Effectiveness of single-dose azithromycin to treat latent yaws: a longitudinal comparative cohort study

Oriol Mitjà, Camila González-Beiras, Charmie Godornes, Reman Kolmau, Wendy Houinei, Haina Abel, August Kapa, Raymond Paru, Sibauk V Bieb, James Wangi, Sergi Sanz, Kingsley Asiedu, Sheila A Lukehart, Quique Bassat

Summary

Background Treatment of latent yaws is a crucial component of the WHO yaws eradication strategy to prevent relapse and the resulting transmission to uninfected children. We assessed the effectiveness of single-dose azithromycin to treat patients with latent yaws.

Methods This population-based cohort study included children (age <20 years) living on Lihir Island, Papua New Guinea, with high-titre (rapid plasma reagin titre ≥1:8) latent or active yaws, between April, 2013, and May, 2015. Latent yaws was defined as lack of suspicious skin lesions or presence of ulcers negative for *Treponema pallidum* subsp *pertenue* on PCR, and active yaws was defined as ulcers positive for *T pertenue* on PCR. All children received one oral dose of 30 mg/kg azithromycin. The primary endpoint was serological cure, defined as a two-dilution decrease in rapid plasma reagin titre by 24 months after treatment. Treatment of latent yaws was taken to be non-inferior to that of active yaws if the lower limit of the two-sided 95% CI for the difference in rates was higher than or equal to –10%. This study is registered with ClinicalTrials.gov, number NCT01955252.

Findings Of 311 participants enrolled, 273 (88%; 165 with latent yaws and 108 with active yaws) completed follow-up. The primary endpoint was achieved in 151 (92%) participants with latent yaws and 101 (94%) with active yaws (risk difference –2·0%, 95% CI –8·3 to 4·3), meeting the prespecified criteria for non-inferiority.

Interpretation On the basis of decline in serological titre, oral single-dose azithromycin was effective in participants with latent yaws. This finding supports the WHO strategy for the eradication of yaws based on mass administration of the entire endemic community irrespective of clinical status.

Funding Newcrest Mining Limited and ISDIN laboratories.

Lancet Glob Health 2017; 5: e1268–74

Published Online October 26, 2017 http://dx.doi.org/10.1016/S2214-109X(17)30388-1

See Comment page e1172

Barcelona Institute for Global Health, Hospital Clinic-University of Barcelona, Barcelona, Spain (O Mitjà MD, C González-Beiras BSc, S Sanz MSc, Prof Q Bassat MD); **Division of Public Health, School of Medicine and Health Sciences, University of Papua New Guinea, Port Moresby, Papua New Guinea** (O Mitjà); **Lihir Medical Centre, International SOS-Newcrest Mining, Lihir Island, Papua New Guinea** (O Mitjà, R Kolmau BSc, H Abel BSc, A Kapa BSc, R Paru BSc); **Lisbon Institute of Hygiene and Tropical Medicine, Lisbon, Portugal** (C González-Beiras); **Department of Medicine** (C Godornes BSc, Prof S A Lukehart PhD) **and Department of Global Health** (Prof S A Lukehart), **University of Washington, Seattle, WA, USA; Disease Control Branch, National Department of Health, Port Moresby, Papua New Guinea** (W Houinei HEO, S V Bieb MaHM); **Office of the WHO Representative for Papua New Guinea, WHO, Port Moresby, Papua New Guinea** (J Wangi MPH); **Biostatistics Unit, Department of Public Health, Faculty of Medicine, University of Barcelona, Spain** (S Sanz); **Department of Control of Neglected Tropical Diseases, WHO, Geneva, Switzerland** (K Asiedu MPH); **Institució Catalana de Recerca i Estudis Avançats (ICREA), Barcelona, Spain** (Prof Q Bassat); **Centro de Investigação em Saúde de Manhiça (CISM), Maputo, Mozambique** (Prof Q Bassat); **and Paediatric Infectious Diseases Unit, Paediatric Department, Hospital**

Introduction

As with venereal syphilis, patients with yaws can develop latent disease that is characterised by seroreactivity without other evidence of primary, secondary, or tertiary disease. Five to six people are estimated to have latent yaws for each case of active disease, and clinical relapse is possible for up to 5 years after infection.[1,2] Clinical diagnosis of yaws has been challenged by the discovery that a substantial proportion of skin lesions contain no *Treponema pallidum* subsp *pertenue* on PCR, but do contain DNA sequences specific to *Haemophilus ducreyi* or are caused by as-yet unknown pathogens.[3–5] Hence, some individuals with reactive yaws serology have skin lesions arising from another cause.

