

The laboratory diagnosis of gonorrhoea and syphilis infection

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(Accepted 19 October 2003)

Journal of Family Planning and Reproductive Health Care 2004; **30**(2): 126–127

Background: epidemiology and clinical features

Following the decline in incidence of gonorrhoea and syphilis in the mid-1980s, there has been a resurgence in cases of both infections during the late 1990s. Since 1994 there has been an ongoing rise in cases of gonorrhoea, with a 9% increase between 2001 and 2002 in the UK. Men who have sex with men (MSM) and black ethnic groups, mainly in urban areas, are disproportionately affected.¹ There is also evidence of increased antimicrobial resistance in worldwide *Neisseria gonorrhoeae* isolates, especially to ciprofloxacin and azithromycin.² A recent study in the UK demonstrated a prevalence of ciprofloxacin resistance in *N. gonorrhoeae* of 9.8%, a two- to three-fold rise from 2001 to 2002, irrespective of overseas sexual contact, sex or area of residence.³ In women, infection is asymptomatic in about 50% of cases, but if symptomatic the commonest symptom is of altered vaginal discharge. In men a purulent urethral discharge is commonly present. Pharyngeal and rectal infection occurs in both sexes and is frequently asymptomatic.

Syphilis is caused by the spirochaete bacterium, *Treponema pallidum*. It is systemic from the outset, infectious in the early stages, and if untreated has a chronic relapsing course. Clinical manifestations of the infection are highly variable and from Victorian times, when up to one in nine Europeans were affected, it has been known as 'the great pretender'. It is transmitted by sexual contact or vertically from mother to child during pregnancy. Initially, a painless, indurated ulcer known as a 'chancere' develops at the site of inoculation. This resolves and may be followed by an erythematous psoriatic rash and orogenital ulcers, mucous patches or condylomata lata (condylomatous lesions in the genital area), which are also transient and often accompanied by systemic symptoms. Untreated the infection becomes latent and neurological, cardiovascular and other sequelae may occur up to 30 years after initial infection in up to 40% of untreated individuals.⁴ If syphilis develops during pregnancy, intrauterine death, stillbirth or congenital syphilis frequently occur. From 1998 to 2002, an 803% increase in cases of early syphilis was observed in the UK, mainly comprised of localised outbreaks in MSM.¹ Outbreaks have been reported from a variety of locations but the two largest and ongoing outbreaks are in London and Manchester in the UK.

Diagnosis of *N. gonorrhoeae* infection

Specimen collection

For women a swab should be taken from the endocervix (rotating it there for a few seconds) and the urethra. Rectal samples should also be taken depending on the sexual history, if a partner has gonococcal infection, or if she is

symptomatic at the site. Pharyngeal samples should be taken if she has practised receptive orogenital sex. For men a urethral swab plus a rectal and/or pharyngeal swab should be taken as indicated by the sexual history and symptoms. The swab should be rolled onto a glass slide to be air-dried and Gram-stained, and then either inserted in transport media or inoculated directly to an agar plate which is suitable for gonococcal culture.

Microscopy

In genitourinary medicine (GUM) clinics microscopy is performed on site as a near-patient test, allowing rapid diagnosis. Under light microscopy (×1000) the typical intracellular Gram-negative diplococci are observed in polymorphonuclear leucocytes. For male urethral samples this has a sensitivity of 89–96%, whereas for female endocervical smears it is only 31–51%.⁵ Microscopy is not appropriate for pharyngeal samples. Although of lower sensitivity, microscopy of rectal smears is of use (particularly in symptomatic cases) to allow rapid diagnosis.

Culture

Culture is a readily available, cheap, sensitive and specific test that also allows sensitivity testing to be performed. Sensitivity testing is essential for the early detection of changes in sensitivity patterns and enables appropriate changes in antibiotic therapy to be initiated. Culture is the diagnostic method of choice in most cases including medico-legal cases such as child abuse and sexual assault. Results are generally available within 72 hours. Selective and non-selective lysed blood agars, typically based on a modified New York City media, are often used since a small proportion of gonococci may be sensitive to vancomycin in selective media.⁶

Nucleic acid detection and amplification tests

Several assays are available including tests able to detect *N. gonorrhoeae* in urine, vaginal, urethral and cervical samples.^{7,8} Such assays are highly sensitive (95%) and specific (99%) in symptomatic patients from areas of high prevalence. For this reason, in low prevalence areas positive cases require confirmation with a second assay.

The Gen-probe assay uses a labelled DNA probe to detect *N. gonorrhoeae* ribosomal RNA in specimens. It is not a nucleic acid amplification test as it does not amplify the target. Sensitivity is around 85%, and with a confirmation assay specificity approaches 98%.

An important limitation of these tests is that they do not allow sensitivity testing which, as discussed earlier, is of increasing importance. Therefore, culture confirmation and sensitivity testing in those patients identified with

gonorrhoea by nucleic acid assay tests is essential. If culture confirmation is not possible then it is crucial to perform a test-of-cure following treatment, ideally using a culture method.

