sufficient in *E. cecorum* native valve endocarditis if treatment is commenced early.

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Actinomycosis canaliculitis

To the Editor,

Lacrimal canaliculitis is a rare medical condition, commonly undiagnosed or misdiagnosed for long periods of time in up

to 60% of patients.¹ It is an infection of the canaliculus of the lacrimal drainage system, often symptomatic with chronic tearing of the eye and irritation in the medial portion of the infected eyelid.² It accounts for only 1.2–2.0% of all lacrimal disease.^{1,3} Examination reveals erythematous protrusion of the lacrimal punctum, erythema of the surrounding skin and mucopurulent discharge.² *Actinomyces*, belonging to the phylum Actinobacteria, are Gram-positive non-acid fast bacilli that can have a fungal mycelia-like appearance.⁴ First described in 1854 by Von Graefe,⁵ *Actinomyces*, typically *Actinomyces israelii*, is the most common aetiological causative organism of canaliculitis, although several organisms have been shown to cause the disease.² *Actinomyces* are commensal flora of the oral cavity, likely causing canaliculitis by either regurgitation or direct inoculation by saliva.^{1,2} Diagnosis of the actinomycotic infection is confirmed by culture, with *Actinomyces israelii* expressed concretions often having unique yellowish 'sulphur granules' composed of aggregates of filamentous branching microorganisms^{2,3} and showing characteristic stellate radiations of the organism.² Due to its rarity, actinomycosis canaliculitis can present a diagnostic challenge, leading to unnecessary suffering for patients, making it essential to recognise the condition early.²

An 88-year-old female presented to the treating ophthalmologist for a second opinion with a 4-year history of tender, injected conjunctiva associated with purulent discharge with episodes lasting for a period of 2–3 weeks. Previously diagnosed with left blocked nasolacrimal duct in 2014, the patient remained symptomatic after surgery and multiple regimes of oral flucloxacillin and chloramphenicol ointment. Medical history included primary open angle glaucoma managed with latanoprost, timolol and brinzolamide, type 2 diabetes managed with metformin and insulin, and hypothyroidism treated with thyroxine. Previous ophthalmic procedures were left phacoemulsification and insertion of intraocular lens and left dacryocystorhinostomy. Visual acuities were right eye 6/9–1 pinhole (Ph) 6/5–1 and left eye 6/60 Ph 6/18 with bilateral intraocular pressures of 15 mmHg. Anterior segment examination was unremarkable. A tender erythematous lump was present in the left medial canthus inferiorly, with exudate in punctum (Fig. 1).

Fig. 1 Pouting punctum, discharge and medial injection of conjunctiva, typical of canaliculitis.

Canaliculitis was initially diagnosed and treated with canaliculotomy performed at the lid margin. The puncta was dilated with a probe passed along the inferior canaliculitis. A small curette was used to remove all the debris from the canaliculus and break down any loculations. The wound was then irrigated with penicillin drops and eye padded overnight. The contents were sent for microbiological and histopathological examination. Heavy growth of *Streptococcus intermedius* was identified, which was sensitive to tetracycline, clindamycin and penicillin. Microscopy showed Gram-positive bacilli resembling *Actinomyces* species, displaying typical branching and filamentous organisms (Fig. 2). Diagnosis was made on microscopic findings. The laboratory did not culture specifically for actinomycoses. Post-operatively the patient was treated with topical tobramycin drops four times daily for one month, with complete resolution at 6 weeks review and no recurrence at her annual review. Tobramycin was selected as the topical agent in this case due to the patient's rural location and lack of manufacturing facilities for penicillin drops.