In 2012, a randomised controlled trial in Papua New Guinea showed that oral azithromycin was as effective as parenteral penicillin in the treatment of active yaws.[6] On the basis of this finding, WHO adopted a revised strategy of treatment with single-dose azithromycin for all community members in yaws-endemic areas, irrespective of clinical status.[7] Treatment of both active and latent yaws is crucial for the success of an eradication programme, and this strategy allows individuals with latent disease to be exposed to curative treatment. Despite implementation of pilot programmes in several equatorial rural provinces of Africa[8] (Aziz A, personal communication) and the South Pacific,[9–12] and plans to expand activities in those regions and worldwide, the efficacy of single-dose azithromycin to treat latent yaws remains to be established.

The objective of treating people with latent yaws is twofold: first, to prevent relapsing episodes and resulting transmission to uninfected children[1] and, second, to prevent progression of the infection to the destructive tertiary stage. Late latent syphilis is treated with benzathine benzylpenicillin for an extended period of time because this antibiotic is active only against dividing cells and organisms divide more slowly in the latent stage, although the validity of this rationale has not been assessed. Thus, single-dose azithromycin might be less effective in treating latent infection than active infection if the bacterium is less metabolically active.

In this study, we assessed the serological response of people with latent yaws to treatment with one dose of oral azithromycin. We compared the findings against those in people with active yaws.

Sant Joan de Déu Barcelona, Barcelona, Spain (Prof Q Bassat);

Correspondence to: Dr Oriol Mitjà, Department of Community Health, Lihir Medical Centre, Post Office Box 34, Lihir Island, New Ireland Province, Papua New Guinea
oriol.mitja@isglobal.org

Research in context

Evidence before this study

We searched PubMed, without language restrictions, for studies of the efficacy of single-dose oral azithromycin to treat latent treponemal disease published up to April 1, 2017, with the search terms "yaws", "syphilis", "latent", and "azithromycin". We identified four studies showing that azithromycin was efficacious in treating early latent syphilis in several regions (North America, Madagascar, Tanzania, and Taiwan). Additionally, studies in Congo, Ghana, Papua New Guinea, the Solomon Islands, and Vanuatu indicated that community-level prevalence of latent yaws fell substantially after mass treatment with azithromycin. In those studies, however, different samples of children were selected for each round of cross-sectional surveys, meaning that no study followed up a consistent group of participants. We did not identify any study that used serological monitoring to assess individual response to treatment of latent yaws with azithromycin.

Added value of this study

In the context of a pilot yaws eradication programme, we investigated the serological response to treatment in a large cohort of people with latent yaws confirmed by serology and PCR. The use of PCR enabled clear differentiation between active and latent yaws, which represents a strong advance over the methods used in previous studies. We followed up the same study cohort for 24 months, which meant that we could reliably determine whether serological titres declined to the expected extent after treatment, including in participants in whom decline was slow.

Implications of all the available evidence

We found that one dose of 30 mg/kg oral azithromycin led to serological cure in a high proportion of participants with latent yaws. When taken together with the previous findings on treatment of latent syphilis, the data suggest efficacy of azithromycin to treat latent treponematoses, including yaws. Studies done in the 1950s showed that, owing to the tendency of latent yaws to relapse early in the course of untreated infection, treatment of latent disease is a crucial part of eradication efforts. Our findings, therefore, support the WHO strategy of mass administration of azithromycin irrespective of clinical status, which allows people harbouring the infection without any skin manifestations (ie, latent infection) to be exposed to treatment.

Methods

Study design and participants

In the context of the yaws eradication programme of Lihir Island, Papua New Guinea,[9] we did a prospective study that involved all children (age <20 years) with active or latent yaws who were residents of the island. Methods of the yaws eradication programme, including details of treatment and clinical assessment, have been previously described.[9] Briefly, we administered mass azithromycin treatment in April, 2013, and repeatedly assessed outcomes in about 16 000 people every 6 months for about 3·5 years.

Participants attended clinical surveys, during which those with ulcers suspected of being due to active yaws were assessed for inclusion in the study. People with a reactive *T pallidum* haemagglutination assay (TPHA) and rapid plasma reagin (RPR) titre of 1:8 or greater at the time of screening were eligible for the study. We excluded people with RPR titres of 1:2 or 1:4 because many children in high endemic areas have a persistently positive low-titre RPR results after successful treatment (ie, serofast status) that cannot be distinguished from true treponemal infection. The criterion for the diagnosis of active yaws was ulcers positive for *T pertenue* on PCR, and for the diagnosis of latent yaws was ulcers negative for *T pertenue*. Asymptomatic individuals (no suspicious skin lesions) from randomly selected villages who were assessed and enrolled during baseline serosurveys of the eradication programme were also included.

