
Technical consultation to review the effectiveness of rectal artesunate used as pre-referral treatment of severe malaria in children

Meeting report,
18–19 October 2022

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Abbreviations

ACT	artemisinin-based combination therapy
AOR	adjusted odds ratio
CARAMAL	Community Access to Rectal Artesunate for Malaria Project
CFR	case fatality rate
CHAI	Clinton Health Access Initiative
CHW	community health worker
CI	confidence interval
COVID-19	coronavirus disease
iCCM	integrated community case management
K13	kelch 13
MMV	Medicines for Malaria Venture
mRDT	malaria rapid diagnostic test
OR	odds ratio
PHC	primary health care
RAS	rectal artesunate
RDT	rapid diagnostic test
RHF	referral health facility
Swiss TPH	Swiss Tropical and Public Health Institute
UNICEF	United Nations Children's Fund
VHW	village health worker
WHO	World Health Organization

Executive summary

Since 2006, the World Health Organization (WHO) has recommended rectal artesunate (RAS) as an effective pre-referral treatment for severe malaria. RAS rapidly clears 50% of malaria parasites or more within 6–12 hours. In a controlled trial reported in 2009, pre-referral RAS was shown to reduce mortality or permanent disability by up to 50% in children under 6 years of age who did not reach a referral facility for more than six hours.

In 2017, the Community Access to Rectal Artesunate for Malaria Project (CARAMAL) was set up to implement and evaluate the introduction of RAS in selected areas of three countries. Preliminary results from CARAMAL were presented to the WHO Global Malaria Programme and the Malaria Policy Advisory Group in 2021. It appeared that these results did not confirm the mortality impact that had been observed in the controlled trial. Consequently, WHO released an information note on RAS in January 2022, suggesting immediate risk mitigation measures.

In October 2022, to provide clarity on the evidence available, WHO convened a technical consultation of independent experts to conduct a formal evidence review of several studies evaluating the effectiveness of RAS as a pre-referral treatment of severe malaria. In addition to the CARAMAL study publications, the review included other studies from early-use countries deploying RAS at the programmatic level. The main objective of the technical consultation was to develop guidance for the safe and effective implementation of this intervention based on the evidence from areas where RAS has already been implemented.

The technical consultation was undertaken over the course of two meetings. During the first meeting on 20–21 September 2022, independent experts reviewed all of the studies and identified questions for the study teams from Swiss Tropical and Public Health Institute (Swiss TPH) and other RAS implementation projects. Responses to the specific questions provided the basis for the second meeting, which was held in person on 18–19 October 2022 and included independent experts and the investigators for the several studies. During this second meeting, the WHO-appointed expert panel recommended additional analyses for the CARAMAL database. The outcomes of the review and results of the additional analyses underpin the conclusions of the technical consultation.

RAS and mortality

The technical review identified several issues in the design of the CARAMAL study, which have left it susceptible to a number of biases and made the results difficult to interpret, particularly in terms of the impact of RAS on mortality and referral completion.

The CARAMAL study design was powered to detect a reduction in the case fatality rate (CFR) among children receiving RAS using pooled data from the three participating countries. However, during the study, it became apparent that the health care systems and baseline CFRs for severe malaria differed substantially between countries. Indeed, the CFR was much lower in Uganda (0.5%) than in the Democratic Republic of the Congo (6.7%) and Nigeria (11.7%). Therefore, each country was analysed separately, even though the study was not designed or powered for such analysis; this substantially reduced the power of the study to detect the effects of RAS.

The primary analysis compared children who received RAS to those who did not. The untreated group included all severe malaria cases in the pre-RAS period, with

potential temporal confounding given the evidence, at least in Nigeria, that the CFR was substantially lower in the pre-RAS period than in the post-RAS period, including among children untreated with RAS in the post-RAS period.

There is no evidence that the increased CFR observed in Nigeria in the post-RAS period was due to RAS. An additional analysis comparing the CFRs in RAS users and non-users only in the post-RAS period to avoid temporal confounding resulted in an odds ratio (OR) of 1.45 (95% CI: 0.68–3.09). The measured differences and temporal confounding between the RAS user and non-user groups (pre- and post-RAS periods) were not suitably accounted for in the analyses. Therefore, it is difficult to interpret the relationship between RAS use and change in CFR.

Implementation research on scaling up the use of RAS for treatment of severe malaria at the community level in Zambia showed that the CFR decreased from 3.1% to 0.1% in the two high-intensity intervention districts and from 10.7% to 1.4% in the other districts. At the end of that study, there were fewer stockouts of RAS, better knowledge of the signs of severe malaria among the community health workers (CHWs) and better knowledge of how to manage severe malaria among health workers at health facilities. Of the 11 486 children identified with suspected severe malaria at the community level, 97% were administered RAS and 96% were referred to a health facility. Besides RAS, several other supporting activities were implemented. The project confirmed that effective implementation of a community-based RAS intervention requires identification and tackling of health system bottlenecks, such as localized drug and commodity shortages, inadequate supervision of community health volunteers and weak referral systems. It also requires attention to barriers that contribute to poor access to health services such as community-managed food banks and emergency saving schemes. In this setting, bicycle ambulances probably had a major effect on the uptake of referral advice. Therefore, the importance of ensuring effective referral and ongoing monitoring of the continuum of care following the roll-out of RAS cannot be underestimated.

Care-seeking for danger signs and referral completion

In the CARAMAL study areas in northern Uganda, results from annual household surveys conducted between 2018 (pre-RAS) and 2019 and 2020 (post-RAS) showed low care-seeking for children under 5 years of age with a febrile illness, with or without danger signs, both in the public (26%) and private (18%) sectors. Despite the presence of over 5000 CHWs in 81% of villages in the study area, only 13% of children with integrated community case management (iCCM) danger signs sought care from CHWs. The study team mentioned the frequent stockouts of RAS as the main reason for low care-seeking from CHWs. Care-seeking at the referral health facility (RHF) was even lower at 2%. While 48.3% of the young children with danger signs received an antimalarial from any source of care, less than 1% received RAS.

Among those who received RAS, a large proportion of older children did not receive the required full dose of RAS. The percentage of children over 3 years of age who received one suppository instead of the required two was 86% in the Democratic Republic of the Congo, 32% in Nigeria and 58% in Uganda.

Children with severe malaria should be referred to a treatment centre whether or not they receive RAS. Overall, referral completion (i.e. reaching a study-designated RHF) was low – at 67% in the Democratic Republic of the Congo, 48% in Nigeria and 58% in Uganda. Additional analyses in the post-RAS period showed that referral completion was not associated with RAS use in the Democratic Republic of the Congo (adjusted OR [AOR]: 1.17; 95% CI: 0.66–2.07) or Uganda (AOR: 0.84; 95% CI: 0.64–1.10).

In Nigeria, while referral completion was not associated with RAS use among young children enrolled in primary health care (PHC) (AOR: 0.93; 95% CI: 0.20–4.26), referral completion was lower among RAS users (73%) than among non-users (95%) enrolled by Nigerian CHWs (AOR: 0.09; 95% CI: 0.0–0.37). The exclusion of referral facilities that were not study-designated is a further limitation of this CARAMAL study assessment, as it may underestimate referral completion. It is also important to note that children who died early could not complete referral.

An important finding of the CARAMAL study was that children with severe malaria often received suboptimal treatment with injectable artesunate and an artemisinin-based combination therapy (ACT) when presenting to a referral facility, particularly in the Democratic Republic of the Congo and Nigeria. If a referral facility provides suboptimal treatment, any beneficial impact of RAS is likely to be significantly reduced or negated. However, in Malawi, a controlled implementation study showed that over 93% of young children with danger signs complied with referral instructions, even with rapid improvement following administration of RAS.

The CARAMAL study did not show RAS, as implemented in the study areas, to be effective in reducing mortality from severe malaria. It seems likely that this finding was because, in Uganda, there was already an effective severe malaria management strategy in place and a low CFR, and, in the Democratic Republic of the Congo and Nigeria, the existing health system framework was not sufficiently strengthened to ensure that children completed referral and received an appropriate full course of antimalarial treatment at a referral centre (hospital). To reduce the CFR for children with severe malaria, there needs to be a functional continuum of care for severely ill children, with a good referral system and referral facilities equipped to comprehensively manage a severely sick child.

Artemisinin resistance

The CARAMAL study also reported that, in a sub study in Uganda, the prevalence of the *kelch 13* (K13) C469Y marker for partial artemisinin resistance increased at day 28 post-RAS in children who failed to complete referral treatment (20%) compared to on day 0 in children presenting at an RHF (6.2%). However, this finding was difficult to interpret, as it was based on a relatively small number of children and convenience sampling was used.

K13 C469Y molecular markers for partial artemisinin resistance were present in Uganda before RAS was deployed and were widely present and increasing in the northern provinces – in some CARAMAL districts (Kole and Oyam, but not Kwanja districts) and in other districts (e.g., Lamwo and Agago districts) where RAS was not deployed. Uptake of RAS and treatment-seeking from CHWs appeared very low in Uganda (less than 1% in household surveys among children with symptoms of severe malaria). Similar increases in resistance marker were not seen in the CARAMAL study sites in the Democratic Republic of the Congo or Nigeria. The impact of pre-referral RAS on the selection of K13 mutations associated with partial artemisinin resistance is likely to be very context specific.

Despite the limitations noted above, this study provides a signal that RAS alone, when not followed by referral and complete treatment with a full course of ACT, may select partial artemisinin-resistant parasites with the K13 C469Y mutation. This mutation was shown to have emerged locally in East Africa and to be associated with increased tolerance to artemisinins in the ring-stage survival assay.

Draft conclusions

- Countries that are already implementing or considering implementation of RAS for pre-referral treatment of severe malaria need to strengthen all aspects of the continuum of care for a severely sick child – from CHWs being adequately trained and stocked for giving RAS in the areas where it is most needed, to ensuring rapid transfer and access to referral facilities where a complete course of post-referral treatment is given as per WHO guidelines for the treatment of severe malaria.
- Support for adequate supply chain management and referral systems from CHWs and facilities to treatment centres is essential for achieving the intended impact of RAS. Barriers to referral completion need to be addressed, as this will improve outcomes not only for severe malaria but also for other severe diseases.
- Effective community sensitization is needed to increase understanding of severe malaria, its causes, how dangerous it is for children, how to recognize danger signs and the need to promptly seek care if such signs are present.
- Countries deploying RAS for pre-referral treatment of severe malaria should review, monitor and, as necessary, strengthen the whole continuum of care.
- Malaria programmes and their partners in the public, nongovernmental organization and private sectors should ensure that health providers adhere strictly to malaria treatment guidelines and make sure that caregivers of children with severe malaria are aware of the importance of completing treatment courses. Intense efforts should be made to ensure that:
 - artemisinin-based monotherapies (both rectal and parenteral) are used for treating severe malaria cases only as per WHO guidelines;
 - RHF treat severe malaria patients with parenteral artesunate and a full course of an effective ACT;
 - appropriate supportive management excludes or treats other concurrent infections that could be causing danger signs in a child with low-density parasitaemia; and
 - initial rectal and/or injectable artemisinin-based monotherapy is always followed by a full oral course of an effective ACT.
- Antimalarial resistance surveillance should be strengthened at the population level across Africa, and most urgently in East Africa, with:
 - prioritization of interventions to holistically address the drivers of resistance selection; and
 - prompt response in line with the WHO Strategy to respond to antimalarial drug resistance in Africa (1) when resistance is detected.

1. Background

Since 2006, the World Health Organization (WHO) has recommended rectal artesunate (RAS) as an effective pre-referral treatment for severe malaria (2). RAS has been shown to be feasible and acceptable at the community level. When given at the appropriate dose, RAS rapidly clears 50% of malaria parasites or more within 6–12 hours and can reduce mortality or permanent disability by up to 50% in treated children under 6 years of age who are referred to and reach a facility in more than six hours (3).

A project to implement and evaluate the introduction of RAS in selected areas of three countries, supported by Unitaid, was approved in April 2017. The Community Access to Rectal Artesunate for Malaria Project (CARAMAL) was led by the Clinton Health Access Initiative (CHAI), implemented by the United Nations Children's Fund (UNICEF) in the Democratic Republic of the Congo, Nigeria and Uganda, and evaluated by the Swiss Tropical and Public Health Institute (Swiss TPH). The Medicines for Malaria Venture (MMV) and WHO also supported the project through enabler grants to ensure the supply of quality-assured RAS and to derive operational guidance from the lessons learned. The CARAMAL multi-country observational study was based on an overall pre-post intervention analysis without comparator and included an individual analysis of outcomes in RAS users compared to non-users.

In April 2021, the WHO Global Malaria Programme, as part of its role in the Unitaid enabler grant, convened a technical consultation to review the lessons learned from the CARAMAL project and other implementation studies on RAS as a pre-referral treatment of severe malaria. At the time of the WHO consultation, the unpublished report by Swiss TPH was based on a preliminary analysis. The report indicated that there was a higher malaria case fatality ratio (CFR) after deployment of RAS, and there appeared to be a significantly higher prevalence of *kelch 13* (K13) mutants in children who received RAS but failed to complete referral and receive the full course of artemisinin-based combination therapy (ACT).

The preliminary CARAMAL results were presented to the Malaria Policy Advisory Group in October 2021. Based on the findings and main conclusions, the Malaria Policy Advisory Group urged the WHO Global Malaria Programme to:

- advise countries that had not yet introduced the intervention to await further guidance before adopting and deploying RAS;
- notify countries that had adopted RAS about the risk of negative effects if the WHO recommendation cannot be fully implemented, including referral for complete treatment, and the need to ensure the quality of care throughout; and
- conduct an evidence review and develop guidance for the conditions under which this tool can be implemented safely and effectively.

In line with the Malaria Policy Advisory Group's recommendations in January 2022, the Global Malaria Programme issued an information note (4), including the same recommendations and making the following commitment:

The WHO Global Malaria Programme, in consultation with other relevant departments, will conduct a formal evidence review and develop detailed guidance on the conditions under which the use of this tool can be implemented safely and effectively. Such guidance will be shared with countries as soon as it becomes available.

In October 2022, the Global Malaria Programme convened a technical consultation to conduct an evidence review of the CARAMAL project and to develop guidance on the conditions under which this tool can be implemented safely and effectively. This document is the report of that technical consultation. The review considered the CARAMAL study publications and report, as well as additional information from early-use countries deploying RAS at the programmatic level.

Evidence review process

WHO convened an independent group of experts to review all CARAMAL published studies and online unpublished pre-prints from the study, as well as other relevant studies conducted in Angola, Malawi, Senegal, Sierra Leone and Zambia evaluating RAS deployment at the programmatic level. The experts included methodologists with expertise in the review of observational studies, biostatisticians, clinical research epidemiologists, clinical pharmacologists, paediatricians, integrated community case management (iCCM) experts and health systems experts with specific professional experience in high-burden malaria-endemic countries (Annex 1). The review was undertaken over the course of two meetings.

The first meeting was convened on 20–21 September 2022 with the objective of conducting an in-depth review of the studies and developing additional questions and areas of clarification directed to the study teams (Swiss TPH and principal investigators of other RAS implementation projects), the responses to which would form part of the background materials for the second meeting. All pre-reads were shared with all experts, and two experts were assigned as lead reviewers for specific sets of studies and to lead the presentation and discussions on the assigned topics. The first meeting was convened remotely and the Rapporteur consolidated the questions to submit to Swiss TPH and the principal investigators of other RAS implementation projects two weeks prior to the second meeting, which was in person. The list of pre-reads reviewed and list of formal questions elaborated are included as Annex 2.

Swiss TPH provided responses to the specific questions, enclosed as Annex 3, and both the questions and responses formed the basis for the second meeting of the WHO technical consultation, which was held in person on 18–19 October 2022.

This consultation was organized in two sessions:

- a one-day “open session” with participation of the principal investigators, observers and interested stakeholders to further discuss the specific and other questions asked by the experts in more detail; and
- a one-day “closed session” only for the experts and the WHO Secretariat to agree on the interpretation of the study findings and develop recommendations to the Global Malaria Programme.

The experts recommended further analysis of the impact and referral data. Following a data transfer agreement and presentation of a statistical analysis plan (Annex 4), Swiss TPH granted access to the CARAMAL database for further analysis. The results of these analyses are included in this report.

The full list of participants and agenda of the technical consultation are provided in Annexes 1 and 5, respectively.

This meeting report is divided into the following sections:

- Methodological reviews of RAS studies: from randomized controlled trial to effectiveness studies
- Effectiveness of RAS as pre-referral treatment in the CARAMAL project
- Pre-referral RAS and referral completion in the CARAMAL project
- Treatment-seeking of children at the community level in Nigeria and Uganda in the CARAMAL project
- Clonal expansion of artemisinin-resistant falciparum malaria in Uganda in the CARAMAL project
- Real-world costs, financing and economic evaluation of RAS
- Observations on RAS implementation studies in Malawi, Sierra Leone and Zambia

Each section of the report includes the related conclusions and draft recommendations.

2. Methodological reviews of RAS studies: from randomized controlled trial to effectiveness studies

Lengeler C, Burri C, Awor P, Athieno P, Kimera J, Tumukunde G, et al. Community access to rectal artesunate for malaria (CARAMAL): a large-scale observational implementation study in the Democratic Republic of the Congo, Nigeria and Uganda. *PLoS Glob Public Health*. 2022. doi:10.1371/journal.pgph.0000464 (5).

2.1 Key outputs of the paper

The paper described the CARAMAL study, the study countries (Democratic Republic of the Congo, Nigeria and Uganda), site selection, health system environment, roll-out of RAS and evaluation methods including data collection from three main sources: a patient surveillance system; three health provider surveys (one pre roll-out and two post roll-out); and three household surveys (one pre roll-out and two post roll-out). Blood samples were also collected for monitoring artemisinin resistance.

The patient surveillance system tracked patients at three contact points: patients provisionally enrolled and assigned a unique study ID at the level of RAS administration (either a community health worker [CHW] or primary health care [PHC] facility); patients enrolled at referral health facilities (RHF) to collect data on severe malaria treatment; and a follow-up visit by study staff at day 28 post-RAS. Of note, during the day-28 home visit, study nurses asked for information on the referral process and antimalarial treatment (including RAS). Sample sizes were estimated to show: a 30% reduction in CFR post roll-out across countries; a 19% decrease from a baseline of 80% in minimum acceptable coverage (availability of RAS and adherence to case management guidelines, including referral) at health provider level pooled across countries; and an increase from 15% to 20% in treatment-seeking pooled across countries.

The paper described the identification of countries and districts and the reported endemicity of malaria cases in children and district populations before the implementation of the study. The numbers of patients enrolled was very heterogeneous across countries and there was a lower-than-expected CFR in Uganda. Enrolment also varied within countries at the CHW or PHC facility level. Despite the fact that the numbers of children < 5 years in the study areas in the three countries (130 000–200 000) were comparable, there were large differences in the number of children < 5 years per community-based provider (690 in the Democratic Republic of the Congo, 284 in Nigeria and 46 in Uganda), reflecting differences in the number of CHWs in the three countries. The total enrolment numbers to the patient surveillance system varied between countries (Democratic Republic of the Congo: 5540, Nigeria: 1505, Uganda: 6713), and there were also differences in the proportion of enrolment between CHWs, PHC and RHF within each country.

The paper also described RAS distribution and dosing. Many children over the age of 3 years were underdosed, with just one suppository administered instead of two (83% in the Democratic Republic of the Congo, 32% in Nigeria and 55% in Uganda).

RAS roll-out was different between countries, as Uganda achieved high coverage after eight months and the Democratic Republic of the Congo achieved high coverage within three months. In Nigeria, coverage was variable, with several peaks and troughs of RAS use that rarely exceeded 60%. Methods of RAS distribution varied between countries, and a key conclusion noted in the paper was that stockouts at the CHW level reduce confidence in CHWs' ability to provide treatment when needed and contribute to low usage at that level.

