Audit of efficacy of Coartem™ to clear plasmodium falciparum malaria parasitaemia at single forty-two day follow-up

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Summary

Background: A follow-up study was undertaken to assess the efficacy of Coartem™ tablets (20 mg artemether and 120 mg lumefantrine – Novartis South Africa (Pty) Ltd) to clear *plasmodium falciparum* malaria parasitaemia at a single 42-day follow-up, with 42 days being chosen in order to detect early emergence of resistance. The study was performed at Ndumo Clinic and Mosvold Hospital in the Ingwavuma District of KwaZulu-Natal, South Africa in January/February 2002.

Method: The study included 37 patients presenting to Ndumo Clinic and two presenting to Mosvold Hospital with uncomplicated malaria diagnosed by symptoms and a positive immunochromographic test (ICT) for *plasmodium falciparum*. The main outcome measures were Trophozoite counts on thick film and polymerase chain reaction parasite analysis of blood spot at day 42.

Results: Only 31 of the 37 recruited patients were confirmed to be suffering from malaria by polymerase chain reaction (PCR). Of the 31, 24 returned for follow-up. One patient had parasitaemia at day 33, but tested negative at day 42 after re-treatment with Coartem™. It was not determined whether this patient was suffering from a recrudescence or re-infection of falciparum malaria. All the other returning patients tested negative for falciparum malaria on blood film and PCR examination.

Conclusions: Coartem™ still appears to be an effective treatment for falciparum malaria. Regular assessment of its efficacy is desirable.

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Introduction

Between 1996 and 2000, the incidence of malaria in South Africa, and in northern KwaZulu-Natal in particular, increased dramatically. For example, there were 5 991 notified malaria cases in South Africa in 1995, whereas there were 61 934 in 2000.[1] Ndumo Clinic, in northern KwaZulu-Natal, is a satellite clinic of Mosvold Hospital situated in an area of high malaria incidence. The number of malaria cases detected at the clinic increased from 637 in 1995 to 30 885 in 2000.[2]

In 2000, a study by Bredenkamp et al at Ndumo Clinic found failure of treatment with sulphadoxine/pyrimethamine (SP), then a standard treatment for uncomplicated malaria, in at least 61% of patients.[3] In January 2001, SP ceased to be issued by the Department of Health Pharmaceutical and Medical Supplies Centre for the treatment of malaria in KwaZulu-Natal, and Coartem™ (20mg artemether and 120mg lumefantrine – Novartis South Africa (Pty) Ltd) became the recommended treatment for uncomplicated malaria in non-pregnant patients older than one year in KwaZulu-Natal.[4]

The failure of SP may have been partly responsible for the increase in malaria between 1996 and 2000. Regular studies of the efficacy of SP prior to 2000 might have detected failure of the drug earlier and permitted an earlier change of treatment of falciparum malaria, decreasing the ensuing epidemic. This view is echoed by Durrheim, Sharp and Barnes in their editorial in the South African Medical Journal.[5]

A standard WHO protocol for the assessment of the therapeutic efficacy of antimalarial drugs requires follow-up

of the patients on at least days 0, 1, 2, 3, 7 and 14.[6] Substantial resources are needed and it is difficult to prevent patients from dropping out.

With a previous audit of SP and chloroquine (CQ) in October 2000,[7] and an audit of Coartem™ in February 2001,[8] a single 14-day follow-up of a sample of patients was used as a quick and simple assessment of the efficacy of the existing regime. These audits demonstrated a high failure rate of the SP/CQ regime, but no resistance to Coartem™. Fourteen days was chosen because it is the latest interval at which post-treatment parasitemia could be confidently attributed to treatment failure, rather than recrudescence, in the absence of sophisticated techniques, such as polymerase chain reaction (PCR), to distinguish different strains of parasite. It takes 9-10 days from infection with plasmodium falciparum to the appearance of parasites in the blood (the prepatent period).[9]

However, according to Professor NJ White, the early stage of development of resistance to a combination drug regime containing an artemisenin derivative is unlikely to be detected before 42 days, even using polymerase chain reaction techniques, due to the number of parasites being too low to be detected before 42 days.[10] A 14-day follow-up, although being recommended as the last day of follow-up in a WHO protocol, is an insensitive test of parasite resistance.[6]

PCR is a sensitive test for the presence of malaria parasite DNA, and is also capable of distinguishing between different strains, so that recrudescence of malaria due to resistance to antimalarial drugs can be distinguished from re-infection. When PCR analysis was offered by the Medical Research Council's Malaria Research Programme, it was decided, for the above reasons, to perform a single 42-day follow-up.

