Master's thesis

NTNU
Norwegian University of Science and Technology
Faculty of Medicine and Health Sciences
Department of Public Health and Nursing

Sigrid Marie Dyrkorn

Safety and immunogenicity performance of RTS,S/AS01 and RTS,S/AS02 malaria vaccines in sub-Saharan African children:

a systematic review and meta-analysis of randomized controlled trials

Master's thesis in Global Health
Supervisor: Francis Ndungu
Co-supervisor: Eva Skovlund
May 2022

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Kunnskap for en bedre verden

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Abstract

Introduction: Despite being preventable and treatable, malaria remains a major global health concern, with 627 000 deaths caused by malaria in 2020. The global burden of *P. falciparum* malaria is borne disproportionally by children in sub-Saharan Africa. The newly implemented RTS,S malaria vaccine is hoped to reduce the levels of mortality and morbidity in this population. This systematic review and meta-analysis aimed to investigate the safety and immunogenicity performance of the RTS,S/AS01 and RTS,S/AS02 vaccines in sub-Saharan African children of different ages.

Methods: PubMed, Ovid Embase, Clinicaltrials.gov, Scopus, and CENTRAL databases were searched for placebo-controlled trials of RTS,S (phase I-III) in sub-Saharan African children. The studies included in the review were quality assessed by Cochrane standards. Heterogeneity between studies was evaluated using Cochran´s Q and I^2 statistics. Fixed effect models were used to estimate weighted mean differences in anti-CS GMTs and risk ratios of the occurrence of SAEs in RTS,S-vaccine groups compared to placebo groups. The studies were sub-grouped based on the age of the participants.

Results: 10 articles reporting 11 studies (n=19838) were included in the meta-analysis. The overall relative risk reduction was 15% (95% CI 9% to 21%, I^2=60,4%) or 24% (95% CI 13% to 35%, I^2=0,0%) of having an SAE after vaccination when vaccinated with RTS,S/AS01 or RTS,S/AS02, respectively, compared to immunization with a placebo vaccine. On average, participants vaccinated with RTS,S/AS01 and RTS,S/AS02 increased the mean anti-CS antibody GMT by 315,3 EU/mL (95% CI 330,97 to 329,66, I^2=99,0%, $p_{heterogeneity}$<0,001) and 127,92 EU/mL (95% CI 116,34 to 139,49, I^2=96,7%, $p_{heterogeneity}$<0,001), respectively, compared to participants vaccinated with a placebo vaccine. For both RTS,S/AS01 and RTS,S/AS02, the mean numerical difference in GMT was higher in the participants aged five months and older than in participants between 0-4 months of age.

Conclusion: The findings from this review suggest that RTS,S/AS01 and RTS,S/AS02 vaccines are safe and well-tolerated in both infants and younger sub-Saharan African children. It is difficult to draw clinically meaningful conclusion based on the pooled mean differences of anti-CS GMTs in the RTS,S-vaccine groups compared to the placebo groups because of the considerable heterogeneity between the estimated effect sizes in the studies. Further evaluation of the vaccines' immunogenicity and efficacy data is needed to conclude whether the performance of the RTS,S-vaccines is affected by the age of the vaccinees.

Sammendrag

Innledning: Til tross for at malaria både kan forebygges og behandles, førte sykdommen, forårsaket av *P. Falciparum* parasitten, til 627 000 dødsfall i 2020. Dødeligheten er høyest blant barn i Afrika sør for Sahara, men den nylig implementerte malariavaksinen RTS,S gir håp om å forhindre alvorlig sykdom og død i denne befolkningsgruppen. Målet med denne systematiske litteraturgjennomgangen og metaanalysen var å undersøke sikkerhet- og immunogenisitetsdata av RTS,S/AS01- og RTS,S/AS02-vaksinene hos spedbarn og barn i Afrika sør for Sahara.

Materiale og metoder: Databasene PubMed, OVID Embase, ClinicalTrials.gov og CENTRAL ble søkt i for å finne randomiserte placebokontrollerte studier av RTS,S (fase I-III) i afrikanske barn og spedbarn. Studiene ble kvalitetsvurdert etter Cochrane-standarder. Heterogenitet mellom studiene ble undersøkt ved bruk av I^2-statistikk og Cochrans Q. Fasteffektmodeller ble brukt for å estimere vektede gjennomsnittlige forskjeller i anti-CS antistoffkonsentrasjon og relativ risk for alvorlige bivirkninger i RTS,S-vaksinegrupper sammenlignet med placebogruppene. I metaanalysen ble studiene delt opp i undergrupper etter alderen til studiedeltakerne.

Resultater: 10 artikler som rapporterte 11 studier (n=19838) ble inkludert i metaanalysen. Den totale relative risikoen for å ha en alvorlig bivirkning etter vaksinasjon ble statistisk signifikant redusert med 15% (95% KI 9% til 21%, I^2=60,4%) eller 24% (95% KI 13% til 35%, I^2=0,0%) ved vaksinering med henholdsvis RTS,S/AS01 eller RTS,S/AS02, sammenlignet med vaksinering med en aktiv placebovaksine. I gjennomsnitt økte deltakere vaksinert med RTS,S/AS01 og RTS,S/AS02 anti-CS-antistoffkonsentrasjon med 315,3 EU/ml (95% KI 330,97 til 329,66, I^2=99,0%, $p_{heterogenitet}$<0,001) og 127,92 EU/ml (95% KI 116,34 til 139,49, I^2=96,7%, $p_{heterogenitet}$<0,001), sammenlignet med deltakere vaksinert med en placebovaksine.