2.2 Review

Sample size: Although the sample size calculations were done based on the assumption that the statistical analyses would be carried out on the pooled data from the three countries together, each country was analysed separately due to the heterogeneity in roll-out and recruitment among the countries and clear differences in the results. This was considered to be an appropriate action, but no country was individually powered at the 80% level to detect a 30% reduction in CFR, assuming a pre-RAS CFR of 6%.

Study areas: The criteria for choosing the study areas were not sufficiently discussed in the paper. In addition, the situation on the ground differed from previous reports. From the presentation and discussions, it was established that a primary requirement for country and district selection was specifically a UNICEF-supported system of CHWs implementing iCCM, not just any system supporting CHWs implementing iCCM. This requirement was not explicitly stated in the paper. The time for referral in terms of the number of hours of travel from the CHWs to the referral facilities was not available and the only information recorded was whether children were referred on the same day of treatment or later.

Research questions: Two questions of equal importance were considered to be not fully addressed by the study and the effectiveness analysis, namely:

- the overall impact of implementing the RAS roll-out strategy on health outcomes; and
- the impact of RAS on the health of children receiving RAS compared to those not receiving RAS, within the post roll-out period only.

For the first question, comparisons of the CFRs (% n/N) pre and post RAS roll-out by country were provided, but no results were presented from an analysis controlling for patient and temporal confounders. For the second question, a comparison between RAS users and non-users in the post roll-out period only was presented, but with no data on CFRs.

The effectiveness analysis done by the study team compared RAS users to non-users over the whole study period, including those enrolled before the RAS roll-out in the non-user group. The justification was that they were interested in the individual health impact of RAS. However, it is difficult to interpret this comparison, as it includes a period when RAS was not available. Furthermore, this analysis was not planned and included in the statistical analysis plan. Determinants of changes over time were not fully accounted for in the analyses and other analytical methods may be able to accomplish this better (such as interrupted time series analyses, which have been used for other pre-post studies). This problem was most apparent in Nigeria, where the CFR among RAS non-users increased substantially after RAS introduction. Such a change between the pre and post roll-out periods was not reported in the published paper. In addition, the analyses presented in the paper's abstract, and tables incorrectly adjusted for the timing of referral and treatment received, as these variables are on the causal pathway and are not confounder variables.

Potential confounders: There was a possible recall bias related to the day-28 home visit, when the study nurses asked information on the referral process and antimalarial treatment (including RAS); this information may have been biased for children who died.

The significant differences in recruitment between providers (CHWs, PHC and RHF) within each country and across countries may also have confounded comparison of the CFRs between the different periods (pre- and post-RAS periods). This may have influenced the findings, especially in Nigeria where sicker children presented at PHC facilities, and the percentage of patients enrolled in PHC facilities was 11% for the pre-RAS period and 25% for the post-RAS period.

Another limitation was that study staff were based only within inpatient areas of referral facilities and therefore may not have captured referred children who had improved enough to be treated as outpatients.

Primary outcome: There was also a consideration of whether the effectiveness of RAS on health outcomes was a suitable primary end-point, or whether the primary end-point and focus should be on successful referral and completion of treatment. This is important, as RAS is part of a continuum of care. After RAS, WHO recommends referral to a health facility for intravenous artesunate followed by a three-day oral course of ACT. Furthermore, some children may have been suffering from other severe illnesses (such as sepsis and pneumonia) (6), highlighting the importance of successful referral to a health facility as the primary outcome.

Availability of RAS and provider: The frequent stockouts and challenges with availability of RAS at the CHW level may have led the CHWs to reserve their stock of RAS for sicker children, creating a bias in the children who received RAS.

It was not possible to adjust for the individuality of health workers and availability of the drug, which may have led to some bias as described above. Some of the models in the analysis examining health outcomes included clustering at the provider level as

a random effect, but this has led to a large change in the odds ratios (ORs) for some outcomes. This issue needs to be explored further to better understand the clustering due to different treatment and referral practices by individual providers and the potential influence that single providers may have had on study findings.

Treatment of severe malaria: The methods used to collect information could have led to potential biases in the data; for example, information on ACTs often came from the prescriptions from study RHF and not the treatment administered; the difference between the two was not recorded. In addition, the data available on injectable antimalarials prior to the RAS roll-out were limited, making it difficult to compare data over the whole study period. There was also the potential for recall bias for the data on “treatment received”, as much of this information was collected from caregivers during the day-28 post-RAS visit.

2.3 Additional data/evidence from responses and presentations

Swiss TPH shared the data with a statistician on the WHO expert panel to undertake further analyses, as detailed in the statistical analysis plan agreed by Swiss TPH and WHO (see Annex 4). The results of these analyses are presented in **Tables 1, 2a** and **2b** below.

Table 1 presents the results for the day-28 mortality outcome. In Nigeria, there was a four-fold increase in the CFR during the post-RAS period compared to the pre-RAS period (OR: 4.30; 95% CI [unadjusted with a random effect term for clustering by the 139 health care providers] 1.89–9.80). However, this finding was attenuated greatly when adjusting for age, sex, danger signs, season, enrolment location and, in particular, month of enrolment (adjusted OR [AOR]: 1.76; 95% CI: 0.55–5.65). This difference highlights the temporal confounding, as the CFR for those not receiving RAS was 12.1% in the post-RAS period and 4.2% in the pre-RAS period. Comparing RAS users to non-users in the post-RAS period, the CFR was 19.7% for RAS users and 12.1% for non-users. However, when adjusting for confounders (including month of enrolment), the OR was 1.45 (95% CI: 0.68–3.09), showing only a moderate difference that was not statistically significant.

In the Democratic Republic of the Congo, the CFRs were similar for RAS users and non-users in the pre- and post-RAS periods (6.6% and 6.7%). In the post-RAS period, there was a two-fold increase in the CFR for RAS users compared to non-users (7.0% versus 4.1%; AOR: 2.39; 95% CI: 1.04–5.49). However, only 10% of children observed during the post-RAS period did not receive RAS (seven deaths reported for 173 children). Therefore, these results should be interpreted with caution.

In Uganda, the CFR was very low overall: 0.5%. The CFR increased from 0.3% in the pre-RAS period to 0.7% in the post-RAS period, mostly driven by those who did not use RAS (1.3% in non-users compared to 0.4% in users in the post-RAS period).

Tables 2a and 2b present the results for the referral completion outcomes. The new analyses did not adjust for variables measured after referral, as these are on the causal pathway. This is a key difference between the analyses presented here and those in Brunner et al., *BMJ Global Health* (7). In Nigeria, referral completion differed greatly between those enrolling at the CHW or PHC level. Therefore, in Table 2b, the results are presented separately for those groups (as also presented in Brunner et al., *BMJ Global Health*). In the Democratic Republic of the Congo, the percentage of children completing referral decreased marginally in the post-RAS (66.3%) versus

pre-RAS (69.0%) period, with a larger difference observed between RAS users (64.7%) and non-users (79.9%) in the post-RAS period. Of note, clustering due to health care provider had a big impact on the results, with great variation in the number of children enrolled per provider (mean of 14, range of 1–175 children). Similar patterns of referral completion were observed in Uganda, but of smaller magnitude in the post-RAS period (RAS users 53.4% versus non-users 56.8%). In Nigeria, referral completion was very low for patients seeing CHWs, but increased in the post-RAS period (22.1% versus 7.3% in the pre-RAS period). For children attending PHC facilities, referral completion was much higher than with CHWs and increased during the post-RAS period (83.3% versus 73.3% in the pre-RAS period). However, in the post-RAS period only, referral completion among RAS users was lower (72.9%) than among non-users (94.9%). There was much heterogeneity in referral completion over the study months and a small sample size per month. Therefore, it was not possible to adequately control for month of enrolment.

Table 1. Effect of RAS on day-28 mortality

	Democratic Republic of the Congo			Nigeria			Uganda		
	n/N (%)	Unadj OR (95% CI)	Adj OR (95% CI)	n/N (%)	Unadj OR (95% CI)	Adj OR (95% CI)	n/N (%)	Unadj OR (95% CI)	Adj OR (95% CI)
Overall	135/2011 (6.7%)	-	-	69/589 (11.7%)	-	-	19/3686 (0.5%)	-	-
Implementation									
Pre-RAS	20/304 (6.6%)	1	1	9/217 (4.2%)	1	1	4/1441 (0.3%)	1	-
Post-RAS	115/1707 (6.7%)	0.94 (0.56, 1.58)	1.58† (0.79, 3.17)	60/372 (16.1%)	4.30 (1.89, 9.80)	1.76† (0.55, 5.65) 3.44* (1.51, 7.86)	15/2245 (0.7%)	2.42 (0.80, 7.30)	-
RAS use overall (results from Hetzel et al., BMC Medicine (8))									
No	27/475 (5.7%)	1	1	30/391 (7.7%)	1	1	12/2018 (0.6%)	1	-
Yes	108/1536 (7.0%)	1.25 (0.81, 1.93)	1.97† (0.88, 4.35) 2.04† (1.16, 3.57)	39/198 (19.7%)	2.95 (1.78, 4.90)	2.57† (1.40, 4.73) 1.58† (0.77, 3.25)	7/1668 (0.4%)	0.70 (0.29, 1.74)	-
Post RAS roll-out									
RAS use									
No	7/173 (4.05%)	1	1	21/174 (12.07%)	1	1	8/624 (1.28%)	1	-
Yes	108/1534 (7.04%)	1.99 (0.88, 4.46)	2.39† (1.04, 5.49)	39/198 (19.7%)	2.01 (0.99, 4.06)	1.45† (0.68, 3.09)	7/1621 (0.43%)	0.33 (0.12, 0.92)	-

† Adj OR – adjusted for age group, sex, danger signs (convulsion, sleepy/unconscious, not able to drink), rainy season, enrolment location (Nigeria only), month of enrolment (included as a continuous variable and assuming a linear association); * **not adjusting for enrolment month**; the values provided in blue are from analyses adjusted for all these variables and not the values presented in the Hetzel et al. paper.

‡ Adj OR – Democratic Republic of the Congo – age (< 1 year), sex, pre/post RAS roll-out, rainy season, danger signs (convulsion), enrolment location; Nigeria – danger signs (convulsion), enrolment location

Health care provider (clustering unit over entire study period, included as a random effect in above logistic regression models):

- Democratic Republic of the Congo – total of 138 providers, average number of patients/provider (min, max) = 15 (1, 176)
- Nigeria – total of 139 providers, average number of patients/provider (min, max) = 4 (1, 42)
- Uganda – total of 159 providers, average number of patients/provider (min, max) = 23 (1, 505)

Enrolment location: Democratic Republic of the Congo – CHW 4.5%, PHC 95.5%; Nigeria – CHW 53.3%, PHC 46.7%; Uganda – all CHW

Table 2a. Effect of RAS on referral completion in the Democratic Republic of the Congo and Uganda

	Democratic Republic of the Congo			Uganda		
	n/N (%)	Unadj OR (95% CI)	Adj OR (95% CI)	n/N (%)	Unadj OR (95% CI)	Adj OR (95% CI)
Overall	1308/1962 (66.7%)	-	-	2111/3664 (57.6%)	-	-
Implementation						
Pre-RAS	203/294 (69.0%)	1	1	898/1433 (62.7%)	1	1
Post-RAS	1105/1668 (66.3%)	0.63‡ (0.40, 0.98) 0.88 [^] (0.67, 1.15)	0.61† (0.34, 1.09)	1213/2231 (54.4%)	0.68‡ (0.57, 0.81) 0.71 [^] (0.62, 0.81)	0.66† (0.51, 0.86)
Post RAS roll-out						
RAS use						
No	135/169 (79.9%)	1	1	351/618 (56.8%)	1	1
Yes	970/1499 (64.7%)	1.02‡ (0.59, 1.80) 0.46 [^] (0.31, 0.68)	1.17† (0.66, 2.07)	862/1613 (53.4%)	0.81‡ (0.63, 1.04) 0.87 [^] (0.72, 1.05)	0.84† (0.64, 1.10)

[^] Crude OR with no adjustment for clustering

[†] Adj OR – adjusted for age group, sex, danger signs (convulsion, sleepy/unconscious, not able to drink), rainy season, month of enrolment (included as a continuous variable and assuming a linear association)

^{‡,†} Health care provider (clustering unit over entire study period, included as a random effect in above logistic regression models):

- Democratic Republic of the Congo – total of 136 providers, average number of patients/provider (min, max) = 14 (1, 175), missing referral outcome for 49
- Uganda – total of 159 providers, average number of patients/provider (min, max) = 23 (1, 505), missing referral outcome for 22

Enrolment location: Democratic Republic of the Congo – CHW 4.5%, PHC 95.5%; Uganda – all CHW

Table 2b. Effect of RAS on referral completion in Nigeria

	Nigeria – CHW		Nigeria – PHC	
	n/N (%)	Unadj OR (95% CI)	n/N (%)	Unadj OR (95% CI)
Overall	40/264 (15.2%)	-	203/249 (81.5%)	-
Implementation				
Pre-RAS	9/124 (7.3%)	1	33/45 (73.3%)	1
Post-RAS	31/140 (22.1%)	2.78‡ (0.91, 8.52) 3.63 [^] (1.65, 7.98)	170/204 (83.3%)	3.30‡ (1.23, 8.89) 1.81 [^] (0.85, 3.87)
Post RAS roll-out				
RAS use				
No	12/60 (20.0%)	1	92/97 (94.9%)	1
Yes	19/80 (23.8%)	1.25‡ (0.40, 3.90) 1.25 [^] (0.55, 2.82)	78/107 (72.9%)	0.10‡ (0.03, 0.35) 0.15 [^] (0.05, 0.40)

[^] Crude OR with no adjustment for clustering

[†] Adj OR – adjusted for age group, sex, danger signs (convulsion, sleepy/unconscious, not able to drink), rainy season

* **not adjusting for month of enrolment**

‡ Health care provider (clustering unit over entire study period, included as a random effect in above logistic regression models):

- Nigeria, CHW – total of 94 providers, average number of patients/provider (min, max) = 3 (1, 30), missing referral outcome for 50
- Nigeria, PHC – total of 29 providers, average number of patients/provider (min, max) = 9 (1, 39), missing referral outcome for 26

2.4. Summary of key findings

The study design, data and analyses are susceptible to many biases and, therefore, it is difficult to draw any conclusions about the impact of RAS on mortality. There is no clear evidence that the increased CFR observed in Nigeria in the post-RAS period was due to RAS, given the OR of 1.45 (95% CI: 0.68–3.09) when comparing RAS users and non-users in the post-RAS period.

The measured differences and temporal confounding between the RAS non-user group (pre- and post-RAS periods) and user group were not suitably accounted for in the analyses, and the comparison presented in the effectiveness paper is difficult to interpret.

The additional analyses showed that there is a large degree of temporal confounding in Nigeria, with a three-fold higher CFR among RAS non-users in the post- versus pre-RAS periods, supporting the conclusion that the increase in CFR observed in Nigeria in the post-RAS period could not be attributed to RAS. Referral completion was low in all three countries and varied greatly by health care provider in Nigeria. Furthermore, in all three countries, there were moderate to small declines in referral completion when comparing RAS users to non-users, highlighting the importance of ongoing monitoring of continuum of care following the roll-out of RAS.

2.5 Conclusions

Countries that are already implementing or considering implementation of RAS for pre-referral treatment of severe malaria need to strengthen all aspects of the continuum of care for a severely sick child – from CHWs being adequately trained and stocked for giving RAS in the areas where it is most needed, to ensuring rapid transfer and access to referral facilities where a complete course of post-referral treatment is given as per WHO guidelines for the treatment of severe malaria.

The expert group recommend that countries deploying RAS for pre-referral treatment of severe malaria continue to strengthen and monitor the whole continuum of care.

3. Effectiveness of RAS as pre-referral treatment in the CARAMAL project

Hetzel MW, Okitawutshu J, Tshetu A, Omoluabi E, Awor P, Signorell A, et al. Effectiveness of rectal artesunate as pre-referral treatment for severe malaria in children under 5 years of age: a multi-country observational study. *BMC Med.* 2022;20:343. doi:10.1186/s12916-022-02541-8 (8).

3.1 Review

The CARAMAL study was designed to evaluate the impact of RAS on severe malaria CFRs when deployed under “real-life” conditions in areas with a high malaria burden. The study was originally designed to compare the CFRs among children with signs of severe malaria in three areas in the Democratic Republic of the Congo, Nigeria and Uganda in the six months prior to RAS introduction to the CFRs in the 18 months after RAS introduction. Operational constraints led to these periods being changed to about 10 months and 15 months, respectively.

The three study sites were chosen from among the 16 countries that were implementing RAS at the start of the CARAMAL study. The main criteria for selecting the three specific study areas were that they should have a high malaria burden, malaria treatment policies in line with WHO recommendations and a functional CHW system. It was also planned to assess the readiness of each site to implement RAS introduction before including the site in the study. However, it was unclear whether this was done; other factors may have determined the inclusion of the Democratic Republic of the Congo and Nigeria (Salim Sadruddin, personal communication).

As noted in section 2, the study was originally powered to detect a reduction in the CFR among children receiving RAS based on pooling the data from the three countries. However, it became apparent during the study that there were large differences in the health care systems in the three countries and in baseline CFRs for severe malaria (an order of magnitude lower in the sites in Uganda [0.5%] than in the sites in the Democratic Republic of the Congo and Nigeria [6.7% and 11.7%, respectively]). Therefore data were not pooled and a separate analysis was done for each country. Furthermore, the primary analysis was not based on the CFRs in pre-RAS and post-RAS periods, but on the CFRs over the whole study period, comparing children who received RAS to those who did not. This effectively included all severe malaria cases in the pre-RAS period in the latter group, potentially producing important temporal confounding; there was evidence, at least in Nigeria, that the CFR in the pre-RAS period was substantially lower than that in the post-RAS period, even among those not receiving RAS in the post-RAS period.

3.2 Summary of key findings

There was no evidence in the three countries that the introduction of RAS decreased the CFR for severe malaria; in Nigeria, the CFR was higher in the post-RAS period than in the pre-RAS period. Nevertheless, as the CFR changed with time independently of RAS, the findings from Nigeria are very difficult to interpret and do not provide any compelling evidence that the introduction of RAS increased the CFR.

An important finding was that children with severe malaria often received suboptimal treatment when presenting to an RHF, particularly in the Democratic Republic of the Congo and Nigeria. This is important because if an RHF provides suboptimal treatment, any beneficial impact of RAS is likely to be significantly reduced.

Areas with a high malaria burden tend to have poor health accessibility due to weak health systems and complex anthropological factors that greatly influence health-seeking behaviour. Some behaviours may have been conditioned by the limited access to formal health care, owing to physical, economic and health governance structural deficiencies. For RAS to impact the CFR for severe malaria in areas with high malaria burden and constrained resources, adequate referral and treatment completion should be accessible to all those receiving RAS as pre-referral treatment. The study demonstrated that for RAS to work, it is essential to have a functional health system with a good referral system and referral facilities equipped to comprehensively manage a severely sick child.

3.3 Conclusions

The study, as implemented, could not provide conclusive proof of the effectiveness of RAS in areas of high malaria burden within the existing health system framework. This work showed that to impact the CFR in children with severe malaria, there is a need for a functional continuum of care for severely ill children.

4. Pre-referral RAS and referral completion in the CARAMAL project

Brunner NC, Omoluabi E, Awor P, Okitawutshu J, Tshefu Kitoto A, Signorell A, et al. Prereferral rectal artesunate and referral completion among children with suspected severe malaria in the Democratic Republic of the Congo, Nigeria and Uganda. *BMJ Glob Health*. 2022;7:e008346. doi:10.1136/bmjgh-2021-008346 (7).