We classified participants with dual positivity on TPHA and RPR into three groups, on the basis of PCR testing: group 1 included asymptomatic people with no ulcers and who were not tested with PCR; group 2 included people with ulcers negative for *T pertenue* on PCR; and group 3 included people with an ulcer and PCR evidence of active yaws. Groups 1 and 2 constituted the latent yaws study population and group 3 was the active yaws comparator group. The Medical Research Advisory Committee of Papua New Guinea approved the study protocol (number 12.36). All eligible people were informed of the study's aims, procedures, and associated risks and provided informed consent (children <15 years gave verbal consent and their parents or guardians provided written consent).

Procedures

All participants with skin ulcers underwent clinical, serological, and PCR assessments, were treated with one directly observed oral dose of 30 mg/kg azithromycin, and were followed up in village community outreach clinics for repeated RPR testing at 6, 12, and 24 months. Follow-up was discontinued when results showed serological cure. Asymptomatic participants also received one oral dose of 30 mg/kg azithromycin and were followed up at 12 and 24 months.

The serological reactivity was determined at the Lihir Medical Centre laboratory by RPR and TPHA testing (both Human Diagnostics Worldwide, Wiesbaden, Germany). To calculate RPR titre, a blood serum sample was diluted in serial ratios until there was no longer antibody reactivity. Real-time (rt)PCR specific for *T pertenue* and *H ducreyi* was done on all lesional swab samples at the University of Washington laboratory, Seattle, WA, USA. Three primer sets for three independent *T pallidum* gene targets—*tp0548*, *tpN47* (also known as

tp0574), and a pertenue-specific region of *tprL* (also known as *tp1031*)—were PCR amplified to detect the presence of *T pallidum* DNA and to confirm the subspecies, as has been described previously.[3] We used Taqman rtPCR targeting the 16S rRNA gene to identify *H ducreyi*.[13]

Definitions

The primary endpoint of the study was serological cure at 24 months, defined as a decrease in the RPR titre by at least two dilutions (fourfold) at any time during the study (eg, change from 1:16 at baseline to 1:4).[14] Participants not cured at 24 months were classified either as having treatment failure, defined as at least two dilutions (fourfold) increase in RPR titre (eg, change from 1:16 at baseline to 1:64), or having non-significant change, defined as either no change in titre or a decrease or increase of one dilution (twofold) from baseline (eg, change from 1:16 at baseline to either 1:8 or 1:32), based on the 24-month RPR titre. The outcome categories were mutually exclusive. The secondary endpoint was the proportion of participants with seroreversion to non-reactive at 24 months (ie, no antibodies detectable by RPR testing).

Statistical analyses

We calculated that a sample size of 165 participants in the latent yaws group and 108 in the active yaws comparator group (sampling ratio 1·5) would give a power of 98% to test the hypothesis of non-inferiority.[15] This sample size accounted for an expected efficacy of 91·5% in the latent yaws group and of 93·5% in the active yaws group, a non-inferiority margin of 10%, and a one-sided type I error rate of 0·05.

We included in the primary analysis participants who were treated and completed the study procedures for outcome assessment at 24 months. Data on participants who were lost to follow-up before serological cure were censored at the time of the last follow-up visit and were not included in the primary analysis.

To analyse the primary endpoint, we estimated the two-sided 95% CIs for the difference in proportions of participants with serological cure by subtracting the active yaws group values from the latent yaws group values. Treatment of latent yaws was deemed to be non-inferior to that of active yaws if the lower bound of the CI was higher than or equal to –10%. We used the same method to analyse the secondary endpoint. We did subgroup analyses to investigate the association between serological cure and time of retesting by use of differences in proportions or mean baseline RPR titre, assessed with the Student's *t* test of geometric means with serum RPR titre as a continuous variable. We did all statistical analyses with Stata version 14.1. This

