Donovanosis (*Klebsiella granulomatis*)

Laboratory case definition

The Public Health Laboratory Network (PHLN) has developed standard case definitions for the diagnosis of key diseases in Australia. This document contains the laboratory case definition for *klebsiella granulomatis*.

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1 PHLN Laboratory Definition

1.1 Condition:

Donovanosis

1.2 Tests

1.2.1 Definitive Criteria

Detection of the histological characteristics of donovanosis in a tissue biopsy specimen.

1.2.2 Suggestive Criteria

PCR detection of *K. granulomatis*-specific sequences in tissue biopsies, smears, swabs or paraffin-embedded tissue blocks.

1.3 Special Considerations

- The laboratory diagnosis by histology or PCR must be supported by appropriate clinical and epidemiological evidence (ie. the patient has a lesion consistent with donovanosis and has travelled to or resided in an endemic community).
- PCR detection of *K. granulomatis*-specific sequences may be upgraded to a definitive diagnosis in the near future when the specificity of the assay is defined in on-going evaluations.

- Microscopic examination of tissue smears has not been included in this laboratory case definition because the technique is insensitive and probably highly non-specific when performed by an inexperienced microscopist.

2 Introduction

Donovanosis is a chronic destructive genital ulcerative disease (GUD).[1-3] The infection is presumably sexually transmitted and is a disease of disadvantage being prevalent among Australian Aborigines and in low-resource countries such as India, southern Africa, South America, and Papua New Guinea. The incubation period is usually 1-4 weeks but can extend to one year. The disease begins as single or multiple subcutaneous nodules that soon erode leaving beefy-red, fleshy, relatively-painless lesions that bleed on contact. This presentation is the most common ulcerogranulomatous form of the disease. Other presentations are described as hypertrophic (ie. an ulcer with a raised cauliflower-like edge), necrotic (ie. a deep ulcer with extensive tissue destruction), or sclerotic (ie. typified by excessive scar tissue formation). Complications of donovanosis include genital destruction, disseminated disease, and secondary skin malignancy.

As with other GUDs, an association with HIV transmission has been postulated and has been the driving force behind an increased interest in this neglected disease. Recently, a diagnostic PCR has been developed to detect the causative organism, *Klebsiella granulomatis*, which cannot be cultivated using routine microbiological techniques, and azithromycin has proven to be an effective and convenient treatment. These breakthroughs raise the possibility of eradicating donovanosis from Aboriginal communities. However, any eradication campaign will require reliable laboratory diagnostics because the accuracy of clinical diagnosis alone has been reported as only 63% in men and 83% in women.[4]

3 Laboratory Diagnosis Of Donovanosis

3.1 Histological examination of tissue biopsies

Histological examination of tissue biopsies provides the most reliable means of diagnosing donovanosis. Histology will also detect malignancy, which must be considered in the differential diagnosis of a non-healing genital lesion. After gentle washing with normal saline, punch or snip biopsies can be collected from an area of active ulceration. The biopsy should be placed immediately in 10% formalin/saline solution for histological processing. Excessive drying of the specimen prior to fixation can cause the mononuclear cells containing the causative bacilli (Donovan bodies, DBs) to rupture making detection of the DBs more difficult.

A variety of stains can be used.[1-3,5,6] Haematoxylin and eosin stains however do not demonstrate DBs well and should be avoided. An overnight Giemsa stain or a silver stain are probably the preferred methods. Alternative stains include a modified Giemsa (Rapidiff), Leishman's or Wright's stain.

3.2 Suitable Specimens

Tissue.

3.2.1 Test Sensitivity and Specificity

The performance characteristics of histological diagnosis for donovanosis are poorly defined. They depend on the disease manifestation (ie. whether the genital lesion contains a high or low burden of DBs), the quality of the biopsy, and the experience and patience of the histopathologist. Donovan bodies may be demonstrated in 60-80% of lesions and examination by an expert histopathologist has a sensitivity of 95-100% compared with clinical diagnosis.[4,5]

3.2.2 Suitable Test acceptance/validation criteria

Certain histological characteristics are typical of donovanosis:

- a massive cellular infiltrate comprising mainly plasma cells with a diffuse sprinkling of polymorphs but a paucity of lymphocytes
- large mononuclear cells (20-90 nm) with an eccentric nucleus and cyst-like compartments containing DBs (ie. cells of Greenblatt)
- epithelial proliferation seen as acanthosis, epidermal microabscesses, elongation and intercommunication of the rete pegs, or pseudoepitheliomatous hyperplasia.