Hetzel MW, Okitawutshu J, Tshefu A, Omoluabi E, Awor P, Signorell A, et al. Effectiveness of rectal artesunate as pre-referral treatment for severe malaria in children under 5 years of age: a multi-country observational study. *BMC Med*. 2022;20:343. doi:10.1186/s12916-022-02541-8 (8).

Lengeler C, Burri C, Awor P, Athieno P, Kimera J, Tumukunde G, et al. Community access to rectal artesunate for malaria (CARAMAL): a large-scale observational implementation study in the Democratic Republic of the Congo, Nigeria and Uganda. *PLoS Glob Public Health*. 2022. doi:10.1371/journal.pgph.0000464 (5).

4.1 Key outputs of the papers

One of the primary outcomes of the analysis of the CARAMAL project was referral completion, defined as a child being brought to one of the study-designated RHF's at any stage during the treatment-seeking process after seeing a community-based provider, as reported by the caregiver or by CARAMAL staff stationed at the RHF.

Overall, 67% (1408/2104) of patients completed the referral in the Democratic Republic of the Congo, compared to 48% (287/600) in Nigeria and 58% (2170/3745) in Uganda. In the Democratic Republic of the Congo and Uganda, RAS users were less likely to complete referral than non-users in the period prior to the roll-out of RAS (Democratic Republic of the Congo: AOR: 0.48; 95% CI: 0.30–0.77; Uganda: AOR: 0.72; 95% CI: 0.58–0.88). However, among those who completed referral in Uganda, RAS users were significantly more likely to complete referral on time than non-users (AOR: 1.81; 95% CI: 1.17–2.79). Timely referral completion was defined as presenting to an RHF on the same or next day after seeing a community-based provider.

In Uganda, 96% of patients visited a second provider after visiting the CHW. However, only 56% followed the CHW's recommendation regarding the RHF, with over 30% choosing to go to a private health facility. A high proportion of patients received injections at the subsequent source of care in Uganda, which is anecdotally reported to have a generally high use of artesunate injections.

The authors raised concerns about the roll-out of RAS leading to lower referral completion in children who were administered pre-referral RAS. They were of the view that alternative effective treatment options should be provided to children who are unable to complete referral.

4.2 Review

Methodological issues: The papers suggested some evidence that children who received pre-referral RAS were less likely to complete referral. Further discussion with the investigators, however, revealed that “referral completion” meant patients going to a designated referral facility, pre-defined in the study, after referral by a CHW or PHC provider.

There was a substantial disparity between the number of children who received RAS and the number who did not in the post-RAS phase, which was an expected finding. This disparity was substantial in Uganda (1631 vs 635) and the Democratic Republic of the Congo (1548 vs 188) and may potentially introduce some bias in analysing referral completion.

In addition, the comparison of referral completion in a pre-RAS vs post-RAS analysis is confounded by the challenges of RAS roll-out and the fact that RAS was not available to everyone soon after roll-out, especially in Uganda and Nigeria.

Evidence review or re-analysis: While there was a negative association between RAS and referral completion in the Democratic Republic of the Congo and Uganda, this association was positive in Nigeria, where referral completion in the post-RAS period was higher than in the pre-RAS period, irrespective of RAS use. However, in Nigeria, children who were administered RAS in a PHC facility were less likely to complete referral to an RHF than children who did not receive RAS. Referral completion by children attending a PHC facility in the Democratic Republic of the Congo and Nigeria was consistently higher than the referral completion by children attending a CHW. Since the management of severe febrile illness by health workers at the PHC level is quite different from that at the CHW level, all analyses should have been stratified by CHW vs PHC enrolment. PHC staff have higher educational background, advanced case management skills, different catchment populations and different service offerings. PHC providers are supervisory structures for CHWs, responsible for the supervision, mentoring, supply chain management and data management of CHWs.

Since children were taken to both public and private facilities, the assessment should have also looked at those who chose to go to health facilities other than the study health facilities to manage children with severe febrile illnesses or should have at least acknowledged this as a limitation.

The review panel noted that multiple factors may have an impact on referral completion. Often the nearest/cheapest place may be the most convenient. Referral to a recommended facility may be more costly (for the family) than going to a closer facility; the study team should have provided multiple nearby options for referral to reflect real-life experience. It is also important to note that children who died early could not complete referral.

While children treated with RAS were less likely to complete referral in the post-RAS period, timeliness of referral completion was better among these children. Time to completion, however, was only measured in days instead of hours.

Additional data/context made available: Brunner et al. (7) stated that, in Nigeria, 314 cases of severe malaria were enrolled by 108 CHWs compared to 275 cases by 31 PHC providers (post-RAS). The paper by Lengeler et al. (5) mentioned that 500 CHWs were involved in managing severe malaria cases in Nigeria (Table 1). This means that 392 CHWs did not see a single case of severe malaria in the 15-month RAS implementation period. Similarly, in the Democratic Republic of the Congo, 82 CHWs managed only 71 cases of severe malaria in the 15-month implementation period.

Informal reasons were given for not completing referral. Reasons included no money, distance and, in Nigeria, improvement of clinical condition after receiving RAS.

It is well known that communities' perception of seizures and their association with "spirits" may prompt individuals to seek treatment from traditional healers instead of

health workers; to address this issue, community sensitization is important. This was seen in the Democratic Republic of the Congo where central nervous system danger signs had a negative effect on referral completion (Brunner et al. (7)). Furthermore, travelling to a distant referral facility to receive ACTs that could be obtained at a local clinic/drug shop may discourage parents to complete referral.

Other factors that could influence referral behaviour include stockouts in health facilities and the perception of the quality of care at the referral facility by the CHW and the child's caregiver. Mapping of RHF is important but often incomplete, and multiple aspects need to be considered when analysing various aspects of the referral system.

The importance of these factors in terms of influencing referral completion in these settings could have been uncovered by a qualitative study exploring the reasons for non-completion of referral.

Implications for previous conclusions: There may be a need to take into consideration additional elements in the analysis of referral completion, stratified by CHW and PHC provider referring the children to a referral facility and by resources and capacity of the referral facility to manage severe malaria. These factors have a strong influence on referral completion.

4.3 Conclusions

There are several health system and individual factors that may have influenced referral completion in this study. These issues do not differ from those related to other community-level interventions that also rely on a functional health system. Barriers to referral completion need to be addressed, as this will improve outcomes not only for severe malaria but also for other severe diseases.

5. Treatment-seeking of children at the community level in Nigeria and Uganda in the CARAMAL project

5.1 Key outputs of the paper – Awor et al.

Awor P, Kimera J, Brunner NC, Athieno P, Tumukunde G, Angiro I, et al. Care seeking and treatment of febrile children with and without danger signs of severe disease in northern Uganda: results from three household surveys (2018–2020). *Am J Trop Med Hyg.* 2022;107:934–8. doi:10.4269/ajtmh.21-1132 (9).

Objective: To determine the treatment-seeking practices and treatment patterns for children under 5 years of age with an acute febrile illness, with and without danger signs of severe disease, in a highly malaria endemic area of northern Uganda

Health services: Approximately 5000 CHWs implementing iCCM, 30 primary health facilities (health centre II), 15 secondary health facilities (health centre III), four tertiary health facilities (health centre IV and referral hospitals) and many small, private, for-profit facilities

Methods: Three household surveys were conducted between November and December each year, in 2018, 2019 and 2020, in three districts in the Lango region, northern Uganda.

Results: Overall care-seeking outside the home was low. Only 51% of caregivers sought care for children under 5 with fever, and 61% sought care for fever with danger signs. Overall, care-seeking from the public sector (26%) and the private sector (24%) was similar. Care-seeking from CHWs for children under 5 with fever was low – only 12% on average (12% in 2018, 6% in 2019 and 14% in 2020), with similar findings (13%) for children with iCCM danger signs. Care-seeking at RHF was very low – only 2% (1%, 1% and 2% over the three survey rounds, respectively). Only 39% of the children received an antimalarial from the different sources of care across the three rounds, and less than 1% received RAS.

5.2 Review – Awor et al.

The study of treatment-seeking behaviour based on annual household surveys showed that overall care-seeking for fever among children under 5 years of age was very low in Uganda. Care-seeking from CHWs was low and RAS administration was very low. Care-seeking at the referral facility was very low. The Uganda Malaria Indicator Survey (2018–2019) showed similar results on care-seeking; despite the presence of community-based providers in the majority (81%) of villages in the Lango region, only 8.4% of children under 5 with fever sought care from CHWs.

Although 5100 CHWs were involved in the CARAMAL project, only 2675 cases were enrolled over nearly 17 months of implementation (April 2019–August 2020). This indicates that many CHWs who were provided with RAS did not see children with severe febrile illnesses. It is possible that a small group of CHWs were involved in pre-referral treatment of severe malaria with likely impact on quality of care, and a large number of CHWs were not active in pre-referral treatment of children with severe febrile illness, despite the financial and human resources invested in the Village Health Team programme.

There is a need to invest in behaviour change communication at the community level to encourage communities to seek care from CHWs. In addition to improving community awareness about the availability of curative services from CHWs, a major factor affecting care-seeking is the quality of care at the CHW level. This includes both case management skills and the availability of diagnostics (rapid diagnostic tests [RDTs] and respiratory rate counting devices) and medicines. The main reason for low care-seeking from CHWs in Uganda mentioned by the study team was frequent stockouts of RAS.

In addition, non-availability of medicine for any one main clinical condition (e.g. pneumonia) has an impact on care-seeking for febrile illness, demanding greater effort to improve the overall drug supply chain for the management of febrile illnesses.

5.3 Key outputs of the paper – Brunner et al.

Brunner NC, Karim A, Athieno P, Kimera J, Tumukunde G, Angiro I, et al. Starting at the community: treatment seeking pathways of children with suspected severe malaria in Uganda. medRxiv. Pre-print, 11 December 2021. doi:10.1101/2021.12.09.21267055 (10).

Objective: This study aimed at identifying treatment-seeking pathways that lead to appropriate management of suspected severe malaria in children under 5 after initial treatment-seeking from a CHW.

The study compared referral recommendations with actual post-referral treatment-seeking actions to understand the extent to which CHWs and caregivers deviated from the official recommendations and the reasons for selecting different types of post-referral providers in order to gain an insight into caregivers' motivations for following or disregarding referral recommendations.

Methods: The study was conducted in the same Ugandan districts as Awor et al.'s (9) study reported above. Data on disease episodes were collected after the implementation of RAS between April 2019 and August 2020. The study analysed treatment-seeking pathways involving 5100 CHWs, 30 PHC providers (health centre II) and 20 RHF (health centre III, IV and hospitals), which provided free malaria diagnosis and treatment for children under the age of 5.

CHWs enrolled children under 5 with fever and at least one danger sign for which RAS was indicated as per Ugandan iCCM guidelines: unusually sleepy or unconscious, convulsions, inability to drink or eat anything, and persistent vomiting. CHWs notified the local study team of the new enrolment via a text message service. CARAMAL study nurses called the CHWs to confirm the eligibility of the child and scheduled a follow-up visit at the child's home 28 days after enrolment. Study nurses were also present at three health centre IVs and one hospital in the study districts to record post-referral case management of community-enrolled children admitted to the inpatient ward. During follow-up on day 28, caregivers were interviewed about signs and symptoms, treatment-seeking history, diagnosis and treatment for their child's illness episode. Information on the child's condition and treatment administered was extracted from the CHW register.

Analysis included children who fulfilled the iCCM eligibility criteria for RAS administration, who tested positive for malaria by any provider seen during the treatment-seeking process, and for whom written informed consent was obtained. Antimalarial treatment was considered complete if a child received an ACT following an injectable treatment.

At follow-up, the health status of the child, referral recommendations by the CHW, adherence to the recommendations and reasons for seeking post-referral treatment were recorded from the caregiver.

Results: Of the 2675 provisionally enrolled children, 2211 children (83%) were included in the analysis. At CHW level, 70% of 2211 severe malaria cases received RAS and 24% received an ACT. Most children (93%) received a referral recommendation from the CHW; 65% were referred to an RHF and, of these, 56% received treatment at the RHF. Many children were brought to a private clinic (33%) even though only 3% were referred to a private clinic by the CHW.

Children who were brought to a private clinic were more likely to receive an injection than children who were brought to an RHF (78% vs 51%, $p < 0.001$). Most children who received an injection also received an ACT (866/1168 = 74%). However, children who only went to a non-RHF provider were less likely to receive an ACT than children who attended an RHF (OR: 0.64; 95% CI: 0.51–0.79; $p < 0.001$). Only 857 (39%) children received complete severe malaria treatment with RAS + injection + ACT.

Caregivers of children under 5 reported that they took the child to a public health facility (RHF [29%] and PHC [31%]) because of experience and professionalism. However, for children brought to a private clinic, nearly half (49%) of caregivers mentioned knowing the provider and 35% mentioned experience and professionalism as the reasons for taking the child to a private provider.

5.4 Review – Brunner et al.

CHWs were diligent in referring 93% of children with signs of severe malaria, and most of the referrals were to a public RHF. However, 33% (698/2119) of the caregivers sought treatment for their child at a private clinic, meaning that a substantial number of caregivers did not follow the CHW's recommendation to go to the RHF.

Fifty-five percent (1168) of children received an antimalarial injection. Of the children receiving an injection, 534 (46%) received it from an RHF and a similar number, 507 (43%), received it from a private clinic. The remaining children received injections from PHC providers and drug shops. In countries with a sizeable private sector, there is a need to explore ways to engage private sector referral facilities, including in the referral system.

The completion of full treatment, including RAS, injectable artesunate and a full course of ACT, was low in Uganda (39%). As stated earlier, the CHWs were diligent in their work. The issue was adherence to the referral recommendation (as discussed above) and quality of care at the next level facility (RHF, private clinic, PHC). Only 40.5% (483/1192) of children referred to the RHF and 40.3% (374/927) of those who went to a non-RHF facility (mostly private clinics) received full treatment.

Distance to the referral facility was mentioned by 53% of the caregivers as the reason for not following the CHW's advice to take the child to the recommended referral facility. In nearly all iCCM programmes, PHC facilities are designated as the CHW supervisory facility (e.g. health centre I in Uganda and similar facilities in other countries) and the government district/county hospital of that district is designated as the referral facility (irrespective of the fact that the district hospital or a similar inpatient facility of the adjacent district may be closer to the community from which the child is referred). iCCM programmes need to consider this when developing a referral pathway for the CHWs/communities.

5.5 Key outputs of the paper – Lee et al.

Lee TT, Omoluabi E, Ayodeji K, Yusuf O, Okon C, Brunner NC, et al. Treatment-seeking for children with suspected severe malaria attending community health workers and primary health centres in Adamawa State, Nigeria. medRxiv. Pre-print, 5 December 2021. doi:10.1101/2021.12.01.21267130 (11).

Objective: The CFRs among Nigerian children with suspected severe malaria during the pre- and post-RAS implementation periods were 4.2% and 16.1%, respectively. The CFR was higher in children first attending a PHC provider (18.5%) than in those first attended to by a CHW (5.7%). The objective of this analysis was to investigate the underlying differences between children who visited CHWs and those who visited PHC providers, specifically focusing on the severity of symptoms, home treatment and treatment-seeking delay.

Methods: The study included children with suspected severe malaria enrolled by community-based providers (CHWs or PHC providers) in three Local Government Authorities of Adamawa State in northeastern Nigeria, involving 500 CHWs, 77 PHC providers and three RHF (cottage hospitals). Children attending a CHW were compared to those attending a PHC provider.

Children under 5 with a history of fever and at least one iCCM danger sign (unusually sleepy or unconscious, not able to drink or feed, vomiting everything, convulsions or yellow eyes) who visited a PHC provider or CHW between June 2018 and July 2020 were enrolled in the study. Follow-up visits were done 28 days later at the child's residence, or by phone during the coronavirus disease (COVID-19) pandemic lockdown. Caregiver interviews were conducted in the local language (Hausa or Fulfulde) to collect the following information: child's health status, signs and symptoms of disease, treatment-seeking perceptions and practices, and medicines the child received. Data were collected electronically on tablets. Treatment-seeking delay was defined as the reported number of days between illness onset and attending the CHW or PHC provider, categorized into two-day periods. Presence of danger signs involving the central nervous system (convulsions, unusually sleepy or unconscious), number of danger signs (out of convulsions, unusually sleepy or unconscious, vomits everything, unable to drink or feed, unable to sit or stand, blood in stool, swelling of both feet), and caregiver-perceived severity were used as proxies for disease severity.

Results: The analysis included 589 children with suspected severe malaria: 314 (53%) enrolled by CHWs and 275 (47%) enrolled by PHCs. Children were enrolled by 139/500 (28%) community-based providers in the study area. The number of enrolled patients per provider varied from one to 42 (median = 2, IQR: 2–4).

Convulsions (79%) and being unusually sleepy or unconscious (70%) were more common in children visiting a PHC provider. Together, these two symptoms involving the central nervous system were reported more frequently in children first visiting a PHC provider (90%) than in children first visiting a CHW (74%) (AOR: 3.5; 95% CI: 1.9–6.1). Children often presented with multiple symptoms: proportionately more children attending a PHC provider (50%) had ≥ 4 danger signs compared to children taken to a CHW (39%) ($p = 0.02$).

Home treatment was more common among patients attending a PHC provider than those attending a CHW (AOR: 1.5; 95% CI: 1.0–2.1). In addition, 14% of children attending a PHC provider and 7% of those attending a CHW had previously been to another provider (AOR: 2.2; 95% CI: 1.1–4.4). There was no substantial difference overall in treatment-seeking delay to the community-based provider between children first visiting a CHW after becoming ill (38% sought care on the same or following day and 32% after 2–3 days) and those first visiting a PHC provider (35% on the same or following day and 41% after 2–3 days).

A total of 456 caregivers provided reasons for taking their child to the CHW or the PHC provider. The most common reasons for visiting the CHW were knowing (76%) and trusting (26%) the provider and low cost (22%). By contrast, reasons for attending a PHC provider included the experience (49%) and medical professionalism (34%) of PHC health workers, and knowing the provider (32%).

5.6 Review – Lee et al.

The comparison of children visiting PHC providers and those visiting CHWs was not appropriate, as the catchment populations of the two service providers differed in most cases. CHWs are located ≥ 5 km from the PHC facility/RHF. Therefore, the patient profiles would be different for the two providers. PHC attendees more often lived in urban areas (16% vs 4%, $p = 0.01$). Most cases going to a PHC provider would come from villages closer to the facility (unless the CHW was non-functional or unavailable when the child needed care, or the child was very sick and the family felt that the CHW may not have the skills to manage the child).

Another difference was the enrolment ratios for PHC providers and CHWs. Out of a total of 500 CHWs, 108 of them enrolled 314 cases. Therefore, the other 392 CHWs did not see a single case of severe malaria during the RAS roll-out phase. This calls into question their availability in the community when children were sick, their skill retention and overstocking of RAS (and ACTs) for the non-performing CHWs. Strict supervision is necessary to ensure the quality of care (skills and drug availability, or overstocking of RAS) at the CHW level. The paper by Lengeler et al. (5) also highlighted problems with the quality of care: “A large proportion of children above 3 years of age did not receive the required full dose of 2 suppositories for their age: DRC [Democratic Republic of the Congo]: 86% of older children received one suppository instead of the required two; Nigeria: 32% of older children received one suppository instead of the required two; Uganda: 58% of older children received one suppository instead of the required two.”