Study Population and Methods

Pre-study calculation of sample size

With the previous follow-up study of Coartem™, shortly after its introduction, no resistance was detected at 14 days follow-up.[8] At the time of the present study, the drug had only been in use for one year so it was not expected (nor hoped) that resistance would be detected. A sample size of 30 with complete returns and no malaria detected at follow-up is large enough to be confident of a failure rate of less than 10% (p=0.05), whereas a sample size of 59 is needed to be sure of a failure rate of less than 5% (p=0.05). The study was intended to be cheap, quick and repeatable. The study required medical staff time, a resource considered valuable, so it was decided that only six days would be spent recruiting patients, whom, it was hoped, could all be followed up over six days beginning 42 days later.

Table I: Malaria treatment guidelines (uncomplicated falciparum malaria), KwaZulu-Natal, 2001[4]

Weight	Number of Coartem™ tablets per dose (given twice daily over three days)
10 - <15 kg	1
15 - <25 kg	2
25 - <35 kg	3
35+ kg	4
65+ kg*	4

Exclusions

Patients with severe or complicated malaria, pregnant women, patients younger than five years and patients treated for malaria during the previous two weeks were excluded from the study.

Recruitment and methods

Thirty-seven self-presenting patients at Ndumo Clinic and two at Mosvold Hospital, who had been diagnosed as suffering falciparum malaria by positive immunochromographic test (ICT) (KAT-Quick Malaria Rapid Test for Plasmodium falciparum – Cape Biotech (Pty) Ltd), were assessed by a medical officer. Those not excluded were asked, or in the case of children, the accompanying adult was asked, if they would be prepared to return for a 42-day check. It was explained that the object of the study was to find out how well Coartem™ was working. Consent was obtained in writing from patients aged 14 or older, and in the case of children younger than 14, from an accompanying adult. The consent form was written in English and Zulu.

A thick blood film and blood sample collected on filter paper for PCR analysis was taken on the day of presentation, after diagnosis of malaria with ICT.

Patients were given the recommended treatment for uncomplicated malaria in KwaZulu-Natal, as shown in Table I.[4]

Patients were provided with a written follow-up date, and offered R50 for travelling expenses and a mosquito net on return. Patients were told to return immediately should their condition deteriorate, and were informed that, if this should happen, they would still receive travelling expenses and a net.

Clinic staff were requested to refer study patients with suspected treatment failure to hospital. Upon their return, the patients had their temperature taken, were clinically assessed, and malaria ICT, thick films and PCR samples were taken. ICT was taken to be able to give the patient a quick result.

Thick films were allowed to dry and then stained with 10% Giemsa (5 ml Giemsa diluted with 45 ml phosphate buffer) for 10 minutes, rinsed with tap water and air dried. The films were examined using 100x oil objective. Malaria parasites were counted in conjunction with 300 white cells. The number of parasites thus counted was multiplied by 25 to give an estimate of the number of parasites per microlitre of blood.

Ethical considerations

The study was approved by the Mosvold Hospital Ethical Committee.

Results

At recruitment

Thirty-nine patients (37 from Ndumo Clinic and two from Mosvold Hospital) were recruited for the follow-up study. No patients refused to return when asked to participate. The age-sex distribution of the selected sample is as given in Figure 1. Five patients weighed more than 65 kg.

Temperature at recruitment

The temperatures of the patients, taken when they were recruited, are shown in Table II.

Parasite count at recruitment

The parasite count measured when the patients were recruited is shown in Table III.

Parasite counts are shown divided into classes as recommended in Essential Malariology.[11] It was noticeable that counts were higher than in previous

Figure 1: Age-sex distribution of patients recruited for malaria treatment follow-up at Ndumo Clinic and Mosvold Hospital, January 2002