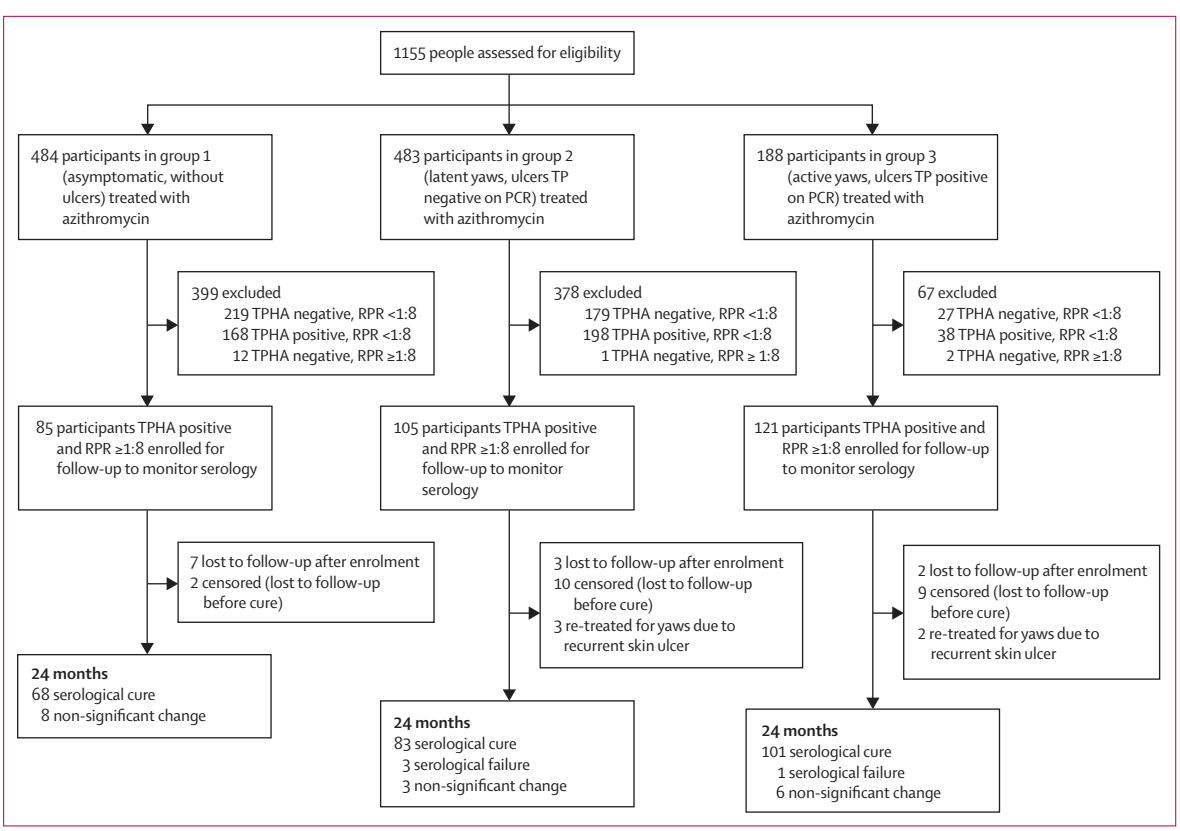

Figure 1: **Study profile**
TP=*Treponema pallidum* subsp *pertenue*. TPHA=*T pallidum* haemagglutination assay. RPR=rapid plasma reagin.

	Latent yaws (n=190)*	Active yaws (n=121)†	p value
Age (years)	10·4 (5·4)	9·8 (6·0)	0·361
Boys	101 (53%)	77 (64%)	0·069
Girls	89 (47%)	44 (36%)	..
RPR titre			
≥1:16	160 (84%)	92 (76%)	0·076
≥1:64	75 (40%)	42 (35%)	0·395

Data are mean (SD) or number (%). RPR=rapid plasma reagin. *Defined as ulcers negative for *Treponema pallidum* subsp *pertenue* on PCR or no suspicious skin lesions. †Defined as ulcers positive for *T pertenue* on PCR.

Table 1: **Baseline characteristics of the study population**

	Latent yaws infection (n=165)*	Active yaws infection (n=108)†	Absolute difference in risk (95% CI)
Serological cure‡	151 (92%)	101 (94%)	−2·0% (−8·3 to 4·3)
Seroreversion§	73 (44%)	63 (58%)	−14·1% (−26·1 to −2·1)

RPR=rapid plasma reagin. *Defined as ulcers negative for *Treponema pallidum* subsp *pertenue* on PCR or no suspicious skin lesions. †Defined as ulcers positive for *T pertenue* on PCR. ‡Decrease in RPR titre by two or more dilutions. §RPR reverted to non-reactive.

Table 2: **Serological cure or seroreversion 24 months after treatment with azithromycin**

	Number of patients	Treatment failure*	Non-significant change†	Number of dilution reductions in RPR titre		
				Two	Three	Four
Latent yaws	165	3 (2%)	11 (7%)	14 (9%)	29 (18%)	108 (66%)
Active yaws	108	1 (1%)	6 (6%)	14 (13%)	30 (28%)	57 (53%)

RPR=rapid plasma reagin. *At least two dilutions increase in RPR titre. †No change or one dilution (twofold) reduction or increase in RPR titre.

Table 3: **Outcomes 24 months after treatment with azithromycin, by clinical stage**

study is registered with ClinicalTrials.gov, number NCT01955252.

Role of the funding source

The funders of the study had no role in study design, data collection, data analysis, data interpretation, or writing of the report. The corresponding author had full access to all the data in the study and had final responsibility for the decision to submit for publication.