5.7 Conclusions from the three studies

Community sensitization and engagement

- There is a need for effective community sensitization to increase the understanding of severe malaria, its causes, how dangerous it is for children, how to recognize early danger signs and the need to immediately seek care when such signs are present.
 - Community education must be done with multiple modalities, considering the literacy level of most members of the community.
 - In educating the community, sociocultural perceptions of disease need to be addressed.
 - There may need to be periodic (annual?) monitoring of treatment-seeking behaviour.
 - Treatment-seeking for severe disease should be targeted at the level of the health system with the best capacity to perform initial management (pre-referral RAS and antibiotics).
 - Sensitization must emphasize the great potential for harm (death, more severe disease) if care-seeking is delayed or referral not completed.
 - Education about home treatment of malaria should be provided (which should not be done if there are danger signs).
 - Strategies for community engagement should include traditional leaders, village leaders and any community-based health service delivery groups.
 - PHC providers must conduct supportive supervision of CHWs.
 - Emergency transport for severe disease should be facilitated.

Referrals

- Community and CHWs must know where to refer children. They should know the closest facility with the capacity to manage severe disease (ideally injectable artesunate plus oral ACT, oxygen and parenteral antibiotics for bacterial sepsis management). Multiple referral options should be available close by in order to allow for consideration of community members' preferences during referral.
- There is a need to consider strengthening both the public and private sector for appropriate management of severe malaria (either triage and referral with pre-referral RAS and antibiotics) or proper treatment with injectable artesunate and a follow-up oral ACT. In all locations, the first dose of oral therapy should be given under direct observation.
- Injectable artesunate should always be followed by a full course of ACT with counselling on complete adherence even if the patient feels better before treatment is completed.
- The district Ministry of Health or relevant health authorities need systems to track the functioning of referral systems and supply chain management at each level along the continuum of care.
- Referral feedback loops must be intact so that CHWs (village health workers [VHWs], CHWs, etc.) know that referrals have been completed, the final diagnosis of their referred patient, and any follow-up needed upon return of the child to the community.

6. Clonal expansion of artemisinin-resistant *falciparum* malaria in Uganda in the CARAMAL project

Awor P, Khim N, Coppée R, Rondepierre L, Roesch C, Khean C, et al. Clonal expansion of artemisinin-resistant *Plasmodium falciparum* in Uganda is associated with substandard treatment practices. SSRN. Pre-print, 17 December 2021. doi:10.2139/ssrn.3974542 (12).

6.1 Key outputs of the paper

The primary objective of the study was to assess the prevalence of artemisinin resistance molecular markers before and following the roll-out of pre-referral RAS in three adjacent districts in northern Uganda (Kole, Kwanja and Oyam), as part of the observational CARAMAL study conducted between April 2018 and April 2020.

Study populations: Filter paper blood samples were collected from a subsample of three groups of children under 5 enrolled with malaria danger signs who had a positive malaria RDT (HRP2/pan-pLDH combo mRDT). Samples were taken on either day 0 or day 28, and assayed for K13 markers associated with artemisinin partial resistance.

Dried blood samples were reported as collected from three groups:

- **Group A (before RAS roll-out, 2018/2019):** Of 248 samples collected from children under 5 with malaria danger signs on day 0 upon presentation at a study-designated RHF, 127 (51.2%) had sufficient DNA to be sequenced for K13 molecular markers. Of the seven samples with a non-synonymous mutation in K13, five (3.9%) had candidate artemisinin-resistant mutations, with no C469Y mutations.
- **Group B (after RAS roll-out, 2019/2020):** Of 367 samples collected from children under 5 with malaria danger signs on day 0 upon presentation at a study-designated RHF, 145 (39.5%) had sufficient DNA to be sequenced for K13 molecular markers. Thirteen samples (9.0%) had non-synonymous mutations that were candidate artemisinin-resistant mutations, including nine C469Y mutations (6.2%).
- **Group C (after RAS roll-out, 2019/2020):** Blood samples were taken at home visits on day 28 from 186 children who were given RAS by a CHW, but who had not attended a study-designated RHF. Of those sampled, 77 (41.4%) had sufficient DNA to be sequenced for K13 molecular markers. Nineteen samples (24.7%) had candidate artemisinin-resistant mutations, including 16 (20.8%) with C469Y mutations.

During the period after RAS roll-out, C469Y mutations were more frequently found in samples collected at home visits 28 days after RAS treatment from 186 children who had not completed referral to a study-designated RHF (OR: 3.9; 95% CI: 1.7–9.5; $p = 0.002$) than in 145 samples collected on day 0 from children who had presented at a study-designated RHF (i.e. Group C vs Group B). A higher frequency of C469Y mutations was reported in Group C patients whose caregivers reported on day 28 that the child had only been given RAS, with or without an injectable artemisinin, than in those reporting that their child had also been given an oral ACT. The prevalence of C469Y mutations in patients treated with injectable/rectal/oral artesunate monotherapy was higher (26%; 9/34) than in those treated with artesunate plus an ACT (16%; 7/43); however, this difference was not statistically significant (OR: 1.9; 95% CI: 0.6–5.6; $p = 0.278$).

Ring-stage survival assay: The effect of the C469Y haplotype on parasite susceptibility to artemisinin derivatives was explored with a ring-stage survival assay (RSA)_{0-3h} on the 3D7 strain edited with both the wild type and C469Y haplotypes. The median ring-stage survival assay values were 3.0% (95% CI: 2.0–4.0) for 3D7-wild type (N = 10) and 9.2% (95% CI: 7.6–10.8) for 3D7-C469Y (N = 8), reflecting a higher tolerance of 3D7-C469Y to dihydroartemisinin (Student's *t*-test, $p < 0.0001$).

Clonal expansion: A neighbour-joining phylogenetic tree showed these Ugandan K13 C469Y mutants branched with isolates collected in East Africa, suggesting local emergence of this lineage. This is consistent with the findings of the discriminant analysis of principal components. A single shared haplotype surrounding the C469Y mutation in the Ugandan isolates indicates a single epidemiological origin of this mutation.

6.2 Key conclusions from the paper

The study documented clonal expansion of artemisinin-resistant *Plasmodium falciparum* in northern Uganda in the context of substandard treatment, such as the use of artesunate monotherapy. The authors acknowledged that no paired day

0 and day 28 samples were collected, so no distinction could be made between recrudescence and reinfection, and ACT therapeutic efficacy could not be assessed. However, a small but significant overall increase in lumefantrine IC50 was recently reported ex vivo in eastern Uganda (13). Awor et al. (12) concluded that the roll-out of pre-referral RAS was not responsible for the emergence or spread of artemisinin-resistant falciparum malaria, as this was well documented before RAS deployment and in districts where RAS had not been introduced. Awor et al. also reported that the population-level use of RAS among all children with suspected severe malaria in the three districts in Uganda was less than 1%. This suggests that the inadequate use of artesunate monotherapy in different formulations (both parenteral and rectal), without completion of referral and follow-up ACT treatment, may exacerbate the selection of artemisinin-resistant strains.

6.3 Review

Methodological issues: The study team assessed the prevalence of molecular markers of artemisinin resistance among multiple subsamples of non-randomized children under 5 enrolled in the observational CARAMAL study in Uganda.

Information on sample size calculation was not provided. In addition, the numbers of children in each group and subgroup were small and convenience sampling was used. Therefore, there could have been several undetected confounders and effect modifiers. Observational, multi-stage sampling, subgroup analysis and small sample sizes suggest that selection bias and incidental findings cannot be excluded.

Samples from day 28 post-RAS were only reported for Group C. A significant increase in mutation prevalence generally occurs when comparing pre-treatment (day 0) and post-treatment (e.g. day 28), as has been seen with markers of lumefantrine tolerance (*pfcr*t Lys76Thr and *pfmdr* Asn86Tyr and Asp1246Tyr) after treatment with artemether-lumefantrine and artesunate-amodiaquine (14–16). However, this observation has not been indicative of decreasing efficacy of artemether-lumefantrine and artesunate-amodiaquine. This sampling method precluded quantification of the extent to which the increase in K13 C469Y prevalence reported in Group C was the result of ascertainment bias, as no paired sampling was done on both day 28 and day 0, rather than C469Y selection from drug pressure, including possible concomitant treatment with oral/injectable artemisinin monotherapies in some children as a result of incomplete treatment (i.e. not followed by a full course of ACT).

One group of children studied, i.e. those receiving pre-referral RAS from a community-based provider who were successfully referred to an RHF, were excluded from the report. The exclusion of this important group that had been managed according to WHO recommendations was justified, as the exact day that the post-RAS sample was collected at the RHF was not recorded; however, even with this caveat, these data should be presented, as they are informative with respect to the patient group of great interest, i.e. those complying with WHO recommendations on RAS administration, referral and completion of treatment.

6.4 Additional evidence reviewed

Asua V, Conrad MD, Aydemir O, Duval Saint M, Legac J, Duarte E, et al. Changing prevalence of potential mediators of aminoquinoline, antifolate, and artemisinin resistance across Uganda. *J Infect Dis.* 2021;223(6):985–94. doi:10.1093/infdis/jiaa687 (17).

Asua et al. (17) analysed the prevalence of K13 molecular markers in multiple districts of Uganda, including Kole, one of the districts where CARAMAL was implemented. In both 2018 and 2019, 50 samples (dried blood spot) per site per year were collected from individuals > 6 months of age (up to 10 years of age for 2018; all ages for 2019) with clinical malaria confirmed by microscopy or HRP2-based RDT. Samples were collected in the period April–June every year. Initial emergence of C469Y in northern Uganda was documented before the RAS roll-out and in districts outside the RAS study area. Across all three Ugandan districts, CARAMAL dispensed < 202 doses of RAS by the time Asua et al. had collected all 2019 samples for their K13 prevalence survey. According to market data provided by MMV (18), the expected annual market for RAS is 1 million units per year, against 25 million doses of injectable artesunate. There is thus no evidence that RAS alone led to the emergence or spread of the K13 C469Y marker of artemisinin partial resistance.

Ampadu HH, Asante KP, Bosomprah S, Akakpo S, Hugo P, Gardarsdottir H, et al. Prescribing patterns and compliance with World Health Organization recommendations for the management of severe malaria: a modified cohort event monitoring study in public health facilities in Ghana and Uganda. *Malar J*. 2019;18:36. doi:10.1186/s12936-019-2670-9 (19).

Achan J, Tibenderana J, Kyabayinze J, Mawejje D, Mugizi H, Rukaaka M, et al. Case management of severe malaria – a forgotten practice: experiences from health facilities in Uganda. *PLoS One*. 2011;6:e17053. doi:10.1371/journal.pone.0017053 (20).

Ampadu et al. (19) conducted a modified cohort event monitoring study involving patients who were prescribed an injectable antimalarial for treatment of presumed severe malaria in eight sites (four each in Ghana and Uganda) between May and December 2016. Injectable artesunate is the most commonly prescribed medicine for the management of severe malaria in Ghana and Uganda. However, adherence to the WHO recommendation of at least three doses of injectable antimalarial in 24 hours followed by a full course of ACT was low – at less than 30%. Compliance was 20 times higher in Ghana than in Uganda, where only 4.8% of patients had a prescription for injectable antimalarial followed by a co-prescription of an oral ACT. This finding contrasts with that of Achan et al. (20), who found that 429 (52%) of 823 Ugandan patients who received parenteral antimalarial also received oral medication.

Therefore, poor compliance with the WHO recommendation of at least three doses of injectable antimalarial in 24 hours followed by a full course of ACT in Uganda was well established before the start of the CARAMAL study.

Awor P, Kimera J, Brunner NC, Athieno P, Tumukunde G, Angiro I, et al. Care seeking and treatment of febrile children with and without danger signs of severe disease in northern Uganda: results from three household surveys (2018–2020). *Am J Trop Med Hyg*. 2022;107:934–8. doi:10.4269/ajtmh.21-1132 (9).

This study described treatment-seeking practices and treatment patterns for children under 5 with an acute febrile illness, with or without danger signs of severe disease, in the highly malaria endemic CARAMAL districts in Uganda. Three household surveys were conducted from November through December each year in 2018, 2019 and 2020. Overall, 30% of the children in the study were reported to have a danger sign. Only half (50.6%) of children under 5 with fever and 61.8% among those with danger signs sought care from a health provider. Only 11.8% sought treatment from a CHW and, in total, only 0.1% received RAS (none with danger signs).

Therefore, the drug pressure exerted by RAS appeared to be negligible during the CARAMAL study.

Brunner NC, Karim A, Athieno P, Kimera J, Tumukunde G, Angiro I, et al.
Starting at the community: treatment seeking pathways of children with
suspected severe malaria in Uganda. medRxiv. Pre-print, 11 December 2021.
doi:10.1101/2021.12.09.21267055 (10).

This study also described treatment-seeking practices and treatment patterns for children under 5 with an acute febrile illness, specifically with danger signs of severe disease, in the same highly malaria endemic CARAMAL districts in northern Uganda as the study by Awor et al. (12).

In contrast to the findings of Awor et al. (12), the larger study by Brunner et al. (10) found a higher use of RAS, reporting that, at the CHW level, 70% of 2211 severe malaria cases received RAS. The substantial discrepancy between the studies by Awor et al. and Brunner et al. conducted in the same districts and health facilities and over a similar time period (2018–2020 vs 2019–2020, respectively) makes assessment of the drug pressure exerted by RAS during the CARAMAL study uncertain. However, the use of RAS in these districts is estimated to be only one twenty-fifth that of injectable artesunate (18).

6.5 Additional data/context made available by study investigators

The study team generously provided additional data on the assessment of prevalence of artemisinin resistance molecular markers in the blood samples collected during the CARAMAL study, both from the fourth Ugandan group excluded from the pre-print manuscript (those who received pre-referral RAS from a community-based provider and were successfully referred to an RHF), data from all four groups stratified by study district in Uganda, and data from all four groups in the Democratic Republic of the Congo and Nigeria.

In Uganda, data stratified by study site showed that the fewest non-synonymous K13 mutations occurred in Kwanja District, where all mutations occurred before RAS deployment (Group A, n = 3/32, 9%). In Kole District (also studied by Asua et al. (17)), there was an apparent increase in non-synonymous K13 mutations both in children seeking treatment directly from a referral facility (Group B, n = 9/59, 15.3%) and in children not completing referral (Group C, n = 5/21, 23.8%), compared to pre-RAS deployment (Group A, n = 2/45, 4.4%). In Oyam District, there was an apparent increase in non-synonymous K13 mutations both in children who were given pre-referral RAS and presented at a referral facility (group not reported in pre-print, n = 2/15, 13.3%) and in children who did not complete referral (Group C, n = 15/54, 27.8%), compared to pre-RAS deployment (Group A, n = 2/50, 4.0%) and in children who sought treatment directly from a referral facility (Group B, n = 4/60, 6.7%). Data on the prevalence of C469Y mutations stratified by district were not provided.

A subset of children in the Democratic Republic of the Congo and Nigeria: In the Democratic Republic of the Congo, a total of 462 blood samples from children enrolled in the CARAMAL study were sequenced for K13 molecular markers, of which eight carried the non-synonymous mutations. In Nigeria, a total of 250 samples were sequenced, of which 10 carried the non-synonymous mutations. Only one of these K13 mutations (M476I, a sample from Nigeria) was known to be associated with

artemisinin partial resistance. There was no difference in the prevalence of non-synonymous mutations between groups in either the Democratic Republic of the Congo (Fischer's exact p-value 0.236) or Nigeria (Fischer's exact p-value 0.188).

Therefore, the impact of pre-referral RAS on the selection of K13 mutations associated with partial artemisinin resistance is likely to be very context-specific, probably related to baseline levels of circulating mutant parasites.

6.6 Summary of the key findings

The pre-print by Awor et al. (12) showed that selection of the K13 C469Y marker was greater by day 28 among those who failed to complete referral treatment than on day 0 among those who presented at an RHF. This mutation was shown to have emerged locally in East Africa and to be associated with increased tolerance to artemisinins in the ring-stage survival assay.

Considering the findings from the consultation, additional data and evidence reviewed, K13 C469Y molecular markers for artemisinin resistance were present before RAS was deployed and were widely present and increasing in northern Uganda, both in some CARAMAL districts (Kole and Oyam, but not Kwanja district) and in non-study districts (e.g. Lamwo and Agago districts). Uptake of RAS and treatment-seeking from CHWs appeared very low in Uganda, but these findings were not reported consistently across CARAMAL studies in the same Ugandan sites. Similar increases in the prevalence of resistance markers were not seen in CARAMAL study sites in the Democratic Republic of the Congo or Nigeria. The impact of pre-referral RAS on the selection of K13 mutations associated with partial artemisinin resistance is likely to be very context-specific.

6.7 Conclusions

- Awor et al. (12) showed that selection of the K13 C469Y marker was greater by day 28 among those who failed to complete referral treatment than on day 0 among those who presented at an RHF. Despite the limitations noted above, this study provided a signal that suboptimal treatment of mRDT-positive children under 5 with danger signs following pre-referral RAS may select partially artemisinin-resistant parasites with the K13 C469Y mutation.
- It is urgent for malaria programmes to ensure that health providers and patients adhere strictly to malaria treatment guidelines. Intense efforts should be made to ensure that:
 - artemisinin monotherapies (both rectal and parenteral) are used for treating severe malaria cases only as per WHO Guidelines.
 - referral is completed in mRDT-positive young children with danger signs given pre-referral RAS; RHF's should treat severe malaria with intravenous artesunate and appropriate supportive management and must exclude/treat other concurrent infections (e.g. pneumonia, septicaemia) that could be causing danger signs in a parasitaemic child; and
 - rectal and parenteral artemisinin is always followed by a full oral course of an effective ACT.
- Antimalarial resistance surveillance should be strengthened at the population level across Africa and most urgently in East Africa, with:

- prioritization of interventions to holistically address the drivers of resistance selection; and
- prompt response when significant resistance is detected.
- Therapeutic efficacy studies in Uganda, and across East Africa, should be conducted urgently and regularly, given the reported prevalence of partial artemisinin resistance markers and increased lumefantrine IC50 values *ex vivo*.
- Further research is needed to understand the role of RAS/parenteral artesunate and incomplete treatment (without referral treatment and a complete course of ACT) in the selection of K13 mutant parasites already present in an area.

7. Real-world costs, financing and economic evaluation of RAS

Lambiris M, Ndongala G, Ssempala R, Balogun V, Musiitwa M, Kagwire F, et al. Real world costs and barriers to the successful implementation of rectal artesunate as pre-referral treatment for severe malaria in Sub-Saharan Africa. medRxiv. Pre-print, 27 May 2022. doi:10.1101/2022.05.24.22275488 (21).

7.1 Key outputs of the paper

The objective of the paper was to examine the real-world costs and financial constraints to implementing pre-referral RAS in the Democratic Republic of the Congo, Nigeria and Uganda.

Primary data were gathered on baseline health system constraints and RAS implementation expenditures. In addition, the equivalent annual cost of RAS implementation per child under 5 at risk of severe malaria from a health systems perspective was calculated, separating neglected routine health system components from incremental RAS introduction costs. The following health system costs were considered: training of CHWs and health workers at the referral level, supervision, supply chain (mainly the cost of RAS in this study; the distribution costs of RAS were included in the supervision budget, as CHWs collected supplies at meetings with their supervisors), behaviour change communication, monitoring and evaluation, and other supportive interventions. The biggest cost is for health systems strengthening, which was found to be by far the biggest component – ranging from 65% to 76% of the total cost. The total RAS start-up costs are, therefore, quite substantial, while the recurring costs for procurement of medicines (RAS, injectable artesunate and ACTs) are relatively very low. The economic analysis did not consider the cost of strengthening the referral system.

While CARAMAL was implemented in remote areas of the three countries, the study sites differed markedly in terms of the incidence of severe febrile episodes and the distribution of children per community-based provider and RHF.