Appropriate clinical and epidemiological data must support the diagnosis because similar histological features may be present in rhinoscleroma, a chronic nasal infection caused by *K. rhinoscleromatis*.

3.2.3 Suitable internal controls

Nil

3.2.4 Suitable external QC program

Not currently available

3.3 Microscopic examination of tissue smears

Microscopic examination of tissue smears provide a rapid convenient but insensitive method of diagnosing donovanosis.[1,3,7] Punch or snip biopsies may be smeared on a glass slide. Alternatively, after removal of superficial debris with a swab, a second swab may be rolled across the lesion then smeared on a slide. Slides may also be pressed directly on the lesion to obtain an impression smear. Smears are air dried and heat fixed before staining. Modified Giemsa stains (RapiDiff) have been used routinely on smears in STD clinics in South Africa.[7]

3.3.1 Suitable Specimens

Slides prepared from tissue, a swab specimen, or impression smear.

3.3.2 Test Sensitivity and Specificity

The performance characteristics of tissue smears are poorly defined and are very dependant on the experience of the microscopist. O'Farrell et al[7] have reported that DBs were seen in 23 (38.3%) RapiDiff-stained smears collected from 60 patients with clinically-diagnosed donovanosis. No specificity data is available. However, specificity is likely to be low when inexperienced microscopists are asked to detect bacilli in genital lesions.

3.3.3 Suitable Test acceptance/validation criteria

Stained smears are examined by light microscopy under oil immersion (1000 x magnification) for Donovan bodies, which appear as pleomorphic bacilli (1-2 x 0.5-0.7 mm) with or without a capsule. Bipolar chromatin densities in the bacilli produce the classic “closed safety pin” appearance.

3.3.4 Suitable internal controls

Nil

3.3.5 Suitable external QC program

Not currently available

3.4 Polymerase chain amplification

Polymerase chain amplification has provided an alternative non-invasive investigation for donovanosis that may be as sensitive and specific as histology. The technique relies on the detection of two unique base changes in the *phoE* (phosphate porin) gene of *K. granulomatis* that eliminate *HaeIII* restriction sites. Detection of these base changes after amplification has been achieved by sequencing[8], restriction digestion and gel electrophoresis,[9] and by colorimetric enzyme immunoassay.[10]

3.4.1 Suitable Specimens

Tissue, swabs, fixed wax embedded tissue blocks.

3.4.2 Test Sensitivity and Specificity

The performance characteristics of the donovanosis PCR remain undefined. Only limited descriptions and evaluations of the PCR have been published[8-10] or performed.[11] These studies have used a variety of specimens (ie. biopsies, tissue blocks and swabs) and a range of techniques (eg. colorimetric PCR, nested PCR). The “gold standard” for comparison in these studies has also varied between a clinical diagnosis and histological confirmation. Thirty-three (80.5%, 95% CI 65.1-91.2%) of 41 donovanosis-positive specimens were detected by PCR if the results of these PCR studies[8-11] are pooled. However, the methodological discrepancies between these studies invalidate the combination of their results. Further large evaluations of the donovanosis PCR are urgently required but preliminary results do suggest that PCR may be as sensitive as histology.

Published studies describe specimens from nine non-donovanosis genital lesions that have produced negative results.[8-10] The specificity of the donovanosis PCR obviously awaits further evaluation, including investigation of a range of enterobacteria and other organisms to ensure that no other bacterium possesses a similar *phoE* gene lacking the *HaeIII* site. However, the donovanosis PCR is likely to be highly specific.

3.4.3 Suitable Test acceptance/validation criteria

A technical collaborative group involving the Clinical Virology Research Unit (CVRU), SASVRC, Brisbane, and PathCentre, Perth, has been established to optimise the donovanosis PCR in Australia. This group proposes to use in-run positive and negative controls (non-template controls), and an

endogenous retroviral sequence (to ensure that the specimen contains amplifiable DNA free of PCR inhibitors).