Diagnosis of syphilis

Demonstrating the presence of T. pallidum

T. pallidum cannot be cultured in vitro, but the presence of the bacterium in the infectious lesions of primary or secondary syphilis may be demonstrated. Dark-field microscopy involves sampling the lesion's exudates and immediate dark-field microscopy demonstrates the motile treponemes. This requires expertise most often available in GUM clinics and does allow immediate near-patient diagnosis. It is, however, unsuitable for oral and rectal lesions since visual differentiation between oral and bowel spirochaetes is not possible. An alternative method, namely fluorescent microscopy, involves air-drying the slide and staining it with rabbit fluorescent anti-*T. pallidum* antibody and then examining the slide for spirochaetes. As this method is specific for *T. pallidum* it is suitable for oral and rectal lesions, but requires expertise and is not widely available. Polymerase chain reaction can be performed on swabs taken from possible syphilitic lesions and placed in transport media. This is a sensitive and specific test for *T. pallidum*, and has recently been shown to be an important adjunct to treponemal serology in diagnosing infectious syphilis during the current UK outbreaks,⁹ although it is not yet widely available.

Serological tests for syphilis

Following infection with *T. pallidum*, antibodies are produced to many of the treponemal antigens. The intensity of reaction reflects clinical symptoms, and by the time symptoms are evident all but 10–15% of those individuals with early primary syphilis will have immunoglobulin G (IgG) and M (IgM) antibodies. Antibody responses decrease with time from infection and following treatment, allowing the response to treatment to be monitored. The antibody response to infection is not protective, and re-infection may occur with a corresponding increase in antibody titres.

Treponemal tests detect antibody specific to *T. pallidum*. They include enzyme immunoassay (EIA), *T. pallidum* haemagglutination assay (TPHA), *T. pallidum* particle agglutination test (TPPA) and fluorescent treponemal antibody with absorption test (FTA-ABS). EIA assays may test for anti-IgG alone or IgG and IgM in combination. They have high specificity, sensitivity and are suited to automation, and so are increasingly being used as the initial screening test. If the EIA is positive it is confirmed with another treponemal test, usually the TPPA or TPHA since both tests are very sensitive and specific, particularly the TPPA. A non-treponemal test is then performed to assist in diagnosing the stage of infection.

Non-treponemal tests include the rapid plasma regain test (RPR) and venereal diseases research laboratory test (VDRL). These tests detect non-specific treponemal antibody, and quantification of the response allows assessment of stage of infection and response to treatment as discussed above. A high RPR or VDRL titre is indicative of recent, active infection and a fall in titre four-fold following treatment is considered a good response to therapy.⁴ False-positive results may occur, for example, during pregnancy, febrile illnesses and in those with autoimmune conditions. This limits the use of non-treponemal tests as initial screening tests and underlines the fact that no serological test should be used for diagnosis in isolation. In addition, when very high levels of non-treponemal antibody are present a false-negative result, the

'prozone phenomenon', may occur. If this situation is suspected clinically then the sample should be diluted and retested, and this is one reason why sending full clinical details to the laboratory with the sample is crucial.

Summary

Following a decline in prevalence during the 1980s and early 1990s, gonorrhoea and syphilis infections are once again posing a threat to public health. In addition, the antibiotic sensitivity pattern for gonorrhoea appears to have changed with an increased prevalence of resistance. Both syphilis and gonorrhoea appear to disproportionately affect MSM and black ethnic minorities, and are concentrated in urban areas. Their diagnosis requires microbiological tests to be performed appropriately, and a rapid diagnosis can often be provided in GUM clinics using near-patient microscopy. Early diagnosis and effective, rapid treatment is crucial in limiting the morbidity for the affected individual and the public health risks resulting from the spread of infection.

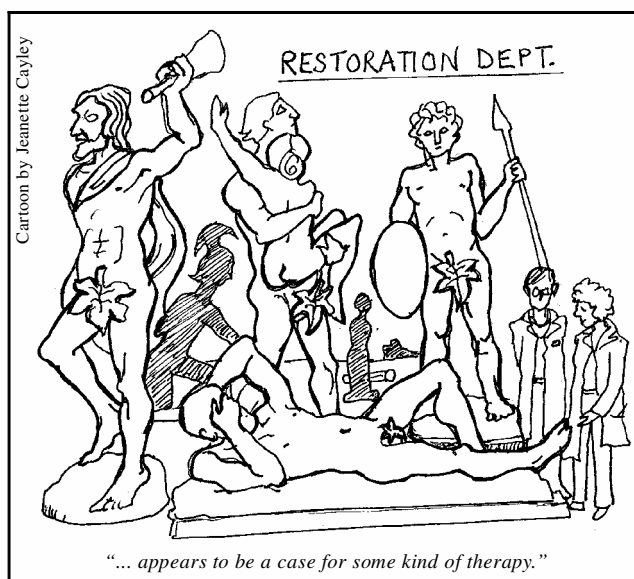
Statements on funding and competing interests

Funding. None identified.

Competing interests. None identified.

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"... appears to be a case for some kind of therapy."