The study team found that the annual costs of preparing the health system for managing severe malaria with RAS was highest in the Democratic Republic of the Congo, followed by Uganda and Nigeria, with the cost of strengthening the neglected routine health system components accounting for most of the overall cost per child. They also noted high monitoring and evaluation costs in the Democratic Republic of

the Congo compared to Nigeria and Uganda. The authors concluded that the high implementation costs reflected the low operational capacity and routine financing gaps in the continuum of care for severe malaria in these settings. Deploying RAS would, therefore, be relatively less expensive in stronger health systems that are already sustainably financed and functioning. They emphasized that investments made to prepare the routine health system components would also benefit the treatment of other common diseases.

7.2 Review

Methodological issues: The primary requirement for study site eligibility was a UNICEF-supported system of CHWs implementing iCCM. However, the three study sites were found to be very different in many ways, including the ratio of CHWs per population, access, relative costs of various activities such as training, and overall functionality of iCCM. It was, therefore, important, as the authors did in their paper, to separate the costs of health systems strengthening from the costs specific to RAS introduction. This will facilitate application of the results to different contexts.

The authors indicated that UNICEF-specific costs were not included in the costing; therefore, the costs presented fully reflect the Ministry of Health perspective. Not including UNICEF-specific expenses in the costing may have underestimated the actual total costs. The UNICEF-specific costs for activities that are critical for the deployment of RAS should have been included in the analysis.

The authors indicated that since population-level data were not available, they did not calculate the costs per child with severe malaria in the population.

7.3 Summary of the key findings

- The findings from the CARAMAL study could be used to advocate for strengthening the health system and ensuring the continuum of care, as this would also benefit other diseases.
- Integrating RAS would be less expensive in functional health systems with regular financing of key activities. However, the health systems are generally weak where RAS is most needed.
- Implementation costs are high due to weak health systems and financing gaps for routine activities in the continuum of care for the correct management of severe malaria.
- While the relative cost of strengthening the health system is high, using the opportunity of introducing RAS to invest in strengthening the health system should benefit the management of many severe illnesses in remote areas, as these also require a functional referral system and continuum of care.
- Unless the health system is strengthened and functioning well, even the best tools/interventions cannot achieve the expected impact.

7.4 Conclusions

- The management of severe malaria in facilities and community programmes providing RAS for pre-referral treatment of severe malaria require investments to ensure the continuum of care across all levels of the health care system.

- The findings from the CARAMAL study could be used to advocate for health systems strengthening, as an improved continuum of care would benefit multiple diseases; without it, the best tools may remain ineffective, and the programme will waste resources.

8. Observations on RAS implementation studies in Malawi, Sierra Leone and Zambia

8.1 Key outputs of the papers from Zambia

Green C, Quigley P, Kureya T, Barber C, Chizema E, Moonga H, et al. Use of rectal artesunate for severe malaria at the community level, Zambia. *Bull World Health Organ*. 2019;97:810–7. doi:10.2471/BLT.19.231506 (22).

Green C, Quigley P, Kureya T, Barber C, Chanda E, Moyo B, et al. et al. Scaling up use of rectal artesunate for severe malaria at the community level, Zambia. *Bull World Health Organ* (in press) (23).

This was a pilot study supported by MMV on the implementation of RAS in the Serenje District of Zambia. All 24 health facilities included in the project had already participated in two previous projects. Besides activities directly linked to the implementation of RAS, there were other supporting activities, including community-managed food banks and emergency saving schemes, bicycle ambulances facilitating referral, and community mobilization. Importantly, volunteers with previous experience in earlier projects and trained in iCCM were preferentially included. Health care workers were given refresher training in severe malaria and in the administration of injectable artesunate, while the National Malaria Elimination Centre and district health teams ensured that drug supplies were available.

With regard to the supply chain in the Zambia study, RAS was distributed through the government supply chain system, which improved the sustainability of the project. District health teams developed a WhatsApp group within the district to facilitate rapid transfer of RAS to facilities that were running short of supplies. CHWs also submitted reports on RAS use to the facilities. The National Health Management Information System, which initially did not include indicators for severe malaria, was improved as part of the study with the integration of RAS clinical indicators. The paper mentioned eight severe malaria-related indicators collected each month. CHWs compiled data on the number of children who were seen, tested for malaria, referred to a facility, used a bicycle ambulance and benefited from community support. Data on outcome indicators in children aged 6 months to 6 years were available from community health volunteers each month and from baseline and end-line surveys.

The second publication described the second phase of the intervention, which continued in Serenje and was extended to nine additional districts. Two of these districts were defined as a high-intensity intervention because more volunteers were trained (resulting in a volunteer to population ratio of one to 250, instead of one to 500 or more in the other districts) over a longer training period, with continuous mentoring support, etc.

In Serenje, the CFR for severe malaria decreased from 8% in 2016–2017 to 0.5% in 2018–2019. When the intervention was extended to nine additional districts, the results of the pilot study were confirmed. The CFR decreased from 3.1% to 0.1% in the two high-

intensity districts and from 10.7% to 1.4% in the other districts. At the end of the study, there were fewer stockouts of RAS, better knowledge of the signs of severe malaria among the CHWs, and better knowledge of how to manage severe malaria among health workers at the health facilities. Of the 11 486 children identified with suspected severe malaria at the community level, 97% were administered RAS and 96% were referred to a health facility.

8.2 Review – Zambia papers

Methodological issues: These two papers monitored the CFR before and after the implementation of RAS and, therefore, did not consider the temporal changes that may have occurred regardless of the intervention, including the implementation of other malaria control interventions. Moreover, the data were collected retrospectively at facility level. In Serenje, during the pilot study, the number of reported cases increased substantially thanks to the intervention, but this may have also increased the number of less severe cases, thereby increasing the denominator and decreasing the CFR. When the intervention was extended to nine additional districts, the severe malaria-related deaths were collected over a 12-month period (September 2020 to August 2021). The actual number of severe malaria cases was not reported, and no additional evidence was presented at the technical consultation meeting.

8.3 Summary of key findings from the Zambia papers

The pilot study was done in a district that had been involved in several other relevant interventions. Besides RAS, several other supporting activities were being implemented. In the extension phase, the effect of the intervention was greater in the districts with more intense support; the schemes were less effective in sites that received less support. The project confirmed that effective implementation of a community-based RAS intervention requires identification and tackling of health system bottlenecks, such as localized drug and commodity shortages, inadequate supervision of community health volunteers and weak referral systems. It also requires attention to barriers that contribute to poor access to health services such as community-managed food banks and emergency saving schemes. The bicycle ambulances probably had a major effect on the uptake of the referral recommendation.

8.4 Key outputs of the paper from Malawi

Oliff MS, Muniina P, Babigumira K, Phuka JC, Rietveld H, Sande J, et al. The five continuum of care criteria that should accompany rectal artesunate interventions: lessons learned from a two-arm cohort intervention study in Malawi. Pre-print, August 2022. doi:10.21203/rs.3.rs-1924718/v1 (24).

This was a single-country two-arm controlled study supported by MMV in remote, hard-to-reach areas of Malawi where pre-referral interventions were provided by CHWs/health surveillance assistants. There were nine village health clinic zones in the control arm and 14 village health clinic zones in the intervention arm. The CHWs in the intervention arm were trained in using a tailor-made, field-tested toolkit, and the community had access to pictorial information, education and communication materials on danger signs and actions to take. These materials were mounted throughout the zone in areas with high levels of foot traffic. The study identified five continuum of care criteria (“5CC Framework”) to reinforce RAS programming: care transitions for the patient; consistency of supplies to village health clinics; comprehensiveness of care received by the patient at the village level and at RHF; connectivity of care between all tiers; and communication between providers from different points of care. There was no difference in treatment-seeking between the

intervention and control arms, possibly due to the Malaria Vaccine Implementation Programme in the control area. Over 93% complied with referral instructions, despite rapid improvement in their child following administration of RAS. Seventy-six percent of caregivers reported that, upon arrival at the referral facility, their child was not admitted and was managed as an outpatient; 70% reported that their child did not receive any form of parenteral care – either an injection or drip. Some referred patients were administered parenteral treatment on an outpatient basis and did not receive three doses of injectable artesunate followed by an ACT, as required by national guidelines. Caregivers' recall indicated that most children were not admitted, even those with ≥ 2 danger signs.

8.5 Review – Malawi paper

Methodological issues: The study was designed as a single-country, two-arm study. However, it was unclear whether the intervention district (Salima) and the control district (Ntchisi) were randomized, and how the village health clinic zones were selected in each district. The sample size was calculated in relation to the incidence of danger signs/severe febrile illness reported at the health facilities in a previous study done in 2018, considering a design effect of 1. A random sample of 173 (exposed) households in Salima and 55 (unexposed) households in Ntchisi were selected, in hard-to-reach areas (> 5 km from the nearest health facility). Based on household interviews and follow-up of febrile children, the study evaluated five continuum of care criteria: care transitions for the patient; consistency of supplies (medicines and referral slips) to village health clinics; comprehensiveness of care received by the patient at the village health clinics and RHF; connectivity of care between all tiers; and communication between providers from different points of care.

8.6 Additional data/context made available – Malawi paper

The study showed problems in the continuum of care and poor lack of compliance by health workers at referral facilities, given that severe malaria treatment guidelines require the patient to be admitted to administer the post-RAS treatment. This suggests that investments made at the village level to save lives and guard against monotherapy through effective danger sign assessment, pre-referral treatment and referral (as demonstrated by the completion rate) can quickly be lost if the patient, who has travelled a long distance at significant expense and is expecting to be admitted, arrives at the referral centre and is not admitted to receive injectable treatment followed by an ACT.

8.7 Conclusions – Malawi paper

This study showed the importance of having referral facilities that are able to manage severe malaria adequately; otherwise, efforts to introduce RAS will be nullified.

8.8 Key outputs of the paper from Sierra Leone

Kamara ARY, Esch K, Larbi K et al. Assessing the roll out of artesunate rectal capsules as pre-referral intervention in five districts in Sierra Leone; Abstract no. 0721 ASTMH 2021 (25).

In Sierra Leone, the implementation of RAS and the training of trainers has been supported by the President's Malaria Initiative, with an efficient system of referrals based on the availability of ambulances supported by the World Bank. RAS was implemented as a pre-referral intervention in peripheral health units in 14 districts.

A convenience sample consisted of 106 peripheral health units in five districts based on urbanicity, facility type and proximity to district hospital in order to ensure representativeness. The paper reported that 90% of patients received RAS as required. A total of 11% of families refused referral, and an ambulance was used in 75% of referrals; 50% of deaths occurred in children initially seen in facilities at least 45 km from the district hospital, i.e. more than a 60-minute driving distance. The project faced delays in replenishing supplies due to the late arrival of RAS. In addition, staff at the referral hospital were not trained to handle severe malaria cases. Over 80% of patients who were admitted to the referral facility were eventually discharged.

The success of this intervention was facilitated by Sierra Leone's robust ambulance system and resulting low financial and geographical barriers to referral. Following the end of the external funding for the ambulance system and its handover to the Sierra Leone Ministry of Health, there have been challenges with paying ambulance staff and maintenance costs, resulting in a reduction in the effectiveness of the system.

8.9 Review – Sierra Leone paper

Methodological issues: There was the conclusion in the paper that there was a 20% decrease in CFR, but the comparator and the specific data used for the analysis were unclear.

8.10 Additional data/context made available – Sierra Leone paper

During the discussion, it became clear that the ambulance system was facing an increasing number of problems, principally due to the availability of funds for maintenance and fuelling. A source of financing for strengthening the health system and deploying RAS is key, and sustainability is critical. In Sierra Leone, once external funding was exhausted, the well functioning ambulance system that supported the continuum of care needed for the effective deployment of RAS was no longer effective because the government was unable to provide the funding needed on a regular basis.

8.11 Conclusions – Sierra Leone paper

- RAS should be part of a continuum of care in a functional health care system and not a standalone therapy.
- RHF's should be able to adequately manage the severe malaria cases referred to them. This ability should extend to public and private health care facilities/practitioners, as, in many countries, patients attend them rather than public facilities.
- Support for an adequate referral system is essential for achieving the intended impact.

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Annex 1. List of participants

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Annex 2. List of pre-reads and list of questions for study teams

List of pre-reads for preparatory meeting of 20–21 September 2022

CARAMAL publications

Lengeler C, Burri C, Awor P, Athieno P, Kimera J, Tumukunde G, et al. Community access to rectal artesunate for malaria (CARAMAL): a large-scale observational implementation study in the Democratic Republic of the Congo, Nigeria and Uganda. *PLoS Glob Public Health*. 2022. doi:10.1371/journal.pgph.0000464.

Hetzel MW, Okitawutshu J, Tshefu A, Omoluabi E, Awor P, Signorell A, et al. Effectiveness of rectal artesunate as pre-referral treatment for severe malaria in children <5 years of age. *medRxiv*. Pre-print, 27 September 2021. doi:10.1101/2021.09.24.21263966.

Brunner NC, Omoluabi E, Awor P, Okitawutshu J, Tshefu Kitoto A, Signorell A, et al. Prereferral rectal artesunate and referral completion among children with suspected severe malaria in the Democratic Republic of the Congo, Nigeria and Uganda. *BMJ Glob Health*. 2022;7:e008346. doi:10.1136/bmjgh-2021-008346.

All other publications reviewed were available as online pre-prints

Signorell A, Awor P, Okitawutshu J, Tshefu A, Omoluabi E, Hetzel MW, et al. Health worker compliance with severe malaria treatment guidelines in the context of implementing pre-referral rectal artesunate: an operational study in three high burden countries. *medRxiv*. Pre-print, 30 November 2021. doi:10.1101/2021.11.26.21266917.

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Lambiris M, Ndongala G, Ssempala R, Balogun V, Musiitwa M, Kagwire F, et al. Real world costs and barriers to the successful implementation of rectal artesunate as pre-referral treatment for severe malaria in Sub-Saharan Africa. *medRxiv*. Pre-print, 27 May 2022. doi:10.1101/2022.05.24.22275488.

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Awor P, Kimera J, Brunner NC, Athieno P, Tumukunde G, Angiro I, et al. Care seeking and treatment of febrile children with and without danger signs of severe disease in northern Uganda: results from three household surveys (2018–2020). *Am J Trop Med Hyg*. 2022;107:934–8. doi:10.4269/ajtmh.21-1132.

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Okitawutshu J, Signorell A, Kalenga J-C, Mukomena E, Delvento G, Burri C, et al. Danger signs and management of suspected severe malaria cases at community level and in referral health facilities: an operational study in the Democratic Republic of the Congo. medRxiv. Pre-print, 2 December 2021. doi:10.1101/2021.11.30.21267082.

Publications from other studies

Kamara ARY, Esch K, Larbi K et al. Assessing the roll out of artesunate rectal capsules as pre-referral intervention in five districts in Sierra Leone; Abstract no. 0721 ASTMH 2021.

Green C, Quigley P, Kureya T, Barber C, Chizema E, Moonga H, et al. Use of rectal artesunate for severe malaria at the community level, Zambia. Bull World Health Organ. 2019;97:810–7. doi:10.2471/BLT.19.231506.

Green C, Quigley P, Kureya T, Barber C, Chanda E, Moyo B, et al. Scaling up use of rectal artesunate for severe malaria at the community level, Zambia. Bull World Health Organ (in press).

Oloff MS, Muniina P, Babigumira K, Phuka JC, Rietveld H, Sande J, et al. The five continuum of care criteria that should accompany rectal artesunate interventions: lessons learned from a two-arm cohort intervention study in Malawi. Pre-print, August 2022. doi:10.21203/rs.3.rs-1924718/v1.

WHO Guidelines

WHO guidelines for malaria, 3 June 2022 – pages 176–178. Geneva: World Health Organization; 2022 (<https://apps.who.int/iris/handle/10665/354781>, accessed 15 March 2023).

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Additional readings

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ANNEX 3. LIST OF QUESTIONS AND CLARIFICATIONS FOR STUDY TEAMS

CARAMAL study: overall comments

- Access to the final CARAMAL report would assist reviewers to understand the full context and findings of this large, important and complex body of work.
- Seven of the manuscripts shared are not yet published or peer reviewed, but kindly provided as pre-prints, mostly dated 2021. Could updated manuscripts resubmitted or responses to reviewers' comments to date be shared?
- Please share as many implementation plans, standard operating procedures, etc. to inform comparisons across sites/studies and WHO field guide for safe and effective RAS implementation. It would also be helpful to have a summary of interventions/inputs (from Ministries of Health, UNICEF, CHAI, etc.), i.e. training (including refresher), supply chain, supervision, mentoring, data management, quality assurance, at CHW, PHC and referral levels individually for the three countries. This should include details on linkages between the referral facility and CHW/PHC.
- Priority requests are highlighted in bold below.

CARAMAL methodological reviews: CARAMAL study

Lengeler et al. (pre-read 1); Hetzel et al. (pre-read 2)

- Is a pre-defined statistical analysis plan available?
 - Pre- vs post-intervention study design, yet the durations pre and post are not matched and most comparisons are of RAS vs no RAS overall.
 - Sample size calculation based on all sites combined, yet results presented by country. Justify.
 - **How were study areas selected for each country (e.g. would > 6-hour delay in reaching RHF be expected)?** Any other significant changes in malaria case management ecosystems at these sites over the study period?
- Additional analyses requested:
- Pre- vs post-intervention (e.g. Table 4 in Hetzel et al.): Why was this not the primary analysis, as this is how study was designed? We would like to see unadjusted and adjusted analyses, based on characteristics of patients presenting, season, etc.
- **Only in post-RAS period (to reduce temporal confounding):** e.g. Nigeria CFR appeared to increase from ~4% to 12% in pre- and post-intervention periods in no-RAS groups (derived from data in Table 4)
 - **RAS vs no RAS (as per Table 3 in Gomes et al. [pre-read 15])**
i.e. risk of death within 0–6 hours and, if survived > 6 hours, if reached clinic in < 6 hours vs ≥ 6 hours, showing the SAME baseline confounders for each country (e.g. age, sex, rainy season, convulsions). In Brunner et al. (pre-read 3) Table 2, time to referral is only reported for following time ranges in minutes: 0 < 15 (reference); 15 < 30; 30 < 60; and ≥ 60, which does not take into account that RAS requires 6–9 hours to have a significant reduction in parasitaemia.

- Among those successfully referred and given injectable treatment: Risk of death following RAS vs no RAS among those who received injectable artesunate and those who received injectable quinine, again adjusting for the SAME baseline confounders for each country.
- **Very large differences noted between unadjusted and adjusted ORs noted, particularly for Democratic Republic of the Congo.**
 - **Justification and method used for variable selection in adjusted analyses are needed.**
 - **These large changes in unadjusted and adjusted ORs may be explained by adjustment for timing of referral and treatment received, which are on the causal pathway from RAS use to death, so not confounding variables. Please reconsider variables included in multivariable analyses and show results without variables on causal pathway.**
- **Hetzel et al., Table 3 (pre-read 2): Sick at day-28 follow-up: Unclear how the investigators handled deaths. It seems deaths are included in the denominator but not in the numerator (not a correct approach). Please review and provide updated tables.**
- High prevalence of incorrect RAS dosage in children > 3 years noted in CARAMAL overview; this should be reported in other relevant analyses on e.g. health care worker compliance and artemisinin resistance.
- Overall, relatively few patients enrolled in Nigeria compared to the other two countries. Why? In the pre-RAS period, there are many more patients from Uganda than from the other two countries, despite similar-sized child populations. Why?
- **Large increase in number of patients in Democratic Republic of the Congo post-RAS compared to pre-RAS noted – how is this explained, and implications for CFRs?**

CARAMAL: referral completion

Brunner et al. (pre-read 3)

Very large differences noted between unadjusted and adjusted ORs (e.g. Tables 3 & 4). e.g. In Table 3, Democratic Republic of the Congo, referral completion is 71.2% for pre-RAS and 75.5% for post-RAS/no RAS use, but the adjusted OR is 0.34 (0.18, 0.66). This effect is in the opposite direction and of very large magnitude.