Canaliculitis is an uncommon inflammation of the proximal lacrimal drainage system that is frequently misdiagnosed. Commonly divided into primary and secondary canaliculitis. Primary canaliculitis is a result of an infection of the canaliculus and proximal lacrimal duct. Secondary canaliculitis is often seen as a complication of punctal or intercanicular plug insertion for treatment of dry eye. Actinomycosis canaliculitis is a chronic infection of the canaliculus that is commonly under-diagnosed; it frequently mimics chronic conjunctivitis and should be considered for all patients with discharge with no known aetiology.⁵ *Actinomyces israelii*, a commensal anaerobic Gram-positive rod, is the most common pathogen responsible for chronic canaliculitis.² Because *Actinomyces* culture success is reported between 25–54%, often isolated as part of a polymicrobial infection, it may be difficult to culture without use of selective media (due to overgrowth of faster growing flora) and the right cultivation conditions (anaerobic).^{3,5,6}

The annual incidence of canaliculitis is 0.59 per 100,000 population in the 40–59-year age group and 1.37 per 100,000 population in the 60–79-year age group.³

Canaliculitis usually presents with discharge and epiphora (93.8%),⁶ eyelid oedema, erythema of the canaliculus, and a 'pouting' punctum, a classic diagnostic finding, present in 75% of cases.^{3,6} This condition is often unilateral, affecting the inferior canaliculus in over 71% of cases and

Fig. 2 *Actinomyces* species identified through Gram staining of extracted contents.

demographically appears most commonly in middle-aged and elderly females, believed to be due to hormonal changes in menopause decreasing tear production and protection against infections.^{3,6,7}

Actinomycosis canaliculitis can be diagnosed with a careful clinical history and examination; without correct diagnosis and treatment the condition will recur.³ Given the difficult nature of diagnosis, dacryocystography may be performed in certain cases to rule out canalicular dilatation, irregularity or filling defects that could present with similar symptomatology as canaliculitis.⁶ Actinomycosis canaliculitis has a median symptomatology of 9 months and median diagnostic delay of 6 months,⁸ however these figures vary dramatically amongst studies from 4.5 months to 34 months.⁶ Despite initial improvement, conservative medical treatment with topical antibiotic drops alone fails to eradicate the infection as it is situated deep within the canalicular wall and drops often can't penetrate through concretions and debris. Anand *et al.* emphasised the importance of early diagnosis as repeated direct pressure to extract the mucopurulent discharge, although effective initially, can lead to nasolacrimal duct obstruction over time.⁹

Typically, treatment requires removal of the canalicular material and concretions, accomplished either by canaliculotomy plus curettage or aggressive curettage of the canaliculus with irrigation.^{2,3} Canaliculotomy involves the insertion of a probe into the canaliculus with an incision made through the adjacent conjunctiva into the dilated canaliculus and a small curette used to remove any concretions.¹ Recent studies have documented that repeat treatment was required in 6.7% of cases when treatment consisted of curettage alone.^{6,10} Studies suggest curettage performed with canaliculotomy under local anaesthetic is the current gold standard treatment, as it doesn't disrupt the canalicular or lacrimal pump systems and removes the concretions.⁶ The probability of recurrence ranges from 26–100% if concretions are present, and remains an important step to reduce risk of recurrence.⁶

Topical penicillin antibiotics drops are frequently used in cases of canaliculitis where *Actinomyces* has been isolated in combination with local irrigation. This therapy has been reported to reduce the risk of recurrence and repair the canaliculus.² Some authors advocate long-term systemic penicillin consisting of intravenous and/or oral therapy and recommend up to 6–12 months of treatment depending on the severity of the disease, possibly due to the ability for *Actinomyces israelii* to form biofilms.⁴ Full recovery will only be achieved after complete elimination of the infection. Actinomycosis canaliculitis has a good prognosis if diagnosed and treated accordingly.

This case highlights the typical features reported in the literature with long delay in diagnosis often leading to inappropriate investigations and treatment that ultimately fail to resolve the symptoms. Aids to diagnosing actinomycosis canaliculitis include a high clinical suspicion in the presence of a chronic relapsing discharge, medial conjunctival injection and a pouting punctum with pus. Treatment should include canaliculotomy with curette to remove all concretions and break down loculations, with lavage of penicillin drops and post-operative topical penicillin.

