. Antibiotic treatment is the first step in treating biofilm. Even if the culture is negative antibiotics should be initiated with a

broad-spectrum agent (quinolone) such as ciprofloxacin 500 mg bid and a macrolide such as clarithromycin extended release 500 mg bid for 4–6 weeks.[14] Intralesional steroids should not be used before antibiotics. Removal of the filler by a 16-gauze needle with syringe and negative pressure or by hyaluronidase should be used to reduce the inflammatory potential of the biofilm.[14] If the indurated area persists treatment with 5-FU injection, up to 50 mg/mL (0.5 cc), alone or in combination with steroids should be initiated and repeated every 2–4 weeks.[14] If induration persists despite 5-FU treatment further options include laser lysis such as intralesional introduction of an optic laser microfiber to produce subsequent heat resulting in a theoretical decrease of bacterial counts on the biofilm, and liquefaction of the filler microparticles. Radiofrequency heating has also been utilized in the same fashion. These minimally invasive techniques could be an intermediate step before attempting surgical excision which should be used as a last option.[14]

Measures to avoid biofilm infection include thorough cleansing of the face before injection, avoid injecting through oral/nasal mucosa and injecting over previous filler or into traumatized tissue.[15] At present there is no evidence or regimen to support the use of systemic antimicrobial agents to prevent biofilm infections in tissue fillers.[13]

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