- **Justification and method used for variable selection are needed.**
- **These differences are likely to be explained by collinearity – to be tested for and reported.**
e.g. Enrolment pre- or during COVID-19 pandemic is likely closely correlated with pre- and post-RAS periods. In Table 4, Nigeria referral completion is 41% for those enrolled during COVID-19 pandemic and 49.5% in pre-pandemic period. This would give an unadjusted OR of 0.71 (0.46, 1.09), but the adjusted OR is 0.09 (0.03, 0.26).
- **Was “time to reach referral hospital” adjusted for? This is part of the outcome variable and there would be no time available for those who were not referred.**

CARAMAL: health workers' compliance and acceptability of RAS

Signorell et al. (pre-read 4); Awor et al. (pre-read 7)

Overall, 42.0% (3356/7983) of admitted children were administered full treatment consisting of a parenteral antimalarial and an ACT, with large variation among study countries (2.7% in Nigeria, 44.5% in Uganda, and 50.3% in Democratic Republic of the Congo).

- In Nigeria, for instance (Signorell et.al. Table 2), there was an indication of very low use of ACTs for in-hospital patients. This is somewhat paradoxical compared to the widespread availability of ACTs following Global Fund support. Can there be further clarification of the status of ACTs at the RHF. What was the effect of injectable artesunate supply on treatment compliance?
- What was the influence of previous training on the compliance of health workers to the completion of treatment?
- **The publications/manuscripts are currently split, and the pieces of relevant information are not properly aggregated to help understand the relationship of some factors and their implications on mortality, especially as reported for Nigeria. To better understand the low compliance to treatment and dosing (Signorell et.al. Fig. 4), can a composite presentation of findings on all the pre-referral issues be made?**
- **Signorell et.al. Fig. 2: Noted that the proportions receiving intravenous artesunate and ACT are increasing in all study sites – so can more deaths in Nigeria in post-RAS period reflect confounding due to indication for RAS and potential other temporal confounding factors?**

CARAMAL: clonal expansion of artemisinin-resistant *Plasmodium falciparum* in Uganda is associated with substandard treatment practices

Awor et al. (pre-read 5).

- **Among the 3686 mRDT-positive Ugandan children enrolled in the CARAMAL study, how were the 801 children selected for enrolment in this molecular substudy? Was the sample size calculated a priori?**
- **Include relevant details known and reported in other CARAMAL manuscripts in terms of antimalarial drug utilization in the study area, e.g. prevalence of underdosing RAS in children > 3 years; daily injectable artesunate use in private clinics rather than three doses in initial 24 hours; injectable artesunate use 6–7x higher in Uganda than in other African countries with similar severe malaria burdens, etc.**
- Given these concerning findings, was any follow-up done after day 28?
- Is an in-depth antimalarial drug utilization review planned in the site and ideally control sites where C469 mutations have increased similarly, but RAS has not been deployed?
- **Please provide figures and any supplementary materials related to this pre-print manuscript.**

CARAMAL: real-world costs and barriers to the successful implementation of RAS

Lambiris et al. (pre-read 6)

- Clarify nature and perspective of the economic evaluation; e.g. would a Ministry of Health perspective differ from a UNICEF perspective?

- Clarify meaning of “full implementation” in the contexts of highly variable health systems. We assume annual health systems strengthening expenditures were those incurred by the CARAMAL study (many of which overlap with routine activities), yet CARAMAL results suggest that these were not optimal for facilitating referral and consolidation of treatment with injectable artesunate plus ACTs.
- **Clarify how the denominator was defined – children under 5 at risk of any malaria or specifically severe malaria/danger signs? If the former, please justify.**
- **Report assumptions and judgements made as costs and costings are context-specific.**
- **Please provide supplementary materials related to this pre-print manuscript.**

CARAMAL: treatment-seeking at community level in Uganda

Awor et al. (pre-read 8)

- Clarify sample size calculation: A minimum of 906 households required in sample size calculation in Lengeler et al. (pre-read 1), but 462 households required per individual survey round reported in Awor et al. (pre-read 8).
- Was the presence of CHWs considered in sampling of villages? Low (12%) treatment-seeking first with VHWs was surprising given the exceptionally small ratio of population per VHW in Uganda (250–500). **Were study sites selected also within this range?**
- **Were any data collected on reasons for not seeking care for children with fever and for not going first to VHWs?** Given the results of the first round of surveys, these data could have been used to inform corrective actions to be taken during RAS implementation in the CARAMAL project and would be helpful in drafting a WHO field guide for safe and effective RAS implementation.
- Were any data collected to assess the impact of COVID-19 pandemic-related restrictions on access to care?
- **Table 3: Recommend**
 - **Separate by Survey Year, as done for Table 2, and by Danger Signs (Yes/No).**
 - **Format/indent to separate *Did something at home* and *Sought treatment outside of home*; then for *Sought treatment outside of home* format/indent again to separate *Type of outside provider visited first* and *Intervention*.**
 - **Suggest separating Primary and Secondary Health Facility.**
 - Could Secondary Facility (HF III) be combined with Referral Facility (HF IV)?
 - **Specify *Private Clinic*; does this include both outpatient clinics and private hospitals?**
 - For the reported antibiotic use, would it be possible to present data on which type of provider provided the antibiotic in supplementary material? Is it possible to determine the reason for provision of an antibiotic (i.e. did some of the children have signs suggestive of pneumonia or bloody diarrhoea)?

- Discussion: Many findings from the Uganda Malaria Indicator Survey 2018–2019 for Lango region are very different from those presented in this manuscript. The authors could discuss key results that are significantly different.

CARAMAL: treatment-seeking at community level in Uganda

Brunner et al. (pre-read 9)

- Clarify sample size calculation.
- In addition to the reasons for choosing a particular provider for referral, information on why 42% of caregivers did not follow the CHW's recommendation to go to an RHF would be important to understand overall CARAMAL results and for a WHO field guide for safe and effective RAS implementation. This group had a high likelihood of child death, so it merits further scrutiny. Are such data or any insights available?
- **Exclusion of children brought to another provider before seeing the CHW (and failure of the caregiver to recall visiting a CHW) are limitations that should be noted in the Discussion.**
- **Table 5: Clarify "other provider" in "Referred by other provider" – if other than CHW, when/how would that contact occur between first provider (CHW) and second provider?**
- The Sankey diagram is rich with information but also difficult to interpret. One reviewer suggested adding denominators for each category, although this may further clutter a complicated figure.
- Are there any preliminary data from (or plan to conduct) qualitative research to better understand why no treatment was given at the RHF or non-RHF providers despite the child being referred for suspected severe malaria?
- **Please clarify differences and similarities between this study and Awor et al. (pre-read 8) above.**
- **Please provide supplementary materials. (Supplementary tables were mentioned in the pre-print but not provided to the review team.)**

CARAMAL: treatment-seeking at community level in Nigeria

Lee et al. (pre-read 10)

- **Clarify enrolment per health care provider, with a breakdown of numbers enrolled by CHWs and the number enrolled by PHCs.** It is stated that children were enrolled from 139 community-based providers in the study area, with the number of enrolled patients per provider ranging from one to 42 (median = 2, IQR: 2–4). Yet, 314 (53%) children were enrolled by CHWs and 275 (47%) by PHCs, which gives an average of 0.68 (314/500) enrolled per CHW and 3.58 (77/275) per PHC. Note: It would be good to have this information for the Democratic Republic of the Congo and Uganda as well. We have seen in iCCM programmes/implementation research studies that large numbers of CHWs do not contribute to enrolment/case registration, which could have serious implications for pre-referral RAS.
- For the children who received artemether-lumefantrine at home, are there data to determine whether their caregivers already had the artemether-lumefantrine at home or first went to a chemist? Also, any data on if they dosed the medicine appropriately and took it with a fatty food?

- Are there plans to do any qualitative research to understand why there appeared to be differential care-seeking between CHWs and PHCs based on type of initial danger signs?
- **Please provide any supplementary materials available.**

Observations on RAS implementation studies in Malawi, Sierra Leone and Zambia

Malawi

Oliff et al. Final draft for submission

The study identified five continuum of care criteria (5CC Framework) to reinforce RAS programming: care transitions for the patient; consistency of supplies (commodity and referral slips) to village health clinics; comprehensiveness of care received by the patient at the village health clinics and RHF; connectivity of care between all tiers; and communication between providers from different points of care.

- Provider and caregiver uptake: Were there other steps in community engagement that influenced uptake of RAS beyond posters?
- Health system supports were not mentioned: How influential was the district health system in supporting RAS uptake and follow-up to treatment?
- How feasible is the integration of the RAS referral slip with the existing Ministry of Health referral note?
- **Please share as many implementation plans, standard operating procedures, etc. to inform comparisons across sites/studies and a WHO field guide for safe and effective RAS implementation.**

Zambia: scaling up use of RAS for severe malaria at community level

Green et al. Final draft for submission

This successful model could provide helpful insights to inform a WHO field guide for safe and effective RAS implementation.

- **Clarify how CFR was calculated pre-RAS.**
- No reference was given to previous case management capacity. How did the study ensure that Community Health Volunteers (CHVs) did not compromise identification of danger signs for referral?
- Did the study observe more utilization or uptake of RAS at community/health facility level or not? What could explain this?
- **Did the study observe improvements in health care-seeking behaviour among caregivers of sick children?**
- Were referral forms given to CHVs developed for the study or were these from the Ministry of Health?
- Supply chain: How was the RAS supply chain managed and how can this be sustained and strengthened?
- Was there any evidence of RAS accountability and mechanisms to ensure rational use of the products?
- Community participation: What is the role of the community governance structures, if any, on service provision at community level that involves the

Community Health Volunteers? What possibilities exist for integration with other community-based interventions?

- How available was community infrastructure to support RAS implementation at community level? Where were storage of commodities and service provided?
- How did other community health governance structures support the introduction of RAS services and referral within an existing platform?
- Was mentorship conducted; if so, who performed this task and how was it conducted?
- Data management: How did the study recognize the existing national health management information system and how can this be strengthened to optimize RAS roll-out?
- **Please share as many implementation plans, standard operating procedures, etc. to inform comparisons across sites/studies and a WHO field guide for safe and effective RAS implementation.**

Sierra Leone

Karama et.al

This successful model could provide helpful insights to inform a WHO field guide for safe and effective RAS implementation.

- **Could the decreased CFR observed reflect increased number of cases referred, including less severe cases? Was any difference in referral rates seen pre- and post-RAS?**
- How feasible would it be to scale up this model across Sierra Leone?
- **Please share as many implementation plans, standard operating procedures, etc. to inform comparisons across sites/studies and a WHO field guide for safe and effective RAS implementation.**

Responses to questions by study teams

WHO Evidence Review Group: Effectiveness of Rectal Artesunate

Preparatory meeting: 20–21 September 2022

⇒ [Swiss TPH responses in blue below, 7 October 2022](#)

Objectives

- To determine factors required to deploy RAS safely and effectively.
- To identify questions for study teams to address ahead of evidence review group meeting on 18–19 October 2022.

Questions and clarifications for study teams

CARAMAL study: overall comments

- Access to the final CARAMAL report would assist reviewers to understand the full context and findings of this large, important and complex body of work.

A scientific report was submitted to the WHO Global Malaria Programme on 8 April 2021 in preparation of the evidence assessment meeting of 27–29 April. However, this report is outdated, many analyses have been re-run and the interpretation has been elaborated much more since the drafting of that report. We, therefore, feel that the context of the study is better reflected in the paper by Lengeler et al., and the updated findings and interpretations are well reflected in the manuscripts.

- Seven of the manuscripts shared are not yet published or peer reviewed, but kindly provided as pre-prints, mostly dated 2021. Could updated manuscripts resubmitted or responses to reviewers' comments to date be shared?

Updated versions of the following manuscripts are available (attached):

- #4 Signorell et al. (updated version, currently under review at PLoS Medicine)
- #5 Awor et al. (updated file as submitted to Lancet Infectious Diseases, including supplementary materials)
- #6 Lambiris et al. (revised version, currently under review at Lancet Global Health)
- #7 Awor et al. (revised version, just accepted by Malaria Journal, pre-typeset)
- #9 Brunner et al. (updated file, including supplementary materials)
- #11 Okitawutshu et al. (final version, now published in Malaria Journal)

We consider reviewers' comments and responses confidential and sharing to go beyond good scientific practice.

- Please share as many implementation plans, standard operating procedures, etc. to inform comparisons across sites/studies and a WHO field guide for safe and effective RAS implementation. It would also be helpful to have a summary of interventions/inputs (from Ministries of Health, UNICEF, CHAI, etc.), i.e. training (including refresher), supply chain, supervision, mentoring, data management, quality assurance, at CHW, PHC and referral levels individually for the three countries. This should include details on linkages between the referral facility and CHW/PHC.

A description of the study sites and implementation activities has been provided in Lengeler et al. (2022). More detailed implementation documents are in the hands of UNICEF and CHAI. UNICEF was responsible for supporting the roll-out of RAS. (See documents sent by Valentina Buj on 29 September 2022).

**CARAMAL methodological reviews: CARAMAL study
Lengeler et al. (pre-read 1); Hetzel et al. (pre-read 2)**

- Is a pre-defined statistical analysis plan available?

Yes, an analysis plan was developed along the project's major thematic areas. The analysis plan was updated in March 2021 with specific timelines in preparation for the WHO CARAMAL Project Evidence Assessment meeting on 27–29 April 2021. The plan was shared with WHO for inputs prior to the April 2021 meeting. (See attached CARAMAL, Analysis plan with timelines, 02.03.2021.pdf).

- Pre- vs post-intervention study design, yet the durations pre and post are not matched and most comparisons are of RAS vs no RAS overall.

This study was observational, accompanying the routine roll-out of RAS through established systems. The implementation did not happen in a research-style controlled setting. The theoretical assumption in a controlled setting would be that from the time of RAS introduction, RAS would be available everywhere and administered to all eligible children. This was not the case (see Lengeler et al. and Hetzel et al.) and, particularly in Uganda and Nigeria, it took several months until sufficient stock was available at all PHC providers. This had to do, among other issues, with the short shelf life of RAS under very hot conditions (originally considered only three months, later extended to six months), requiring a very regular re-stocking of a large number of remotely located community-based providers, in order to avoid drug wastage. Pre-RAS data collection started later than anticipated due to delays in contract signing and ethical approval procedures; implementation of RAS was also dependent on local regulatory approval and logistics. The project only had limited influence on these factors. Nevertheless, the duration of the pre-RAS vs post-RAS study periods was largely according to the initial study protocol.

We have provided a comparison of patient health outcomes in the pre-RAS vs post-RAS periods in Hetzel et al. (see Table 3).

As a significant number of children did not receive RAS in the early post-implementation period, a pre vs post analysis has little meaning with regard to the health effect of RAS. The primary analysis presented is therefore the comparison between RAS users and RAS non-users, reflecting that the effect of RAS is based on the medicine's administration rather than its mere availability in a warehouse. The analysis was done over the entire study period to include all users and non-users of RAS, adjusted for the RAS implementation period (pre vs post) to capture the additional effect of potential differences of relevance to the health outcome between the study period. We consider this analysis the most adequate to assess the effect of RAS on health outcomes.

- Sample size calculation based on all sites combined, yet results presented by country. Justify.

Based on initial information provided to the study team in the proposal development stage, the three study sites were expected to be more similar in terms of malaria case fatality. However, considering the large observed differences in baseline CFR and the differences in RAS coverage, referral rates and post-referral treatment coverage (all a reflection of the different health systems), a pooled analysis would have been trivial, as it would have provided values for a non-existing scenario. Even though this does come at the “cost” of losing power (particularly in Uganda, where fatal outcomes were extremely rare), we decided to conduct a stratified analysis by country that allows assessing the situation in the three distinct settings.

- How were study areas selected for each country (e.g. would a > 6-hour delay in reaching RHF be expected)? Any other significant changes in malaria case management ecosystems at these sites over the study period?

The study site selection is described in detail in Lengeler et al. 2022. The primary requirement was a UNICEF-supported system of CHWs implementing iCCM, and a record of a sufficient number of (severe) malaria cases to meet the study sample size. The decision to select UNICEF as sole implementing partner was made by the funding agency.

As per our study results (see e.g. Brunner et al. 2022, BMJ Global Health), referral delay was frequently more than one day.

To the best of our knowledge, there were no other major changes in malaria case management coinciding with the introduction of RAS beyond the selected supportive interventions described in Lengeler et al.

- Additional analyses requested:

- **Pre- vs post-intervention** (e.g. Table 4 in Hetzel et al.): Why was this not the primary analysis, as this is how study was designed? We would like to see unadjusted and adjusted analyses, based on characteristics of patients presenting, season, etc.

The rationale for focusing on a user vs non-user analysis is provided above. In short, if a significant number of patients in the post-intervention period did not receive RAS, then the measured effect would be “diluted” by the incomplete post-implementation coverage.

Nevertheless, the unadjusted effect of post-RAS vs pre-RAS on health outcomes is presented in Hetzel et al. (see Table 3). We consider further adjusted analyses comparing the health outcomes between pre- and post-implementation periods not to be very meaningful and potentially misleading, for the above-mentioned reasons.

- **Only in post-RAS period** (to reduce temporal confounding): e.g. Nigeria CFR appeared to increase from ~4% to 12% in pre- and post-intervention periods in no-RAS groups (derived from data in Table 4).

In Nigeria, over the entire study period, the CFR was higher among patients enrolled at PHCs than in patients enrolled by CHWs (CORPs). Due to the PHC health worker strike at the onset of the project, fewer patients were enrolled at PHCs during the pre-RAS period. A higher number of PHC enrolments post-RAS contributed to the overall higher CFR during that period. At the same time, we did observe an increase in CFR over the course of the project among enrolments from both provider types. To account for these differences, we adjusted for the type of enrolling provider, for study period, and for seasonality in our analyses.

- RAS vs no RAS (as per Table 3 in Gomes et al., Lancet 2009 [pre-read 15])
i.e. risk of death within 0–6 hours and, if survived > 6 hours, if reached clinic in < 6 hours vs ≥ 6 hours, showing the SAME baseline confounders for each country (e.g. age, sex, rainy season, convulsions). In Brunner et al. (BMJ Global Health, pre-read 3) Table 2, time to referral is only reported for the following time ranges in minutes: 0 < 15 (reference); 15 < 30; 30 < 60 and ≥ 60, which does not take into account that RAS requires 6–9 hours to have a significant reduction in parasitaemia.

It is important to note again that CARAMAL was an observational study with different aims than the controlled trial by Gomes et al. We did not replicate the study protocol of Gomes et al. and can therefore not replicate the analyses presented in the Lancet paper. Specifically, we did not assess time of death or time between RAS administration and referral completion in hours. Assessing the exact time of death as done by Gomes et al. would have required a continuous close follow-up of the patient, which is neither possible nor desired in an observational study. It also raises serious ethical issues if a patient is closely followed to document the exact time of death yet without intervening when the patient's condition worsens.

Brunner et al. (BMJ Global Health) presents the modelled travel time between a patient's household and the nearest referral facility as a theoretical value and proxy for accessibility (details provided below). It does not necessarily represent the actual time between RAS administration and arrival at a referral facility, which is a product of many additional potential delays.

- Among those successfully referred and given injectable treatment: Risk of death following RAS vs no RAS among those who received injectable artesunate and those who received injectable quinine, again adjusting for the SAME baseline confounders for each country.