Results

Of 1155 individuals assessed for eligibility, 484 were asymptomatic (group 1), 483 had skin ulcers negative for *T pertenue* on rtPCR (group 2), and 188 had skin ulcers positive for *T pertenue* on rtPCR (group 3). Among these, 311 children had a reactive TPHA and an RPR titre of at least 1:8, all of whom were enrolled in the study (figure 1). The mean age of participants was 10·1 years (SD 5·7), 178 (57%) were boys and 133 (43%) were girls. The numbers of patients who had baseline RPR titres of at least 1:16 or at least 1:64 in the latent yaws group did not differ significantly from those in the active yaws group (table 1).

12 (4%) of 311 participants enrolled could not be traced for any follow-up visit, 21 (7%) were censored at 6 or 12 months, and five (2%) were excluded after being re-treated for presumed symptomatic reinfection or relapse (figure 1). Therefore, 273 participants were included in the analyses of serological cure at 24 months. 252 patients were classified as having serological cure and 21 as having treatment failure or non-significant change outcomes at 24 months.

The risk difference for serological cure between the latent versus the active yaws groups met the prespecified criteria for non-inferiority (table 2). In the sensitivity analysis of participants who were tested three times (ie, excluding group 1), non-inferiority was maintained (83 [93%] *vs* 101 [94%], risk difference −0·3%, 95% CI −7·2 to 6·7). Additionally, the proportions of participants with serological cure at 24 months were similar in groups 1 and 2 (68 [90%] *vs* 83 [93%], p=0·383), which supports the adequacy of combining asymptomatic and PCR-negative participants in the latent group. 14 participants with latent yaws did not achieve serological cure (table 3). Three or four dilution decreases in RPR titre were seen frequently in both the latent yaws and active yaws groups (table 3). The proportion of participants with seroreversion was significantly lower in the latent yaws group than in the active yaws group (table 2).

We did a post-hoc analysis of individuals with baseline RPR titres of 1:2 or 1:4 in groups 2 (n=54) and 3 (n=21), who were excluded from the main analysis. In group 2, 28 (52%) participants achieved a two-dilution decrease in RPR titre or seroreversion at 24 months, compared with 15 (71%) in group 3 (risk difference −19·6%, 95% CI −43·0 to 3·9). Some individuals with baseline RPR titres of 1:2 or 1:4 who had persistently unchanged RPR titres at 24 months were probably actually serofast, which supports the appropriateness of excluding this group of patients from our analysis.

At retesting visits, the latent yaws group included only group 2 participants at 6 months, but group 1 and 2 participants at 12 months. The proportions with serological cure did not differ significantly between the latent and active yaws groups in the subgroup analysis of time of retesting, either at 6 months (58 [83%] *vs* 59 [88%], risk difference −5·2%, 95% CI −17·0 to 6·6) or at 12 months (125 [86%] *vs* 74 [90%], −4·0%, −12·6 to 4·5; figure 2A). The proportion of participants with seroreversion was lower in the latent yaws group than in the active yaws group at 6 months (12 [17%] *vs* 30 [45%]; risk difference −27·6%, 95% CI −42·4 to −12·8) and 12 months (47 [32%] *vs* 44 [54%], −21·2%, −34·5 to−8·0; figure 2C).

Mean baseline RPR titre was 40·1 (SD 43·3) in participants with serological cure, compared with 34·9 (24·0) in participants who did not achieve serological cure (p=0·721). We saw no difference in serological response at 24 months between participants with latent or active yaws by baseline RPR titre (figure 2B). The mean baseline RPR titre was 33·3 (SD 35·4) in participants

with seroreversion compared with 43·8 (SD 43·8) in participants without seroreversion (p=0·034). The proportion of participants with seroreversion at 24 months was lower among those with latent yaws than among those with active yaws by RPR titre at baseline (figure 2D).

Discussion

The primary endpoint of serological cure at 24 months was seen in 92% of patients with latent yaws after treatment with single-dose azithromycin, which was similar to that among participants with rtPCR-confirmed active yaws. These results expand on the data showing efficacy with 30 mg/kg single-dose azithromycin for treatment of early syphilis, including in the early latent stage.[16–19] Before this study, mass treatment with azithromycin had been shown to reduce the prevalence of latent yaws significantly at the community level.[5,9–12] People with latent yaws, however, had not been consistently and prospectively followed up over a long period of time to monitor serological cure at the individual level.