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SARS-CoV-2 N-gene mutation leading to Xpert Xpress SARS-CoV-2 assay instability

To the Editor,

Real-time reverse transcription polymerase chain reaction (RT-PCR) assays have formed the backbone of COVID-19 diagnosis since the start of the SARS-CoV-2 pandemic. Assay performances have remained relatively stable over the course of the pandemic to date, but any genomic variants in the primer binding or probe regions of assays have the potential to compromise performance. One notable example is the spike protein H69/70del mutation, associated with the emergence of the alpha variant (B.1.1.7).¹ H69/70del results in false negative S-gene results in the Applied Biosystems TaqPath COVID-19 assay (ThermoFisher Scientific, USA), but the two other gene targets of the assay are not affected. This altered assay performance became a proxy for detection of the alpha variant.¹ Surveillance for mutations causing diagnostic assay failures for specific gene targets is crucial,

especially if only one gene target is used in a particular assay.

There have been relatively few COVID-19 cases in Australia relative to global case numbers, but there is currently (1) a sustained outbreak of COVID-19 infections stemming from community transmission of the delta variant of concern (VOC) in June (Winter) 2021, and (2) rising case numbers of the omicron VOC since early December 2021. The Xpert Xpress SARS-CoV-2 assay ('GX'; Cepheid, USA) is one of several diagnostic assays currently used for detection of SARS-CoV-2 in Australia and globally. The GX assay has two targets within the envelope (E) and nucleocapsid (N) genes of SARS-CoV-2. If a sample is positive, the expected behaviour is for both gene targets to be detected with similar cycle threshold (Ct) values.¹ A change in the performance of the GX assay was recently observed across multiple assay cartridges from different cartridge lots, with large differences in cycle threshold (Ct) values between the E and N gene targets in a subset of delta SARS-CoV-2 samples. Samples were classified as possessing an altered GX profile (GX^{alt}) if there was a >3 Ct value difference between the E and N gene targets of the assay. A single category change to presumptive positive was adopted when a sample was E-gene positive (Ct value >30) but was N-gene negative.

The altered GX profile was first observed in August of 2021 in 9.5% (10/105) of cases, increasing to 20% (18/88) and 15% (3/20) of cases in September and October, respectively. Retrospective analysis of all positive cases from July 2021 confirmed that 0% (0/110) of cases exhibited the altered GX profile (GX^{alt}). Although the altered GX profile was defined as any sample with a greater than three Ct value difference between E-gene and N-gene targets, the differential was often far greater (median 10 Ct, IQR 9–11 Ct). Throughout the period of testing, no false negatives were observed.

Whole genome sequencing of isolates was undertaken as part of routine genomic sequencing, as previously described.² A candidate causative SNP for the GX^{alt} profile was identified at genomic position 29179 of the SARS-CoV-2 genome (G29179T) through comparative analyses. This SNP was not observed in any non-GX^{alt} samples.

Most commercial assays use closed-source, proprietary primer sequences. However, definitively linking SNPs to altered assay performance requires knowing the primer sequences, or precise target locations on the reference genome. Accordingly, we cloned the GX assay primers into a pcDNA3.1V5 His plasmid backbone (#K480001; ThermoFisher Scientific Australia), then sequenced purified clone products using Sanger sequencing. Thus, we confirmed that the G29179T point mutation is located 5 nt upstream from the 3' end of the N-gene target region of the GX assay (Fig. 1). In correspondence, Cepheid agreed that 'the data appear to support this (altered assay performance)' (personal communication).

At the time of carrying out this study, the G29179T SNP had not yet been linked to assay instability. However, during the editorial process, three studies from Western Australia, South Korea, and the United States of America have found that the G29179T SNP leads to N-gene target detection failure in the GX assay,^{3–5} strongly and independently