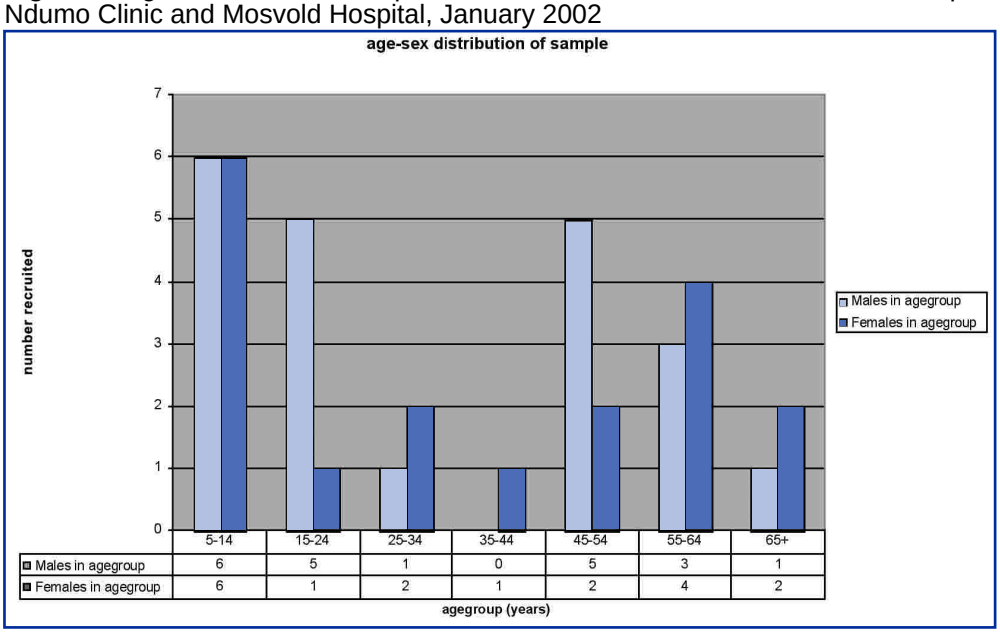

Table II: Temperature of patients at recruitment, Ndumo Clinic and Mosvold Hospital, KwaZulu-Natal, January 2002.

Temperature at recruitment (°C)	Number of patients
37 or less	19
37.1 - 37.5	3
37.6 - 38.0	1
38.1 - 39.0	9
39.1 or higher	7
Total	**39**

Table III: Patient parasite counts at recruitment, Ndumo Clinic and Mosvold Hospital, KwaZulu-Natal, January 2002

Parasite count per µl blood	Number of patients at recruitment
less than 100	11
101-200	0
201-400	0
401-800	0
801-1600	0
1601-3200	4
3201-6400	1
6401-12800	2
12801-25800	3
25801 and over	18
Total	**39**

Table IV: Patient follow-up results, Ndumo Clinic and Mosvold Hospital, KwaZulu-Natal, March 2002

Falciparum malaria confirmed by PCR at recruitment	31(100%)
Film and PCR negative at day 42-45	22(71%)
Film and PCR negative at day 49 after re-treatment on day 7 (although film negative)	1(3%)
Film positive at day 33. Film & PCR negative on day 42 after re-treatment with Coartem™ on day 33	1 (3%)
Patients lost to follow-up	7 (23%)

studies in 2000 and 2001.[7, 8]

PCR results at recruitment

Eight of the recruited patients (seven from Ndumo, one from Mosvold) were both PCR-plasmodium falciparum negative and film-negative, and were therefore not confirmed malaria cases. The other 31 patients at recruitment, two of whom were film negative, were PCR positive, and therefore were confirmed cases.

Follow-up results

The results of PCR at the follow-up of the 31 patients with confirmed malaria are summarised in Table IV:

Of the 31 (30 from Ndumo, one from Mosvold) patients with confirmed falciparum malaria by PCR at recruitment, 24 (23 from Ndumo, one from Mosvold) returned for the follow-up. Unfortunately, seven patients did not return.

Of the 24 returning patients who had initially been confirmed by PCR to be suffering from malaria:

- Twenty-one (20 from Ndumo, one from Mosvold) returned for only the 42-day follow-up. Four of these patients weighed more than 65 kg. All were negative on film and PCR test.
- One woman, aged about 70 years, attended on day 33. She was symptomatic and was attended to by clinic staff, but declined hospital referral. Her ICT was positive and blood film revealed a parasite count of 2080/µl. Unfortunately, a blood spot was not taken for PCR analysis. PCR was attempted using the blood on the slide to try to distinguish between treatment failure and re-infection, but falciparum malaria DNA was not obtained from the small amount of blood on the slide. She was retreated with Coartem™ and was seen again nine days later, when blood film and PCR were both negative.
- One girl aged 10 returned at seven days, when she was seen by clinic staff. Her ICT was positive, but a blood film was negative. She was, however, retreated with Coartem™. She was tracked down to re-attend for further follow-up at day 49 after her first treatment, when she was thick film negative and PCR negative.
- One 45-year-old woman returned on

day 11 feeling unwell. She declined hospital transfer. ICT was positive but blood film was negative. She was not retreated and, when she returned on day 42, was thick film negative and PCR negative.