We have run the analyses as requested, comparing the CFRs between RAS users and RAS non-users only among those patients who completed referral and received parenteral antimalarial treatment, adjusting for baseline characteristics as in the full model, for each country. Results are presented below.

We would like to caution that this is a post hoc subgroup analysis focusing on a group of patients that is likely strongly biased and unlikely to represent the majority of cases. Children who don't complete referral and don't receive parenteral antimalarials may be significantly different from those completing referral and receiving parenteral treatment. Factors determining whether or not a patient completes referral (e.g. socioeconomic characteristics or distance to the referral facility) are likely also associated with the health outcome.

Democratic Republic of the Congo

Mixed effects model for outcome "dead on day 28" with random effect, adjusted for same baseline characteristics as in Table 4, limited to patients completing referral and receiving injectable quinine or artesunate at a referral facility (N = 1028; 31 observations dropped due to collinearity).

RAS use: adj OR: 2.7; 95% CI: 0.7–11.1; p = 0.17

Nigeria

Mixed effects model for outcome “dead on day 28” with random effect, adjusted for same baseline characteristics as in Table 4, limited to patients completing referral and receiving injectable quinine or artesunate at a referral facility (N = 134, 38 observations dropped due to collinearity)

RAS use: adj OR: 1.3; 95% CI: 0.4–4.5; p = 0.71

Uganda

Mixed effects model for outcome “dead or sick on day 28” with random effect, adjusted for same baseline characteristics as in Table 4, limited to patients completing referral and reporting the administration of an injectable antimalarial (N = 739). Due to the small number of events, it was not possible to run this model for the outcome “dead on day 28”.

RAS use: adj OR: 0.5; 95% CI: 0.3–1.1; p = 0.08

- Very large differences between unadjusted and adjusted ORs noted, particularly for Democratic Republic of the Congo.

Justification and method used for variable selection in adjusted analyses are needed.

Covariates in the multivariable analyses were chosen a priori and ranked by importance based on existing knowledge of potential association with a patient’s health outcome. We chose this approach over an automated variable selection, which is inadvisable due to numerous widely recognized statistical problems. We made one post hoc modification in the adjustment for severity with the inclusion of a binary variable for convulsions.

We limited the number of covariates to avoid overfitting of models with a small number of events. This led to models with fewer adjustments in Uganda, where the number of death events was small, and in Nigeria, where the overall number of study participants was low.

We provided three different analyses of two day-28 health outcome measures (dead and dead or sick), provided in Hetzel et al., Table 4:

1. firstly, an unadjusted analysis providing the crude OR;
2. secondly, an analysis adjusted only for background characteristics of the patient (which might make them different at the time of entry into the study and which might be relevant in terms of how well they respond to RAS: sex, age, convulsions as proxy of central nervous system symptoms of severity, enrolment location) and potential temporal confounders that could influence the measured effect of RAS (beginning of RAS roll-out, season). We also performed a separate analysis limited to the pre-COVID-19 period to assess if part of the (lack of) effect is due to COVID-19-related measures. (We found that this was not the case; see supplement to Hetzel et al. 2022).
3. thirdly, an analysis further adjusted for referral completion and post-referral treatment, both factors that are expected to influence the health outcome of a patient, in addition to, or independently of the effect of RAS, and the effect of which we wanted to assess (referral completion and post-referral antimalarial treatment).

- These large changes in unadjusted and adjusted ORs may be explained by adjustment for timing of referral and treatment received, which are on the causal pathway from RAS use to death, so not confounding variables. Please reconsider variables included in multivariable analyses and show results without variables on causal pathway.

We are aware of the rather large difference in effect size between the first adjusted analysis and the analysis further adjusting for referral and post-referral treatment in the case of Democratic Republic of the Congo for the outcome “death”. The effect sizes for RAS in the Nigeria and Uganda models are hardly affected by the adjustments. It should be noted that, albeit the difference in effect size between the Democratic Republic of the Congo models appears rather large, these differences are not statistically significant. The difference between the first and the second adjusted model is primarily a result of the addition of the post-referral treatment variable. The difference between the smallest (unadjusted) and largest (adjusted for baseline characteristics, referral and post-referral treatment) OR estimates is large but not statistically significant ($p = 0.058$).

In Table 4, we have presented three models for each of the outcome measures, where the first “Adjusted” does not include referral completion and post-referral treatment. However, we believe the adjustments for referral completion and post-referral treatment, both potentially on the causal pathway, are both adequate and relevant, considering that both factors may impact the health outcome, in addition to, or independent of the effect of RAS. In short, the final health outcome is a product of the effect of RAS and what happens thereafter (which is essentially referral and post-referral treatment).

- **Hetzel et al., Table 3: Sick at day-28 follow-up: Unclear how the investigators handled deaths. It seems deaths are included in the denominator but not in the numerator (not a correct approach). Please review and provide updated tables.**

We understand the reviewer’s point, as it may appear counter-intuitive to include dead children in the denominator for calculating the % sick on day 28. Yet, both approaches have their merit:

- In the existing table, we calculated the % dead and the % sick out of all children enrolled into the study. This allows a direct comparison of the percentages for the two outcomes.
- Excluding dead children from the denominator represents the % sick out of all children alive on day 28. We have provided this analysis in the amended table below. The problem with this calculation is that the proportions will no longer be “aligned”. For example, in Nigeria, we would then have 11.7% dead and 6.5% sick, suggesting that there were less than twice as many dead than sick, which is not true (since there were 34 sick and 69 dead).

	DEMOCRATIC REPUBLIC OF THE CONGO			NIGERIA			UGANDA			BETWEEN-COUNTRY P-VALUE
	N/N	(%)	P-VALUE*	N/N	(%)	P-VALUE*	N/N	(%)	P-VALUE*	
Case fatality rate										
Overall	135/2011	(6.7)		69/589	(11.7)		19/3686	(0.5)		<0.001
Implementation										
Pre-RAS	20/304	(6.6)		9/217	(4.2)		4/1441	(0.3)		
Post-RAS	115/1707	(6.7)	0.92	60/372	(16.1)	<0.001	15/2245	(0.7)	0.14	
RAS use										
No	27/475	(5.7)		30/391	(7.7)		12/2018	(0.6)		
Yes	108/1536	(7.0)	0.34	39/198	(19.7)	<0.001	7/1668	(0.4)	0.45	
Sick at day-28 follow-up										
Overall	242/2011	(12.0)		34/589	(5.8)		589/3686	(16.0)		0.002
Implementation										
Pre-RAS	40/304	(13.2)		20/217	(9.2)		299/1441	(20.8)		
Post-RAS	202/1707	(11.8)	0.59	14/372	(3.8)	0.007	290/2245	(12.9)	0.003	
RAS use										
No	72/475	(15.2)		25/391	(6.4)		428/2018	(21.2)		
Yes	170/1536	(11.1)	0.04	9/198	(4.6)	0.30	161/1668	(9.7)	<0.001	
Sick at day-28 follow-up among children alive on day 28										
Overall	242/1876	(12.9)		34/520	(6.5)		589/3667	(16.1)		0.009
Implementation										
Pre-RAS	40/284	(14.1)		20/208	(9.6)		299/1437	(20.8)		
Post-RAS	202/1592	(12.7)	0.600	14/312	(4.5)	0.021	290/2230	(13.0)	0.003	
RAS use										
No	72/448	(16.1)		25/361	(6.9)		428/2006	(21.3)		
Yes	170/1428	(11.9)	0.045	9/159	(5.7)	0.526	161/1661	(9.7)	<0.001	

*chi-square test, accounting for clustering at provider level, **among children alive on day 28

- High prevalence of incorrect RAS dosage in children > 3 years noted in CARAMAL overview; this should be reported in other relevant analyses on e.g. health care worker compliance and artemisinin resistance.

Unfortunately, the RAS dosage was assessed only in a subsample of patients.

- Overall, relatively few patients enrolled in Nigeria, compared to the other two countries. Why? In the pre-RAS period, there are many more patients from Uganda than from the other two countries, despite similar-sized child populations. Why?

This observation is correct. The malaria epidemiology, the health systems, and the operational and physical environments differ substantially between the three study settings. A description of the settings is provided in Lengeler et al. and in UNICEF reports. Briefly, in Uganda, health providers are more accessible due to shorter distances and a very large number of health workers. This facilitated patient access and study set-up. In Nigeria, severe malaria cases often go directly to a referral facility (cottage hospital) rather than attending a CHW (locally "CORP") who was not previously known to deal with cases of severe childhood

illness. In the early study phase, there was an unexpected health worker strike in PHCs in Nigeria, limiting the extent to which we could enrol at PHCs (described in Brunner et al., BMJ Global Health). In the Democratic Republic of the Congo, many places were difficult to access due to lack of transport infrastructure, causing delays in the sensitization of PHC health care workers leading to a longer lead-time until patient recruitment started everywhere. CHWs are few and care for severe illness is mostly sought at PHCs and referral facilities.

- **Large increase in number of patients in the Democratic Republic of the Congo post-RAS compared to pre-RAS noted – how is this explained, and implications for CFRs?**

See point above regarding operational and logistical challenges in the Democratic Republic of the Congo. There may be other reasons for the increase in enrolled patients, including more patients seeking care at a primary provider compared to a hospital (though this is purely speculative). Comparing community and hospital enrolments, in the post-RAS period, comparably more children were enrolled at community level (57% vs 40% at referral facility level, see Lengeler et al.). Overall, the CFR was 6.7% for children enrolled at community level vs. 1.9% for children enrolled at referral facilities (Lengeler et al. 2022; please note that referral facility enrolments were not included in the health outcome analysis in Hetzel et al.). However, there was no difference in the CFR between the pre-RAS and post-RAS periods in children enrolled at community level (6.6 vs. 6.7%, see Hetzel et al., Table 3).

CARAMAL: referral completion

Brunner et al. (pre-read 3)

Very large differences noted between unadjusted and adjusted ORs (e.g. Tables 3 & 4). e.g. In Table 3, the Democratic Republic of the Congo, referral completion is 71.2% in pre-RAS and 75.5% for post-RAS/no RAS use, but the adjusted OR is 0.34 (0.18, 0.66). This effect is in the opposite direction and of very large magnitude.

Please note that the unadjusted ORs can be found in the supplementary material. The unadjusted ORs may not be identical with what would be calculated from the raw data due to the introduction of random effects (accounting for clustering; see methods section). In the Democratic Republic of the Congo, we noted that the introduction of random effects altered the effect size significantly. Subsequently, we closely examined associations between the outcome, independent variables and the cluster variable. We did not find any problematic associations.

In the example above, the difference between the adjusted and unadjusted ORs is marginal (unadjusted OR: 0.42 (0.23–0.77), adjusted OR: 0.34 (0.18–0.66)). As outlined in the discussion, post-RAS/no RAS use data in the Democratic Republic of the Congo are hardly comparable to the other two study groups (pre-RAS, post-RAS/RAS use) because of the small number of cases in the post-RAS period that did not receive RAS.

- Justification and method used for variable selection are needed.

Determinants were selected prior to analysis based on results of previous studies. Determinants known to be associated with treatment-seeking but not included in the models were not available (e.g. socioeconomic status).

- These differences are likely to be explained by collinearity – to be tested for and reported.
e.g. Enrolment pre- or during COVID-19 pandemic is likely closely correlated with pre- and post-RAS periods. In Table 4, Nigeria referral completion is 41% for those enrolled during COVID-19 pandemic and 49.5% in pre-pandemic

period. This would give an unadjusted OR of 0.71 (0.46, 1.09), but the adjusted OR is 0.09 (0.03, 0.26).

Please note that the unadjusted ORs can be found in the supplementary material and deviate from ORs calculated from raw data due to the introduction of random effects (accounting for clustering; see methods).

- Was “time to reach referral hospital” adjusted for? This is part of the outcome variable and there would be no time available for those who were not referred.

We adjusted for the distance between the patient’s household and the referral facility (expressed as hypothetical travel time based on modelled data using Malaria Atlas Project friction rasters. Please see the methods section under “Outcomes and explanatory variables” for more detail).

Please note that there is a difference between this theoretical value and the actual delay to reach the RHF. To use “Time to Referral Health Facility” as a determinant, we had to calculate theoretical values because it would not be available for children who did not complete referral (as the reviewer correctly notes). To examine actual behaviour, we only used data of children who completed referral.

CARAMAL: health workers’ compliance and acceptability of RAS

Signorell et al. (pre-read 4); Awor et al. (pre-read 7)

Overall, 42.0% (3356/7983) of admitted children were administered full treatment consisting of a parenteral antimalarial and an ACT, with large variation among study countries (2.7% in Nigeria, 44.5% in Uganda, and 50.3% in the Democratic Republic of the Congo).

- In Nigeria, for instance (Table 2), there was an indication of very low use of ACTs for in-hospital patients. This is somewhat paradoxical compared to the widespread availability of ACTs following Global Fund support. Can there be further clarification of the status of ACTs at the RHF. What was the effect of injectable artesunate supply on treatment compliance?

During annual cross-sectional health care provider surveys, adequate supplies of ACTs were available at all cottage hospitals (referral facilities) in Nigeria. The survey did not assess to whom or how they were dispensed. It should also be noted that in some cases in which an ACT was not administered, it was prescribed to the patient. Whether or not the caregivers then purchased and the child completed a full dose of ACT could not be systematically verified in our study.

Please note that only the Democratic Republic of the Congo profited from direct injectable artesunate supply in the frame of the project (27 000 vials in 2019, see Lengeler et al.). We attribute the increase in treatment compliance in the post-RAS period in the Democratic Republic of the Congo at least in part to this supportive intervention (due to the replacement of injectable quinine and oral quinine).

- What was the influence of previous training on the compliance of the health workers to the completion of treatment?

This was not measured in a systematic way. We would assume some positive effect of repeated health worker trainings in the frame of RAS implementation on the compliance with treatment guidelines.

- The publications/manuscripts are currently split, and the pieces of relevant information are not properly aggregated to help understand the relationship

of some factors and their implications on mortality, especially as reported for Nigeria. To better understand the low compliance to treatment and dosing (Signorell et.al. Fig. 4), can a composite presentation of findings on all the pre-referral issues be made?

Due to the large amount of data, it was impossible to present everything in one manuscript. Also, depending on the specific aspect of interest, a different combination of information may be relevant. We are currently working on a different presentation for the review group meeting in October.

- Signorell et.al. Fig. 2: Noted that the proportions receiving intravenous artesunate and ACT are increasing in all study sites – so can more deaths in Nigeria in post-RAS period reflect confounding due to indication for RAS and potential other temporal confounding factors?

Note that the proportion of children receiving intravenous artesunate + ACT slightly **decreases** in Uganda.

Over the entire study period, the CFR was higher among patients enrolled at PHCs than in patients enrolled by CHWs (CORPs), likely because patients attending PHCs were more severely ill than those seen by CHWs. Due to the PHC health worker strike at the onset of the project, fewer patients were enrolled at PHCs during the pre-RAS period. A higher number of PHC enrolments post-RAS contributed to the overall higher CFR during that period. At the same time, we saw an increase in CFR over the course of the project among enrolments from both provider types.

In Nigeria, referral completion in the post-RAS phase irrespective of RAS use was higher compared with the pre-RAS phase (probably because more children were enrolled at PHCs from where referral completion was generally higher than from CHWs, and because of the project's emphasis on referral completion). However, among PHC enrolments, those who had received RAS were significantly less likely to complete referral than those not receiving RAS in the post-RAS phase. In Nigeria, administration of a parenteral antimalarial was associated with survival (Hetzl et al.).

We cannot exclude that concomitant infections and morbidities may have contributed to the increase in CFR in the post-RAS period. The COVID-19 pandemic for instance had a negative effect on referral completion in Nigeria (Brunner et al.).

CARAMAL: clonal expansion of artemisinin-resistant *Plasmodium falciparum* in Uganda is associated with substandard treatment practices

Awor et al. (pre-read 5).

- Among the 3686 mRDT-positive Ugandan children enrolled in the CARAMAL study, how were the 801 children selected for enrolment in this molecular substudy? Was the sample size calculated a priori?

During defined time periods (Group A and B included children consecutively enrolled between August 2018 and February 2019, and Group C and D during November 2019 and August 2020), filter paper samples were collected from all eligible children in the four study groups. Out of the collected filter papers, some contained an insufficient amount of DNA for the sequencing analyses. The final dataset includes all samples that had sufficient DNA for analysis and a confirmation of consent from the caregiver of the child.

- Include relevant details known and reported in other CARAMAL manuscripts in terms of antimalarial drug utilization in the study area, e.g. prevalence of underdosing RAS in children > 3 years; daily injectable artesunate use in private clinics rather than three doses in initial 24 hours; injectable artesunate use 6–7x higher in Uganda than in other African countries with similar severe malaria burdens, etc.

This information is only relevant for the study group sampled after treatment (Group C in the manuscript) on day 28. As this group includes children who did not complete referral to one of the monitored official referral facilities, we don't have detailed treatment data available but only information reported retrospectively by the caregiver.

- Given these concerning findings, was any follow up–done after day 28?

Children who tested positive by mRDT on day 28 were referred to a health facility. K13 analyses were done in batches after completion of the patient follow-up. There was no individual patient follow-up done thereafter. Authorities, including the national malaria programme, were always kept informed about the findings.

- Is an in–depth antimalarial drug utilization review planned in the site and ideally control sites where C469 mutations have increased similarly, but RAS has not been deployed?

We are unaware of any such plans.

- Please provide figures and any supplementary materials related to this pre–print manuscript.

All figures and additional material are available in the version submitted to the *Lancet Infectious Diseases* (updated document provided).

CARAMAL: real-world costs and barriers to the successful implementation of RAS

Lambiris et al. (pre-read 6).

- Clarify nature and perspective of the economic evaluation; e.g. would a Ministry of Health perspective differ from a UNICEF perspective?

The RAS implementation was done as a full joint activity between UNICEF and the relevant government entities (national and local). UNICEF-specific expenses were not included in the costing. Hence, the costs presented fully reflect the Ministry of Health perspective.

- Clarify meaning of “full implementation” in the contexts of highly variable health systems. We assume annual health systems strengthening expenditures were those incurred by the CARAMAL study (many of which overlap with routine activities), yet CARAMAL results suggest that these were not optimal for facilitating referral and consolidation of treatment with injectable artesunate plus ACTs.

This is a good point raised by the reviewer. After the initial survey of health facilities in the project area (done before implementing RAS – see specific reports), a number of weaknesses were identified. At this point, the project partners, together with the local health authorities, reviewed the situation and decided to implement a small number of interventions aimed at correcting some essential deficiencies – for example the provision of injectable artesunate in the health facilities where this was not available. There was a fine line between correcting some system deficiencies, while not transforming the

health system in such a way that it would be neither reflecting the reality, nor sustainable in the future. From the start, the CARAMAL project was aiming to describe how RAS implementation would fare in a real-world setting, and not in an idealized environment, as the latter had already been done in the frame of the only existing randomized controlled trial in Ghana and the United Republic of Tanzania.

The fact that CARAMAL undertook some initial health systems strengthening is not incompatible with later findings of major health system deficiencies. For example, CARAMAL never set up a system for transporting and financially supporting patients in need of referral. That would have been totally unsustainable. Later, one finding was that referral rates were low in some settings – as it is in many malaria endemic areas. Other examples were the lack of a full ACT treatment in severe malaria cases – while ACT was actually in stock in the health facilities (and hence this was not identified as an issue). Or the absence of medical doctors in Nigerian referral facilities – while there were doctors posted there during our initial assessment.

- Clarify how the denominator was defined – children under 5 at risk of any malaria or specifically severe malaria/danger signs? If the former, please justify.

