The many facets of *Haemophilus ducreyi* infection: a multidisciplinary look at an evolving pathogen

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Dr. Morris M. Gollow (1925 – 2011)

- Emigrated to WA in 1956 from the UK
- Inaugural President of the Australasian College of Venereologists (1988-1991)
- Awarded the Member of the Order of Australia for services to Medicine, particularly in Venereology
- Professor Yvonne Cossart presented the first Gollow lecture in 1990, entitled ‘Molecular Biology in Medicine’
- This is the 23rd Gollow lecture - the first dedicated to a bacterial STI

Introduction

- Chancroid (soft chancre, ulcus molle) is caused by the fastidious Gram-negative bacillus *Haemophilus ducreyi*
- Associated with poverty, sex work, travelling carnivals and commonly affected soldiers in World War I

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History

- First described by the ancient Greeks
- Léon Bassereau distinguished chancroid from syphilis in 1852 using ‘confrontation’
- Augusto Ducrey described the unique morphology of *H. ducreyi* and the phenomenon of autoinoculation in 1889
- Benzacon first isolated *H. ducreyi* in 1900
- Hammond and others developed selective media in the 1970s
- Orle et al. published the first M-PCR for GUD pathogens in 1996

Clinical Features

- Infection commences at sites of microabrasions
- Tender erythematous papules develop at 4-7 days - may evolve into the pustular stage and then the ulcerative stage
- Human studies of experimental *H. ducreyi* infection have demonstrated that a delivery dose of +/- 30 CFU results in:
  - papule formation rate of 95%
  - pustule formation rate of 69%
- Characteristically, the ulcers of chancroid are multiple, painful, purulent and deep with ragged undermined edges and evidence of bleeding points in the base
- Associated with lymphadenopathy and bubo formation
Epidemiology

- Previously highly prevalent cause of GUD, particularly in several resource-poor countries of Africa, Asia, Latin America and the Caribbean
- Higher prevalence among certain groups of individuals:
  - lower socioeconomic groups
  - female sex workers
  - male partners of sex workers
  - sex with crack cocaine-using women
  - men in comparison with women
  - uncircumcised males
- Asymptomatic carriage of H. ducreyi plays little or no role in disease transmission

Chancroid & HIV-1 Transmission

- Patients with GUD are substantially more likely to be co-infected with HIV-1 than those with non-ulcerative STIs
- Re-analysis of data from longitudinal studies of female sex workers/men in Nairobi showed that GUD can produce high co-factor effects per sexual exposure for HIV-1 transmission:
  - 10-50 fold for male-to-female
  - 50-300 fold for female-to-male
- Chancroid may have been responsible for a high proportion of heterosexually-acquired HIV-1 infections in sub-Saharan Africa
- Strong correlation between HIV-1 seropositivity and serological evidence of previous chancroid

Decline of Chancroid

- Over the past 10-20 years, there has been a substantial decline in the prevalence of chancroid in several countries in South-east Asia and Africa
- Simultaneous rise in HSV-2 infections which now account for most GUD cases globally
Kenya
Chancroid virtually disappeared in Nairobi by the end of the 1990s followed by a marked reduction in HIV-1 prevalence among sex workers
Moses et al., Sex Transm Infect 2002; 78 (suppl 1):i114-i120; Kaul et al., JAMA 2004; 291:2555-2562

Uganda
H. ducreyi was detected in only 1% of genital ulcer swabs obtained from the Rakai Community Cohort between 2002-2006
Suntoke et al., Sex Transm Infect 2009; 85:97-101

South Africa
Aetiological surveys undertaken since 2007 have repeatedly demonstrated that chancroid accounts for < 1% of genital ulcers

France
In a prospective study of 278 GUD cases enrolled at a STI clinic in Paris, only eight bacteriologically-confirmed cases of chancroid were detected from 1995-2001 and none in period 2002-2005
Hope-Rapp et al., Sex Transm Dis 2010; 37:153-158

United States of America
Only 24 cases of chancroid were detected in the USA in 2010, which represents a 99.4% decline in the number of reported cases since 1990

Decline of Chancroid
Public Health Success Story in the Absence of a Vaccine

WHY?
Rationale for the Decline of Chancroid

\[ R_0 = \beta D c \]

Interventions which reduce the number of sexual exposures:
- Better health-seeking behaviour
- Improved access to services
- Improved quality of sexual health services
- High levels of effective treatment
- Reduction in high-risk sexual behaviour
- Increased condom use
- Better sexual health care for sex workers and their partners

Source: May and Anderson, Nature 1987; 326:137-142

Condom Promotion and Provision
- Sexual Health & HIV Prevention Education
- STI Symptomatic Management
- Periodic Presumptive Therapy (PPT)

Bacteriological Diagnosis

- Microscopy should not be used to diagnose chancroid
- For many years, culture was the ‘gold standard’ method
- ≥ 2 different media should be employed to improve the sensitivity of culture
- *H. ducreyi* has fastidious growth requirements and optimal recovery is obtained through the use of freshly-made media and attention to the incubation conditions
- Vancomycin (3 µg/ml) is added to prevent enhanced recovery
- Culture is only 75% sensitive (at best) in comparison with molecular assays


Effect of Monthly Azithromycin on GUD among Women at High Risk, South Africa

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Effect of PPT Programme for Women at High Risk on GUD in Miners, South Africa