The RPR titre data indicate that successful treatment for active and latent yaws often results in three or four dilution decreases, but seroreversion was seen with this non-treponemal test in less than 50% of participants with latent yaws. As reported for syphilis,[14] participants with high baseline titres in this study had greater declines than did those with lower titres at baseline, but were less likely to have seroreversion. We stress that non-treponemal tests can show reactivity at low titres in a high percentage of people, even a long time after treatment, and, therefore, do not necessarily indicate untreated yaws. Similar persistent reactivity at low titres is seen after treatment of syphilis with azithromycin or benzathine benzylpenicillin, and is referred to as serofast status. This evidence supports the inclusion of serosurveys in yaws eradication programmes to assess interruption of yaws transmission at least 24 months after mass treatment, alone or with the use of an RPR titre cutoff below which further testing is needed before serology is classified as positive.

Our study has several limitations. First, the RPR test is read by naked eye and there is a degree of inter-observer variability in interpretation of test results that can lead to variation in RPR titre of plus or minus one dilution.[20] We attempted to minimise variation by having all RPR testing done by one technician at a central laboratory with simultaneous retesting of serum samples taken before and after treatment and stored frozen. Second, because of the observational nature of our study design, there was no untreated control group. As the duration of untreated syphilis increases, titres might decline by two or more dilutions.[21,22] A similar effect could have contributed to the results for yaws in our study. Additionally, there was no control group of participants treated with benzathine benzylpenicillin. We did, however, include a comparator group of children with active yaws infection, and a study of single-dose azithromycin compared with benzathine benzylpenicillin for active yaws showed non-inferiority.[6] Third, by excluding people with baseline RPR titres of 1:2 or 1:4, the latent sample might be biased in favour of early latent infection or people between the primary and secondary stages. Serological cure at 24 months was seen in only 52% of participants with low-titre RPR latent yaws, which might reflect stable non-treponemal antibody titres in the serofast state. However, we cannot rule out that the lower serological cure exhibited was related to infections later in the natural history. This pattern, though, would not affect the WHO yaws eradication programmes because late latent yaws, in which lesions are not present, is not an important contributor to incident infections, but it would have implications for individuals' health and clinical management. Finally, group 1 had a different follow-up schedule from groups 2 and 3 (two *vs* three occasions for testing) and, therefore, serological cure might have been underestimated in group 1. Although this difference could bias the non-inferior result towards inferiority for treatment of latent yaws, we were still able to show non-inferiority. Furthermore, non-inferiority was maintained in a sensitivity analysis of only groups 2 and 3. Of note, in people with latent yaws (or syphilis) it can take 12–24 months for the full amplitude of RPR titre fall to be expressed after successful

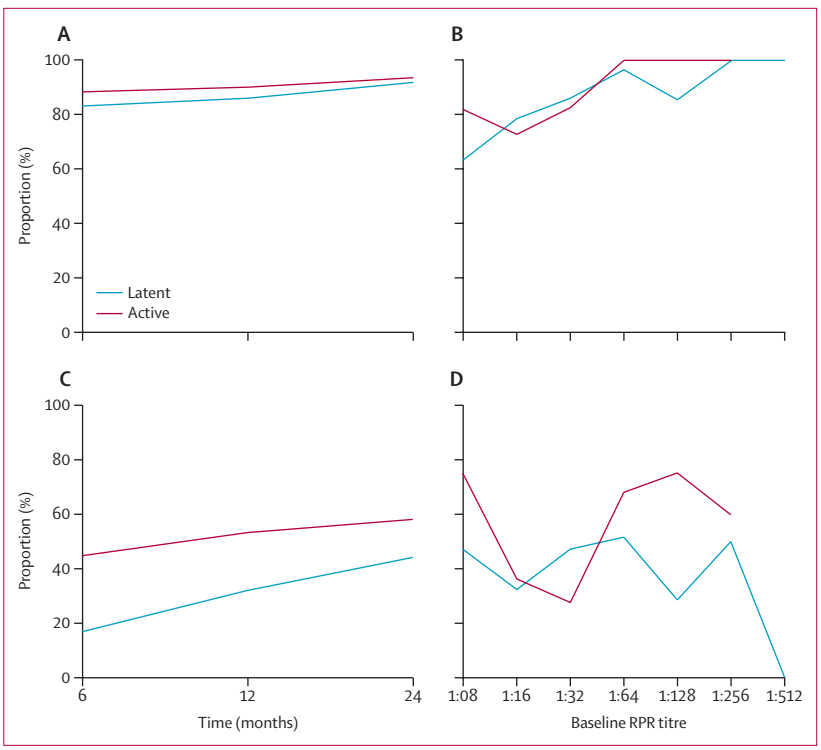

Figure 2: **Serological cure or seroreversion by infection status**
Serological cure (decrease in RPR titre by two or more dilutions) was determined as a function of (A) time of retesting after treatment and (B) baseline RPR titre. Seroreversion (RPR reverted to non-reactive) was determined as a function of (C) time of retesting after treatment and (D) baseline RPR titre. We report proportions of patients with latent or active yaws with serological cure at 6, 12, and 24 months. Asymptomatic participants were not followed up at 6 months and, therefore, data are included in the latent yaws group at 12 and 24 months only. RPR=rapid plasma reagin.

treatment and, therefore, serology at 12 and 24 months is more reliable than assessment at 6 months, which group 1 missed.

