Seronegative primary syphilis: associated clinical and laboratory factors. A cross-sectional clinic-based study.

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Background: syphilis serology

Serology mainstay of syphilis diagnosis for > 100 years, since Wasserman test developed in 1906.

Two different syphilis serology algorithms: traditional or reverse.

Background: syphilis serology

* Towns et al STI 2016

Traditional algorithm

1. Non-treponemal test (RPR or VDRL)
2. Reflexive confirmatory treponemal antibody test (EIA, CLIA, TPPA)

- RPR negative in up to 20% primary syphilis. *

Reverse algorithm

1. Treponemal antibody-led (EIA/CLIA/TPPA)
2. Reflexive RPR/VDRL

- syphilis reinfections problematic, persistent positive treponemal antibodies
- RPR used to diagnose reinfections and treatment success

Background: direct detection

Dark-field Microscopy (DFM)	• DFM less common nowadays. • Few centres retain this expertise.
Polymerase chain reaction assay (PCR)	• *T. pallidum* PCR assays available since 2000s. • Useful adjunct to serology. • *T. pallidum* PCR may be positive before development of treponemal and non-treponemal markers.

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Aim:

To identify clinical or laboratory factors associated with discordant, *T. pallidum* PCR-positive and serology negative primary syphilis cases.

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Methods:

- Retrospective audit.
- Identified primary syphilis cases with positive *T pallidum* PCR & negative syphilis serology.
- Identified any follow up serology.
- Examined clinical and laboratory associations.
- Day 1 denotes day of swab collection and serology.

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Methods: Pathology testing

- *T. pallidum* PCR & herpes simplex virus PCR on anogenital lesions
- Selected cases had dark-field microscopy performed on-site.

Methods: Pathology testing

Serology & PCR assays performed at the Victorian Infectious Diseases Reference Laboratory (VIDRL).

Serology

All cases

RPR	rapid plasma reagin
TPPA	*T. pallidum* particle agglutination assay
EIA/CLIA	enzyme immunoassay/ chemiluminescent immunoassay

Some

EIA IgM	

PCR

All cases

polA assay *

* Leslie et al J Clin Micro 2007

Most

47kDa assay

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Results: cohort

814 primary syphilis cases

38 (4.7%) serodiscordant

35 MSM, 2 heterosexual men, 1 woman

3 HIV-positive MSM on cART, CD4 > 500 & VL< 50

- positive *T. pallidum polA* PCR
- negative syphilis serology.