ICT rapid test results at follow-up

Rapid tests were performed at follow-up to give a quick result to the returning patients. However, 13 of the 24 returning patients had weakly positive tests at their final test, and one patient had a strongly positive test, although all samples taken at the same time were film and PCR negative. It appears that follow-up rapid tests should be interpreted cautiously, even at 42 days.

Conclusions

This study did not prove treatment failure with the use of Coartem™ for *plasmodium falciparum* malaria. The one patient among the 24 returning patients who had parasites on day 33 may have been re-infected. Her blood was film and PCR negative nine days later (day 42) after re-treatment with Coartem™.

Twenty-three patients, one of whom received two treatments, showed no evidence of early emergence of resistance to Coartem™.

Four returning patients weighed more than 65 kg but were successfully treated, despite a lack of data on the efficacy of Coartem™ in patients weighing more than 65kg.[4]

Discussion

A WHO protocol stipulates 14 days as the last day for antimalarial treatment failure.[6] However, authorities on malaria, such as Professor NJ White (Welcome – Mahidol – Oxford Malaria Research Unit, Mahidol University, Thailand), consider this to be an insensitive means of detecting early stages of malaria parasite resistance. If parasites are present at 14 days, then the parasite has already developed a high degree of resistance to the drug and the drug has already become virtually useless. Hence, a 14-day follow-up, rather than being an early warning system for the development of drug resistance, is actually a too-late warning system. For this reason, a 42-day follow-up was chosen to detect late recrudescence, which occurs in the early stages of the development of drug resistance.

The study encountered a number of difficulties:

- Enhanced malaria control in the area had reduced the number of cases to such an extent that recruitment in a short period of time became difficult. Whereas it would have been possible to recruit more than 100 patients in a single day in March 2000, by January 2002 only 37 patients could be recruited in a week using a rapid malaria test, and only 31 of these were confirmed to be malaria cases by microscopy and PCR.
- The discrepancy between the rapid test performed by malaria control programme personnel and microscopy and PCR may be due to a number of reasons. However, the study did not set out to assess the rapid test and the authors are therefore not in a position to comment further on this discrepancy.
- Ndumo Clinic does not have permanent medical staff. It had been envisaged that suspected early treatment failures before 42 days would be referred to Mosvold Hospital, 50 km away. However, the returning patients declined to be referred. As a result, there were instances of re-treatment and the failure to obtain PCR specimens from one patient, which would have permitted differentiating between a recrudescent infection and re-infection. Better contingency plans involving the permanent clinic nursing staff should have been made for early returns.
- There was a substantial drop-out rate – seven of the 31 confirmed cases, which weakens the results. Although a six-week follow-up is a sensitive test of recrudescence, it is also a long interval for a patient to keep to, even with a financial incentive.

Despite these weaknesses, it should be kept in mind that, in 2000, there was at least a 61% resistance rate to sulphadoxine/pyrimethamine, then the first-line drug for malaria treatment.[3] This level of resistance would probably have been detected in a study involving just 10 out of the tens of thousands of cases per year occurring in northern KwaZulu-Natal at the time. Twenty-three of the 24 returning patients showed no trace of recrudescence at 42 days, from which some comfort may be drawn.

Preventing patients from dropping out is a difficulty which becomes even greater in a study involving multiple follow-up visits, as required by the WHO protocol.[6] Malaria tends to occur in poor rural areas and the resources required to trace non-returnees at their homes are substantial. These resources may be a deterrent to regular checks on parasite resistance. Had regular studies into malaria parasite drug resistance been performed in KwaZulu-Natal from 1997 to 1999, when the malaria situation was deteriorating rapidly, the failure of sulphadoxine/pyrimethamine might have been detected earlier and the epidemic might have been curtailed. Simple, cheap studies, repeated at regular intervals, would have a good chance of detecting changes in parasite sensitivity to antimalarial drugs.

The authors believe that regular monitoring of antimalarial drug efficacy is indicated in view of the history of emerging resistance to previous treatments.[3, 5]

Competing interests

None declared.

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