In common with other yaws-endemic regions studied, we found that a substantial proportion of skin lesions clinically attributed to yaws contained *H ducreyi* DNA.[4] Isolates of *H ducreyi* obtained from skin lesions of children in Vanuatu and Samoa were sensitive to azithromycin in vitro,[23] and the antibiotic is recommended for the treatment of sexually transmitted *H ducreyi* infections.[24] On Lihir Island, 2 weeks after treatment with azithromycin, around 90% of all ulcers containing *H ducreyi* were totally re-epithelialised or greatly improved.[25] Hence, azithromycin has the double effect of treating *H ducreyi* ulcers and latent yaws.

Failure of targeted treatment of people with active yaws and their contacts with penicillin by WHO and UNICEF in the 1940s and 1950s was thought to be due to continued untreated asymptomatic infection because of the tendency for untreated latent yaws to relapse early.[26,27] Treatment of latent disease is, therefore, a crucial part of eradication efforts. The availability of a well tolerated oral agent prompted WHO to develop an eradication strategy that involved treatment of all members of yaws-endemic communities, irrespective of clinical status. Our findings provide clear evidence that one high dose of azithromycin is effective for treatment of latent yaws, and support WHO's strategy for yaws eradication. However, a study done on Lihir Island showed that one round of mass treatment might be insufficient because of relapse of latent yaws in people who were absent at the time of treatment (unpublished). Our results showing the effectiveness of single-dose azithromycin for treating latent yaws support relapse from untreated latent yaws, rather than from azithromycin treatment failure, playing a part in persistent transmission of the disease. More than one round of treatment would increase the likelihood of treating each person at least once.

Contributors

OM and QB designed the study. CG-B, AK, and RP supervised the field work and gathered data and samples. HA was primarily responsible for the serological studies. CG and SAL were responsible for the PCR studies. OM and SS did the statistical analyses. OM, WH, and SAL wrote the first draft of the paper, with revisions and input from SVB, JW, and QB. All authors contributed to revisions and approved the final version.

Declaration of interests

We declare no competing interests.

Acknowledgments

We thank the study participants and their parents or legal guardians, the International SOS field team in Lihir Island, the staff at the Lihir Medical Centre laboratory, the University of Washington laboratory, and the National Department of Health of Papua New Guinea for guidance and oversight of the study and continued cooperation. We thank Sergi Gavilán, Barcelona Institute for Global Health, Barcelona, Spain, for project management and general administrative support, and Laia Bertran, Barcelona Institute for Global Health, for knowledge management, liaison between institutions, and acquisition of funding. This study was funded by Newcrest Mining and ISDIN laboratories. The laboratory work was supported by the US Public Health Service National Institutes of Health (grant R01AI42143 to SAL), and WHO provided generic azithromycin.

References

1 Hackett CJ, Guthe T. Some important aspects of yaws eradication. *Bull World Health Organ* 1956; **15:** 869–96.

2 Perine PL, Hopkins DR, Niemel PLA, St John R, Causse G, Antal GM. Handbook of endemic treponematoses: yaws, endemic syphilis and pinta. Geneva: World Health Organization, 1984.

3 Mitjà O, Lukehart SA, Pokowas G, et al. *Haemophilus ducreyi* as a cause of skin ulcers in children from a yaws-endemic area of Papua New Guinea: a prospective cohort study. *Lancet Glob Health* 2014; **2:** e235–41.

4 Marks M, Chi K-H, Vahi V, et al. *Haemophilus ducreyi* associated with skin ulcers among children, Solomon Islands. *Emerg Infect Dis* 2014; **20:** 1705–07.

5 Ghinai R, El-Duah P, Chi KH, et al. A cross-sectional study of 'yaws' in districts of Ghana which have previously undertaken azithromycin mass drug administration for trachoma control. *PLoS Negl Trop Dis* 2015; **29:** e0003496.

6 Mitjà O, Hays R, Ipai A, et al. Single-dose azithromycin versus benzathine benzylpenicillin for treatment of yaws in children in Papua New Guinea: an open-label, non-inferiority, randomised trial. *Lancet* 2011; **379:** 342–47.