Median symptom duration = 3 days

Lesion sites: 30 penile, 7 perianal, 1 vulval

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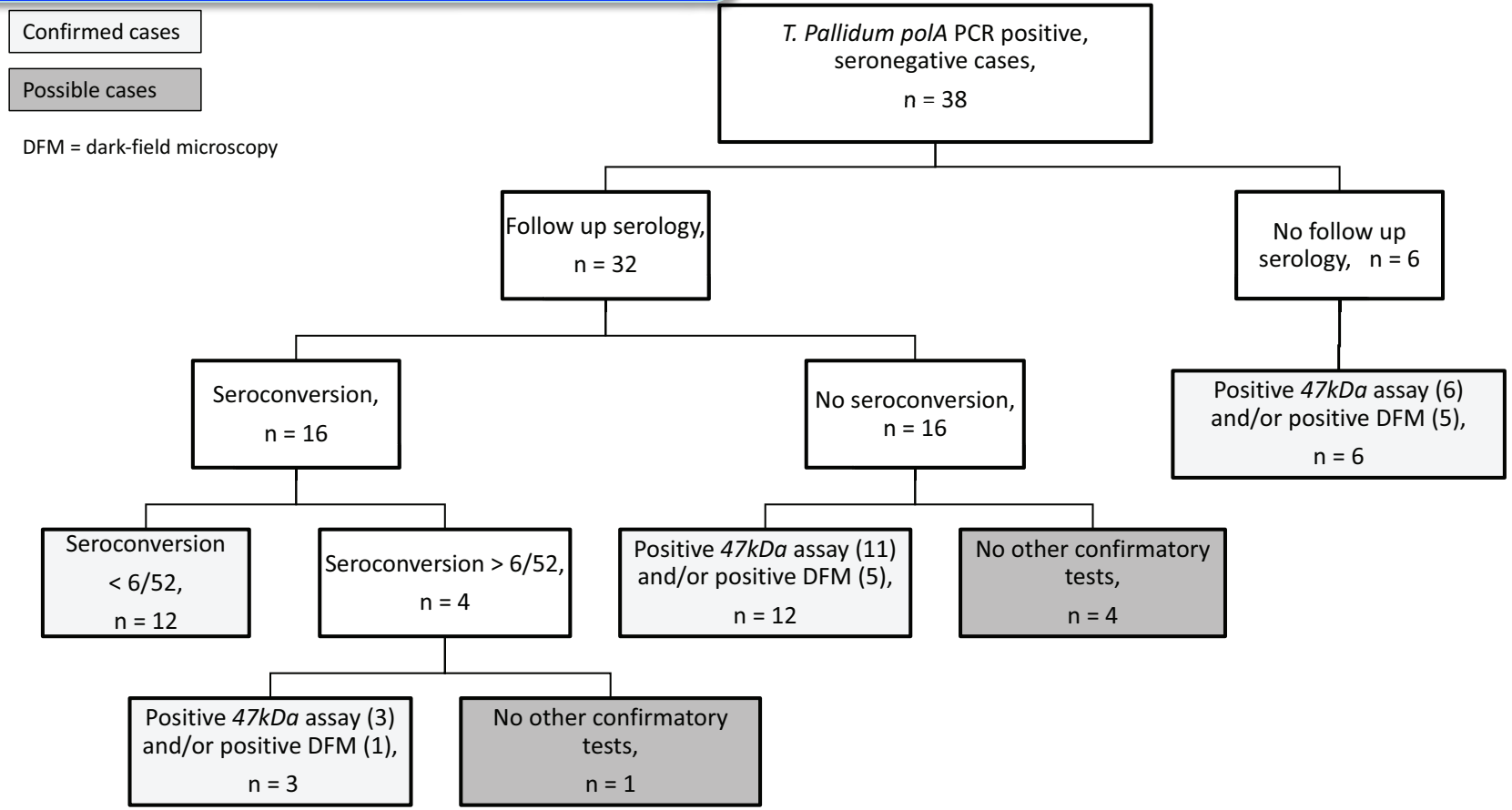
Results: overview



Results: Cases that serocoverted

50% (16/32) seroconverted on repeat serology

12 within 6 weeks

4 by days 57, 87, 212 and 229

Of the 16 that seroconverted, the following markers were positive:

TPPA: 100% (16/16),
EIA or CLIA: 62.5% (10/16),
EIA IgM: 46% (6/13) and
RPR: 38% 6/16 (range 1 – 8)

3 positive *47kDa* (1 positive DFM)

1 had no *47kDa* or DFM performed

1 RPR of 1:1
3 negative RPR

Results: Cases that did not seroconvert

16/32 (50%) cases did not seroconvert on repeat serology

27% (6/16) treated between days 8 & 18.

63% (10/16) treated on day 1

- Clinical chancre
- Positive dark-field microscopy
- Contact of syphilis

75% (12/16) confirmed with positive *47kDa* result and/or positive DFM

25% (4/16) cases had positive *T. pallidum polA* PCR results alone, but no other confirmatory results performed

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Results: Cases with no follow-up serology

6 cases with no follow-up serology

All 6 received syphilis treatment on day 1.

All 6 cases were positive on both the *T. pallidum polA* and *47kDA* assays.

5/6 were also positive on dark-field microscopy.

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Comparing seroconversion vs. no seroconversion

- 88% of cases that seroconverted had delayed syphilis treatment. (Administered between days 7 and 20)
- Seroconversion was significantly associated with delayed treatment.
- If treated on day 1: 12.5% seroconverted compared with 87.5%, if treated after day 1, ($p = 0.009$) .

Discussion

- Earlier treatment of primary syphilis can prevent the development of serological markers.
- *T. pallidum* PCR can identify primary syphilis lesions before development of serological markers and improve diagnosis of primary syphilis.
- Serology alone will miss a proportion of primary syphilis infections and should be repeated if a diagnosis of syphilis is being considered or if risk factors are present.

Key messages

- Primary syphilis may be diagnosed by *T. pallidum* PCR before the evolution of any serological markers.
- Half of the seronegative syphilis cases in this study subsequently seroconverted, most within six weeks.
- Treatment on day one more likely to prevent seroconversion than delayed treatment.

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