3.4.4 Suitable internal controls

Positive controls may be wild-type DNA or a synthetic standard (ie. a plasmid containing the amplified product)

3.4.5 Suitable external QC program

The collaborative group is establishing an external QC program that will be coordinated by the CVRU at SASVRC.

3.5 Alternative diagnostic methods

Culture and serological investigations remain research tools and will only be reviewed briefly here. The causative organism was cultured on 14 occasions between 1942-1962 in embryonated chick eggs.[3] Cell culture techniques have been applied recently to the cultivation of *K. granulomatis*. A South African group has cultivated the organism in human monocyte cultures while the Menzies School has successfully used HEp-2 cells in a technique identical to that used to culture *Chlamydia*.[12] However, difficulties in transportation of viable specimens and problems with bacterial overgrowth confound the routine use of culture for detection of donovanosis.

Seroreactivity of donovanosis patients with cultured antigen was used in the 1940s to confirm that the causative organism had been isolated in the embryonated chick eggs. Serological diagnosis of donovanosis was then formally employed by Maddocks et al[13] in PNG in the 1970s. In complement fixation tests (CFTs) using *K. granulomatis* antigen obtained from US researchers, 27 (96.4%) of 28 DB-positive cases were positive as were 9 (64.3%) of 14 clinically-suspected cases who were DB-negative. Less sensitive results were obtained when *K. pneumoniae* was used as the antigen. None of 36 negative controls reacted in these CFTs. Furthermore, Freinkel et al[14] working in South Africa have performed donovanosis serological testing using an indirect immunofluorescence technique with dewaxed protease-treated DB-positive tissue sections as the antigen. Their technique had a sensitivity of 88-100% and specificity of 98-100% when testing sera from 55 histologically-confirmed cases and 781 controls. These investigations suggest that serology may be an effective diagnostic for donovanosis if an antigen source were available and an assay developed and properly evaluated.

4 Summary

Histological examination of tissue biopsies remains the “gold standard” for donovanosis diagnosis but is an invasive test. Preliminary investigations suggest that PCR on swab or tissue specimens may have similar performance characteristics as histology. However, until the results of further evaluations are available, donovanosis PCR must be considered as an alternative test when biopsy is not possible. Though of some utility as a rapid test when used by experienced operators, tissue smears cannot be generally recommended as having adequate sensitivity or specificity. Culture and serology are research tools only at present.

5 Acknowledgments

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5 References

1. Bowden F. Donovanosis. In: S. Morse, K. Holmes, R. Ballard (ed.) Atlas of STDs and AIDS. Mosby International (in press).
2. Hart G. Donovanosis. Clin Infect Dis 1997; 25: 24-32.
3. Richens J. The diagnosis and treatment of donovanosis (granuloma inguinale). Genitourin Med 1991; 67: 441-452.
4. O'Farrell N, et al. Genital ulcer disease: accuracy of clinical diagnosis and strategies to improve control in Durban, South Africa. Genitourin Med 1994; 70: 7-11.
5. Sehgal VN, et al. The histopathological diagnosis of donovanosis. Br J Vener Dis 1984; 60: 45-47.
6. Sehgal VN, et al. Tissue section Donovan bodies – identification through slow-Giemsa (overnight) technique. Dermatologica 1987; 174: 228-231.
7. O'Farrell N, et al. A rapid stain for the diagnosis of granuloma inguinale. Genitourin Med 1990; 66: 200-201.
8. Bastian I, et al. Amplification of Klebsiella-like equences from biopsy samples from patients with donovanosis. Clin Infect Dis 1996; 23: 1328-30.
9. Carter J, et al. Diagnostic polymerase chain reaction for donovanosis. Clin Infect Dis 1999; 28: 1168-69.
10. Carter J, et al. A colorimetric detection system for Calymmatobacterium granulomatis. Sex Transm Inf 2000; 76: 134-136.
11. Harnett G, Smith D (PathCentre, Perth, WA), pers. comm.
12. Carter J, et al. Culture of the causative organism of donovanosis (Calymmatobacterium granulomatis) in Hep-2 cells. J Clin Microbiol 1997; 35: 2915-7.
13. Maddocks I, et al. Donovanosis in Papua New Guinea. Br J Vener Dis 1976; 52: 190-196.
14. Freinkel A, et al. A serological test for granuloma inguinale. Genitourin Med 1992; 68: 269-72.