7 WHO. Summary report of the consultative meeting on eradication of yaws, 5–7 March 2012, Morges, Switzerland. Geneva, Switzerland. 2012. http://apps.who.int/iris/bitstream/10665/75528/1/WHO_HTM_NTD_IDM_2012.2_eng.pdf (accessed Sept 13, 2017).

8 Coldiron M, Oblava D, Mouniaman-Nara I, Pena J, Blondel C, Porten K. The prevalence of yaws among the Aka in the Congo. *Med Sante Trop* 2013; **23:** 231–32.

9 Mitjà O, Houinei W, Moses P, et al. Mass treatment with single-dose azithromycin for yaws. *N Engl J Med* 2015; **372:** 703–10.

10 Fegan D, Glennon MJ, Kool J, Taleo F. Tropical leg ulcers in children: more than yaws. *Trop Doct* 2016; **46:** 90–93.

11 Chi QH, Danavall D, Taleo F, et al. Molecular differentiation of *Treponema pallidum* subspecies in skin ulceration clinically suspected as yaws in Vanuatu using real-time PCR and serological methods. *Am J Trop Med Hyg* 2015; **92:** 134–38.

12 Marks M, Sokana O, Nachamkin E, et al. Prevalence of active and latent yaws in the Solomon Islands 18 months after azithromycin mass drug administration for trachoma. *Plos Negl Trop Dis* 2016; **10:** e0004297.

13 Orle KA, Gates CA, Martin DH, Body BA, Weiss JB. Simultaneous PCR detection of *Haemophilus ducreyi, Treponema pallidum*, and herpes simplex virus types 1 and 2 from genital ulcers. *J Clin Microbiol* 1996; **34:** 49–54.

14 Romanowski B, Sutherland R, Fick GH, Mooney D, Love EJ. Serologic response to treatment of infectious syphilis. *Ann Intern Med* 1991; **114:** 1005–09.

15 Chow S, Shao J, Wang H. Sample size calculations in clinical research, 2nd edn. New York, NY: Chapman & Hall/CRC Biostatistics Series, 2008.

16 Hook EW 3rd, Martin DH, Stephens J, Smith B, Smith K. A randomized comparative pilot study of azithromycin versus benzathine penicillin G for treatment of early syphilis. *Sex Trans Infect* 2002; **29:** 486–90.

17 Riedner G, Rusizoka M, Todd J, et al. Single-dose azithromycin versus penicillin G benzathine for the treatment of early syphilis. *N Engl J Med* 2005; **353:** 1236–44.

18 Hook EW 3rd, Behets F, Van Damme K, et al. A phase III equivalence trial of azithromycin versus benzathine penicillin for treatment of early syphilis. *J Infect Dis* 2010; **201:** 1729–35.

19 Yang CJ, Tang HJ, Chang SY. Comparison of serological responses to single-dose azithromycin (2 g) versus benzathine penicillin G in the treatment of early syphilis in HIV-infected patients in an area of low prevalence of macrolide-resistant *Treponema pallidum* infection. *J Antimicrob Chemother* 2016; **71:** 775–82.

20 Larsen SA, Creighton ET. Rapid plasma reagin (RPR) 18-mm circle and card test. In: Larsen SA, Pope V, Johnson RE, Kennedy EJ, eds. A manual of tests for syphilis, 9th edn. Washinton DC: American Public Health Association, 1998: 193–207.

21 Eng J, Wereide K. TPI test in untreated syphilis—a serological re-examination of 50 patients belonging to the Oslo study of untreated syphilis. *Br J Vener Dis* 1962; **38:** 223–29.

22 Lukehart SA, Baker-Zander SA, Sell S. Characterization of the humoral immune response of the rabbit to antigens of *Treponema pallidum* after experimental infection and therapy. *Sex Transm Dis* 1986; **13:** 9–15.

23 Gangaiah, D, Webb KM, Humphreys TL, et al. *Haemophilus ducreyi* cutaneous ulcer strains are nearly identical to class I genital ulcer strains. *PLoS Negl Trop Dis* 2015; **9:** e0003918.

24 Lautenschlager S, Kemp M, Christensen JJ, Vall Mayans M, Moi H. 2017 European guideline for the management of chancroid. *Int J STD AIDS* 2017; **28:** 324–29.

25 González-Beiras C, Kapa A, Vall Mayans M, et al. Single dose azithromycin for the treatment of *Haemophilus ducreyi* skin ulcers in Papua New Guinea. *Clin Infect Dis* 2017; published online Aug 16. https://doi.org/10.1093/cid/cix723.

26 Zahra A. Yaws eradication campaign in Nsukka Division, Eastern Nigeria. *Bull World Health Organ* 1956; **15:** 911–35.

27 Rein CR. Treatment of yaws in the Haitian peasant. *J Natl Med Assoc* 1949; **41:** 60–65.