Genital ulcer prevalence was measured in local miners through cross-sectional sampling on two occasions 9 months apart

Before PPT = 5.8% (n=608)  After PPT = 1.3% (n=928)  p < 0.001

Source: Steen et al, Sex Transm Dis 2000;27:1-8

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Molecular Diagnosis

- Several single in-house NAATs exist for *H. ducreyi* detection
- The first M-PCR assay to detect *H. ducreyi*, *T. pallidum* and HSV was developed by Roche in the 1990s
- This assay has been used to study the aetiology of GUD in the USA, India, Lesotho, Madagascar, South Africa and Thailand
- A novel M-PCR assay, with the added advantage of an internal control, can additionally detect *Klebsiella granulomatis*
- Commercially produced Seeplex® M-PCR panels are available:
  - STD4 ACE Detection test (*H. ducreyi*, *T. pallidum*, HSV-1/2 and *C. albicans*)
  - STI Master Panel 5 test (*H. ducreyi*, *T. pallidum*, *C. trachomatis* L1-L3, *Streptococcus agalactiae* and cytomegalovirus)


Serological Investigations

- The humoral immune response to *H. ducreyi* infection develops as the disease progresses through the ulcerative stage
- Lack of IgG antibody responses to either *H. ducreyi* LOS or whole cell antigen up to the pustular stage in the human experimental model
- Due to low sensitivity, serological techniques have no place in the diagnosis of chancroid among GUD patients
- Useful for sero-epidemiological studies within communities

Antimicrobial Susceptibility

- Historically susceptible to a wide range of antimicrobial agents
- *H. ducreyi* acquired several resistance mechanisms over time
- Plasmid-mediated resistance has been described for tetracycline, chloramphenicol, sulphonamides, penicillin and aminoglycosides
- Chromosomally-mediated antimicrobial resistance has been described for trimethoprim, penicillin and fluoroquinolones
- Upward drift in ciprofloxacin and erythromycin MICs in 1990s
- Important to monitor antimicrobial susceptibility of *H. ducreyi* clinical isolates as part of on-going chancroid eradication strategies


Treatment Recommendations

- Ciprofloxacin 500 mg stat. or 12 hrly for 3 days, p.o.
- Azithromycin 1g stat., p.o.
- Erythromycin 500 mg 6 hrly for 7 days, p.o.
- Ceftriaxone 250 mg stat., i.m.
- HIV-infected patients require follow-up
- If fluctuant buboes present, aspiration or excision & drainage


Model Systems to Study Pathogenesis

- Human cell cultures
- Temperature-dependent rabbit model
- Subcutaneous chamber mouse model
- Yorkshire cross pig model
- Pigtail macaque model
- Human model

Lewis, *JSTD AIDs* 1999; 20:73-79
Human volunteers infected with delivery device applied to skin overlying the deltoid

Followed up for 14 days post-inoculation - papules develop at 24 hours and may evolve into pustules over the next 2-5 days

Three possible clinical endpoints:
- Papules resolved at all sites
- Pustule developed that was painful or >4mm in diameter
- 14 days passed since inoculation

At any endpoint, volunteers receive ciprofloxacin 500 mg p.o.

Model used to assess virulence of various isogenic mutants and to determine inflammatory sequelae in early chancroid

WHO launched a worldwide yaws eradication campaign in 2012

H. ducreyi

14 days passed since inoculation

Samples collected from 41 ulcers

12/41 (29.3%) had serological evidence of yaws

1,497 children examined

63 (4.2%) had exudative lesions

Samples collected from 41 ulcers

12/41 (29.3%) had serological evidence of yaws

No evidence of T. pallidum pertenue or M. ulcers was detected in any ulcer

H. ducreyi detected in 13/41 (31.7%)

Skin Ulcers due to H. ducreyi among Children in the Solomon Islands

Yaws is endemic in the Solomon Islands (15,000 cases/year)

WHO launched a worldwide yaws eradication campaign in 2012

Mass distribution of azithromycin and skin ulcer monitoring

RT-PCR used to detect H. ducreyi in skin ulcer samples collected during a cluster-based survey in the Western and Choiseul provinces in 2013

Chronic Skin Ulcers due to H. ducreyi

Molecular techniques recently identified H. ducreyi as the causative agent for chronic skin ulceration in lower limbs of children visiting Samoa and in adult residents in Vanuatu and Papua New Guinea
Skin Ulcers due to *H. ducreyi* among Children in Papua New Guinea

- Prospective cohort study in 5 yaws-endemic villages (population 3,117 persons) during yaws elimination campaign in 2013
- 54/73 (74.0%) evaluated ulcers positive for *H. ducreyi*
- 31/73 (42.7%) evaluated ulcers positive for *T. pallidum pertenue*
- 12 ulcers had dual *H. ducreyi* and *T. pallidum pertenue* infections

![H. ducreyi skin ulcers (A & B)](image)

![Primary yaws (C & D)](image)

**Reasons for the Delayed Recognition of this Association**

- Lack of collecting/testing specimens with molecular techniques
- Lack of facilities for successful *H. ducreyi* culture
- Fastidious nature of *H. ducreyi*

‘Molecular Biology in Medicine’
Inaugural Gollow Lecture - Yvonne Cossart, 1990

**Implications for WHO’s Yaws Eradication Strategy in the Pacific Region?**