Two calculations were done, as shown in Fig. 2 of the Lambiris et al. publication, namely cost per child < 5 years at risk and per child treated with RAS. We did not perform a calculation per child with severe malaria (in the population), as this population-level denominator was not available.

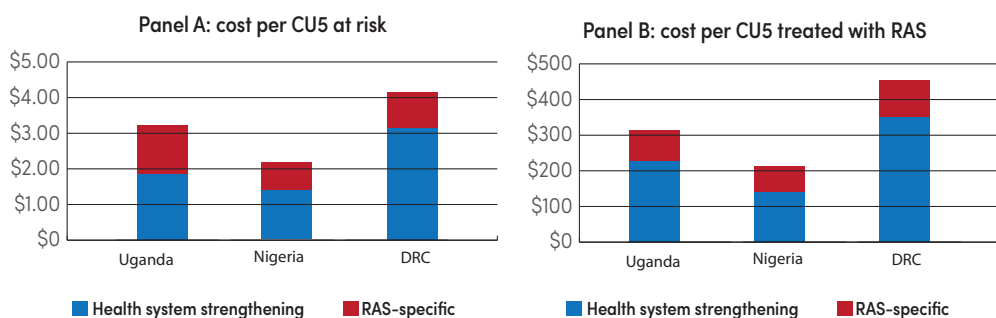


Figure 2 : Health system strengthening vs “RAS-specific” equivalent annual cost of RAS implementation, Panel A: per child under 5 at risk of severe malaria; Panel B: per child under 5 treated with RAS

Note: Costs are calculated as equivalent annual costs and in 2019 real USD. Start-up costs were annualised over 10 years. The denominator in Panel A is the total number of children in implementation areas, or otherwise: all children at risk of severe malaria. Number of CU5 covered by the implementation in Nigeria was calculated as the total number of CU5 in Adamawa state multiplied by the proportion of settlements in Adamawa covered by the iCCM programme, i.e. areas where the project was rolled out (24.7%). The denominator in Panel B was based on the total number of children recruited at the study sites either from a CHW or a PHC (where, according to guidelines, a child with suspected severe malaria should be given RAS and referred; this assumes that once HSS is sufficiently funded over the 10-year annualisation period, RAS is stocked regularly and available). Since RAS was implemented in additional districts or local government areas in Uganda and Nigeria, compared to the areas where patients were enrolled, the number of children treated was scaled up proportionally. For number of CU5 treated with RAS see Supplementary Table S1 1 and Lengeler and Burri et al. (2022).

- Report assumptions and judgements made as costs and costing are context-specific.

Our report is the reflection of the costs incurred during the implementation of the CARAMAL project, plus the cost associated with the CHW programme in the three settings. All costing units were either calculated from existing accounts, or obtained from reliable national and local sources. Few assumptions were made and they are reported specifically in the methods section.

- Please provide supplementary materials related to this pre-print manuscript.

We have attached an updated (re-submitted) version of Lambiris et al. including the supplementary materials.

- If feasible, provide additional analysis reporting either incremental cost outcome analysis and willingness-to-pay or budget impact analysis.

This can unfortunately not be done for lack of time and resources.

CARAMAL: treatment-seeking at community level in Uganda

Awor et al. (pre-read 8)

- Clarify sample size calculation: A minimum of 906 households required in sample size calculation in Lengeler et al. (pre-read 1), but 462 households required per individual survey round reported in Awor et al. (pre-read 8).

About 1020 households were visited each survey round/year. All households had a child under 5. Some children were sick two weeks prior to the survey (with a febrile illness) and received a treatment-seeking questionnaire for that illness. Analysis for this paper was restricted to children who had been ill within two weeks prior to the survey.

Another subset of children had not been sick two weeks prior to the survey. Their caretakers received a vignette-based questionnaire related to severe and simple malaria in children. So we do have responses from all 1020 households either related to an actual illness (two weeks prior to the survey) or based on a vignette.

We could not present the different types of results from the different data sources all together. We have sufficient sample size to present results from children who were sick two weeks prior to the survey. This is the focus of this paper.

- Was the presence of CHWs considered in sampling of villages? Low (12%) treatment-seeking first with VHWs was surprising given exceptionally small ratio of population per VHW in Uganda (250–500). Were study sites selected also within this range?

All villages have CHWs. There are nearly 4000 CHWs in the study area. Sampling of villages was random.

- Were any data collected on reasons for not seeking care for children with fever and for not going first to VHWs? Given the results of the first round of surveys, these data could have been used to inform corrective actions to be taken during RAS implementation in the CARAMAL project and would be helpful in drafting a WHO field guide for safe and effective RAS implementation.

Our survey instruments did not collect information on why a particular provider was not visited.

The data were presented to the national malaria programme and CARAMAL implementing partners in a timely manner, every year. Challenges at the CHW

level were mainly related to frequent stockouts. This data were also made available every quarter to the programme (through quarterly reviews of CHW service delivery).

- Were any data collected to assess the impact of COVID-19 pandemic-related restrictions on access to care?

We only analysed trends in access to services. Only small reductions in access to care were observed in Uganda.

In the manuscripts by Brunner et al. (BMJ Global Health) and Hetzel et al., the effects of COVID-19 on the respective outcomes (referral completion and case fatality) were assessed.

- Table 3: Recommend

- Separate by Survey Year, as done for Table 2, and by Danger Signs (Yes/No).
- Format/Indent to separate Did something at home and Sought treatment outside of home; then for Sought treatment outside of home format/indent again to separate Type of outside provider visited first and Intervention.
- Suggest separating Primary and Secondary Health Facility.
- Could Secondary Facility (HF III) be combined with Referral Facility (HF IV)?
- Specify Private Clinic; does this include both outpatient clinics and private hospitals?
- For the reported antibiotic use, would it be possible to present data on which type of provider provided the antibiotic in supplementary material? Is it possible to determine the reason for provision of an antibiotic (i.e., did some of the children have signs suggestive of pneumonia or bloody diarrhoea)?

This manuscript has already been published in the format shared.

- Discussion: Many findings from the Uganda Malaria Indicator Survey 2018–2019 for Lango region are very different from those presented in this manuscript. The authors could discuss key results that are significantly different.

Noted.

CARAMAL: treatment-seeking at community level in Uganda

Brunner et al. (pre-read 9)

- Clarify sample size calculation.

The sample size for the overall CARAMAL study was calculated to detect a difference in case fatality (see Hetzel et al.). This study was a post hoc analysis using post-RAS data from Uganda. Given the descriptive nature of the study and the large sample size, we believe that the presented results are of sufficient precision and reliability.

- In addition to the reasons for choosing a particular provider for referral, information on why 42% of caregivers did not follow the CHW's recommendations to go to an RHF would be important to understand overall CARAMAL results and for a WHO field guide for safe and effective RAS

implementation. This group had a high likelihood of child death, so it merits further scrutiny. Are such data or any insights available?

We have this information for 553 children who were sent to an RHF by the CHW but the advice was not followed according to the caregiver. The following reasons are not mutually exclusive: 51% did not follow the advice because the RHF was said to be too far away. The reason in 53% of the non-compliant cases was no available transport. Another important reason was the lack of money (23%).

- Exclusion of children brought to another provider before seeing the CHW (and failure of the caregiver to recall visiting a CHW) are limitations that should be noted in the Discussion.

Noted.

- Table 5: Clarify “other provider” in “Referred by other provider” – if other than CHW, when/how would that contact occur between first provider (CHW) and second provider?

The reviewer is correct. The reason for going to a second provider, if “referred by other provider” was named, is the referral by a CHW. We will correct this in the final version of the manuscript which is currently under review.

- The Sankey diagram is rich with information but also difficult to interpret. One reviewer suggested adding denominators for each category, although this may further clutter a complicated figure.

The purpose of the Sankey diagram is not to provide precise estimates of the percentage at each step but to visualize the complexity and dynamics in treatment-seeking processes.

We are aware that the Sankey diagrams are difficult to interpret. However, we can now provide a link to the interactive versions of the Sankey diagrams: <https://public.tableau.com/app/profile/swisstph.caramal>
Hovering over the nodes and flows opens a text box which includes the denominator and other information.

- Are there any preliminary data from (or plan to conduct) qualitative research to better understand why no treatment was given at the RHF or non-RHF providers despite the child being referred for suspected severe malaria?

Qualitative inquiries into reasons for not performing certain procedures at the referral facilities were beyond the scope and capacity of our project. Anecdotally, the reasons for not providing severe malaria treatment at post-referral level may include that the child was no longer presenting with severe symptoms/no longer perceived to have severe malaria/not diagnosed at the RHF as having severe malaria. Note that the treatment compliance analysis (Signorell et al.) however only included children with a diagnosis of severe malaria at the referral facility.

- Please clarify differences and similarities between this study and Awor et al. (pre-read 8) above.

Awor et al. (pre-read 8) presents treatment-seeking data from cross-sectional household surveys where the majority of recently sick children surveyed had a mild febrile illness. It includes children no matter which provider they attended.

Brunner et al. (pre-read 9) includes all children included in the patient surveillance system, children with a severe febrile illness attending a community-based provider of care.

Differences:

- Awor et al. analysed data collected in household surveys. In this study, we used data originating from the patient surveillance system.
 - Awor et al. analysed treatment-seeking for fever in children at community level, i.e. captured all types of first sources of care. In this study, we only included children who were brought to a CHW first. As treatment-seeking from CHW is not very common, the continuation from there is poorly understood.
 - Awor et al. only looked at the first source of care. In this study, we examined the whole treatment-seeking pathway, which is rare in the scientific literature.
 - In addition, we also report referral adherence and reasons for going to a chosen provider.
- Please provide supplementary materials. (Supplementary tables were mentioned in the pre-print but not provided to the review team.)

Updated version including supplementary materials attached.

CARAMAL: treatment-seeking at community level in Nigeria

Lee et al. (pre-read 10)

- Clarify enrolment per health care provider, with a breakdown of numbers enrolled by CORPs and the number enrolled by PHCs. It is stated that children were enrolled from 139 community-based providers in the study area, with the number of enrolled patients per provider ranging from one to 42 (median = 2, IQR: 2–4). Yet, 314 (53%) children were enrolled by CORPs and 275 (47%) by PHCs, which gives an average of 0.68 (314/500) enrolled per CORP and 3.58 (77/275) per PHC. Note: It would be good to have this information for the Democratic Republic of the Congo and Uganda as well. We have seen in iCCM programmes/implementation research studies that large numbers of CHWs do not contribute to enrolment/case registration, which could have serious implications for pre-referral RAS.

In Nigeria, in total, we looked at 139 community health providers (CORP n = 108, PHC n = 31). In terms of children enrolled per provider, CORPs enrolled a median of 1 child (range = 1–38, IQR = 1–2) while PHCs enrolled a median of 5 children (range = 1–42, IQR = 1–12).

We conducted this analysis for Nigeria specifically because of the striking difference in CFRs between CORP and PHC enrolments. Regarding attendance at CORPs, evidence from our enrolment suggests large differences in how “active” individual CORPs are: some see many patients, some others hardly any. This in turn had implications particularly for RAS supply, as the more active CORPs quickly ran out of supplies and needed to be re-stocked more regularly than others – a challenge for supply chain management.

- For the children who received artemether-lumefantrine at home, are there data to determine whether their caregivers already had the artemether-lumefantrine at home or first went to a chemist? Also, any data on if they dosed the medicine appropriately and took it with a fatty food?

We don't have data to answer this question with certainty but from the way the questions were phrased (“You have indicated that you had some form of treatments at home. I will now ask questions about the treatment you had at home” and then “Did {child_name} take any medicines that you had at home?”), we would assume they had the drugs at home. Also, only 8% of CORP

enrolments and 14% of PHC enrolments explicitly mentioned seeing another provider before the enrolling provider.

We do not know if medicines were dosed appropriately or if medicine was given with a fatty food. These data were not collected as we expected poor reliability of this information.

- Are there plans to do any qualitative research to understand why there appeared to be differential care-seeking between CORPs and PHCs based on type of initial danger signs?

This is beyond the capacity of the (already concluded) project, but it would certainly be an interesting undertaking.

- Please provide any supplementary materials available.

Please note that this manuscript is currently being revised.

* * *

Attached documents:

- #4 Signorell et al. (updated version, currently under review at PLoS Medicine)
- #5 Awor et al. (updated file as submitted to Lancet Infectious Diseases, including supplementary materials)
- #6 Lambiris et al. (revised version, currently under review at Lancet Global Health)
- #7 Awor et al. (revised version, just accepted by Malaria Journal, pre-typeset)
- #9 Brunner et al. (updated file, including supplementary materials)
- #11 Okitawutshu et al. (final version, now published in Malaria Journal)
- CARAMAL, Analysis plan with timelines, 02.03.2021.pdf

Annex 4. Statistical analysis plan

Further analyses of the CARAMAL Study

Version: 1.0

Authors: Professor Julie A Simpson

Melbourne School of Population and Global Health,

University of Melbourne, Australia

Dr Elizabeth George

MRC Clinical Trials Unit

University College London, United Kingdom of Great Britain and Northern Ireland

Date: 31 October 2022

1. Primary objective

The primary objective of these analyses is to measure the effectiveness of rectal artesunate (RAS) on the primary outcome mortality and the secondary outcome, referral.

This is an extension of the analyses carried out by Swiss TPH, completing some aspects of the CARAMAL project SAP dated 02.03.2021.

2. Estimands

The estimands for the primary objective are:

- a) Post-RAS period compared to pre-RAS period.
- b) RAS users versus non RAS users in the post RAS period only (defined for each country based on the actual RAS rollout).

The patient population will be those enrolled in the patient surveillance system (PSS) by CHW or at a PHC that met the eligibility criteria.

3. Outcomes

Primary outcome – day 28 mortality (1-dead, 0-alive)

Secondary outcome – referral completion to referral health centre with study staff present (1=yes, 0=no)

4. Statistical analyses

4.1 Initial descriptive analyses will be performed to reproduce Table 1 of Hetzel et al (BMC Medicine) and Table 2 of Brunner et al (BMJ Global Health).

4.2 For the primary outcome, day 28 mortality, logistic regression modelling will be performed and the estimated Odds Ratio (95% CI) presented for the above estimands. The regression analyses will be performed separately for the data from Democratic Republic of the Congo, Nigeria and Uganda. The following unadjusted and adjusted

analyses will be performed to further understand the contribution of clustering due to provider level and confounding.

- a) Logistic regression with intervention arm (post-RAS versus pre-RAS period; and in a separate model, RAS users versus RAS non-users in post-RAS period only)
- b) Logistic regression with intervention arm and random effect for clustering due to provider level
- c) Logistic regression with intervention arm and random effect for clustering due to provider level, and adjustment for the confounders at enrolment: age, sex, danger signs (convulsions, unusually sleepy/unconscious, not able to drink/feed – these will be assessed for collinearity and maybe only one or two danger signs will be included as confounders), month, rainy season (Democratic Republic of the Congo: October–April, Nigeria: May–October, Uganda: April–October) and location (community health worker and primary health centre; for Nigeria only).
- d) Sensitivity analysis – analyses a), b) and c) will be repeated excluding the data from the post-RAS periods when coverage was low, that is, for Democratic Republic of the Congo the first 2 months, for Uganda the first 6 months and for Nigeria the first 7 months. These analyses will be exploratory.

4.3 For the secondary outcome, referral completion, logistic regression modelling will be performed and the estimated Odds Ratio (95% CI) presented for the above estimands. The regression analyses will be performed separately for the data from Democratic Republic of the Congo, Nigeria and Uganda. The following unadjusted and adjusted analyses will be performed to further understand the contribution of clustering due to provider level and confounding.

- a) Logistic regression with intervention arm (post-RAS versus pre-RAS period; and in a separate model, RAS users versus RAS non-users in post-RAS period only)
- b) Logistic regression with intervention arm and random effect for clustering due to provider level
- c) Logistic regression with intervention arm and random effect for clustering due to provider level, and adjustment for the confounders at enrolment: age, sex, danger signs (convulsions and/or unusually sleepy/unconscious), month, rainy season (Democratic Republic of the Congo: October–April, Nigeria: May–October, Uganda: April–October), and location (community health worker and primary health centre; for Nigeria only).
- d) Logistic regression with intervention arm and random effect for clustering due to provider level, and adjustment for the confounders at enrolment: age, sex, danger signs (convulsions and/or unusually sleepy/unconscious), month, rainy season (Democratic Republic of the Congo: October–April, Nigeria: May–October, Uganda: April–October), location (community health worker and primary health centre; for Nigeria only), enrolled on a workday.

All statistical analyses will be performed in Stata Version 16.

Annex 5. Meeting agenda

Chairperson: Olugbenga Mokuolu

TUESDAY, 18 OCTOBER 2022		
	Day 1	Open
09:00 – 09:10	Welcome by a.i. Director Global Malaria Programme	Andrea Bosman
09:10 – 09:20	Introduction of participants	Chairperson
09:20 – 09:30	Objectives of the meeting	Peter Olumese
09:30 – 10:00	Methodological review of CARAMAL multi-country study and effectiveness of RAS as pre-referral treatment (1, 2)	Manuel Hetzel
10:00 – 10:30	Discussion	
11:00 – 11:30	Pre-referral RAS and referral completion in CARAMAL project (3)	Manuel Hetzel
11:30 – 12:00	Discussion	
12:00 – 12:20	Health workers' compliance and acceptability of RAS (4, 7)	Aita Signorell
12:20 – 12:50	Discussion	
13:50 – 14:10	Treatment-seeking of children at community level in Nigeria and Uganda in CARAMAL project (8, 9, 10)	Phyllis Awor
14:10 – 14:40	Discussion	
14:40 – 15:00	Clonal expansion of artemisinin-resistant falciparum malaria in Uganda in CARAMAL project (5)	Manuel Hetzel
15:00 – 15:30	Discussion	
15:30 – 15:50	RAS costs and barriers to implementation (6)	Mark Lambiris
15:50 – 16:15	Discussion	
16:30 – 17:15	Observations on RAS implementation studies in Malawi, Sierra Leone and Zambia	Tendayi Kureya Anitta Kamara John Phuka
17:15 – 17:45	Discussion	
17:45 – 17:50	Closure of the open session of the meeting	Peter Olumese

WEDNESDAY, 19 OCTOBER 2022		
	Day 2	Closed
09:00 – 09:20	Recap of Day 1	Rapporteur
09:20 – 09:40	Discussion and conclusions on methodological aspects reviews of RAS studies	Julie Simpson Lizzie George
09:40 – 10:00	Discussion and conclusions on effectiveness of RAS as pre-referral treatment in CARAMAL project	Peter Smith Bernhards Ogutu

10:00 – 10:30	Discussion and conclusions on pre-referral RAS and referral completion in CARAMAL project	Evelyn Ansah Humphreys Nsona
11:00 – 11:20	Discussion and conclusions on health workers' compliance and acceptability of RAS	Olugbenga Mokuolu Elizabeth Chizema
11:20 – 11:40	Discussion and conclusions on treatment-seeking of children at community level in Nigeria and Uganda in CARAMAL project	David Hamer Salim Sadruddin
11:40 – 12:00	Discussion and conclusions on financing and economic evaluation of RAS	Evelyn Ansah Freddy Kitutu
12:00 – 12:30	Discussion and conclusions on clonal expansion of artemisinin-resistant falciparum malaria in Uganda in CARAMAL project	Freddy Kitutu Karen Barnes
12:30 – 13:00	Discussion and conclusions on RAS implementation studies in Malawi, Sierra Leone and Zambia	Catherine Falade Umberto D'Alessandro
14:00 – 15:00	Discussion on recommendations to WHO in relation to RAS guidelines, information notes and implementation guidance	ALL
15:00 – 15:15	Next steps	Peter Olumese
15:15 – 15:30	Closure of the meeting	Andrea Bosman

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