Konklusjon: Funnene fra denne systematiske litteraturgjennomgangen og metaanalysen antyder at vaksinene RTS,S/AS01 og RTS,S/AS02 er trygge for både spedbarn og unge afrikanske barn. De estimerte samlede gjennomsnittsforskjellene i anti-CS antistoffkonsentrasjon mellom vaksinegruppene og placebogruppene kunne ikke bidra til å gi klinisk meningsfulle konklusjoner på grunn av høy heterogenitet mellom resultatene til studiene. Ytterligere studier av immunogenisitet og effekt av vaksinene er nødvendig for å avgjøre om ytelsen av RTS,S-vaksinen påvirkes av alderen til dem som blir vaksinert.

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Contents

List of figures xii
List of tables xii
Abbreviations xiii
1 Introduction 14
1.1 Global burden of malaria 14
1.1.1 *Plasmodium falciparum* 14
1.1.2 Treatment and prevention 15
1.2 Malaria vaccines and vaccine trials 15
1.2.1 Malaria vaccines 15
1.2.1.1 RTS,S/AS01 (Mosquirix) 16
1.2.1.2 RTS,S/AS02 17
1.2.2 Vaccine trials 17
1.3 Rationale 18
2 Materials and Methods 19
2.1 Overview 19
2.2 Designing the Search Strategy 19
2.3 Databases, search strategies, and data searches. 20
2.3.1 Manual data search 23
2.3.2 Secondary data search 23
2.4 Eligibility criteria for studies 23
2.5 Abstract Screening and full-text screening 23
2.6 Outcome measures 24
2.7 Data extraction 24
2.8 Quality assessment of included studies 25
2.9 Data analysis 25
2.10 Ethical considerations 26
3 Results 27
3.1 Study selection and characteristics 27
3.1.1 Overview of data selection 27
3.2 Characteristics of the included studies 29
3.3 Quality assessment 30
3.3.1 Bias arising from the randomization process 30
3.3.2 Bias due to deviations from the intended interventions 30
3.3.3 Bias due to missing outcome data 31
3.3.4 Bias in measurement of the outcome 31

3.3.5 Bias in selection of the reported result 31
3.3.6 Overall risk of bias 31
3.4 Meta-analyses 33
3.4.1 Meta-analyses of SAEs 33
3.4.2 Meta-analyses of anti-CS GMTs 35
3.4.3 Analysis of publication bias 36
3.4.4 Sensitivity analyses 39
4 Discussion 40
4.1 Discussion of methods 40
4.1.1 Search strategy and databases 40
4.1.2 Data analysis 41
4.2 Discussion of results 42
4.2.1 SAEs 42
4.2.2 Anti-CS GMTs 43
4.3 Strengths and limitations 45
5 Conclusion 46
6 References 47
7 Appendices 51

List of figures

Figure 3.1: PRISMA flow-chart of studies identified, excluded, and included at different stages during the review work. 12 studies were quality assessed, and 1 study was excluded from the meta-analysis for having a high risk of bias. 11 studies, reported in 10 articles were included in the meta-analysis. 28
Figure 3.2: Risk of bias summary of the quality assessed studies. The risk of bias in each domain and the overall risk of bias are classified as low risk of bias (green), showing some concerns (yellow), or having a high risk of bias (red). 32
Figure 3.3: Forest plot depicting the overall estimated risk ratios of SAEs in the RTS,S/AS01 group compared to the placebo group. 34
Figure 3.4: Forest plot depicting the overall estimated risk ratios of SAEs in the RTS,S/AS02 group compared to the placebo group. 34
Figure 3.5: Forest plot depicting the weighted mean differences in GMTs between RTS,S/AS01, and placebo groups. 35
Figure 3.6: Forest plot depicting the weighted mean differences in GMTs between RTS,S/AS02, and placebo groups. 36
Figure 3.7: Funnel plot of SAEs in RTS,S/AS01 studies. 37
Figure 3.8: Funnel plot of SAEs in RTS,S/AS02 studies. 37
Figure 3.9: Funnel plot of GMTs in RTS,S/AS01 studies. 38
Figure 3.10: Funnel plot of GMTs in RTS,S/AS02 studies. 38

List of tables

Table 2.1: Databases with their respective search strategies. 21
Table 3.1: Vaccine efficacy results from the included studies 30

Abbreviations

RCT	Randomized controlled trial
ACT	Artemisinin-based combination therapy
TBV	Transmission-blocking vaccine
MVI	Malaria Vaccine Initiative
EPI	Expanded Programme of Immunization
CHMI	Controlled human malaria challenge
GSK	GlaxoSmithKline Biologicals
RTS,S	RTS,S/AS01 and RTS,S/AS02 vaccines
AS	Adjuvant system
RTS,S/AS01	The RTS,S vaccine with adjuvant system 1
RTS,S/AS02	The RTS,S vaccine with adjuvant system 2
SAE	Serious adverse event
CSP	Circumsporozoite protein
Anti-CS	Antibodies against circumsporozoite
GMT/GMC	Geometric mean titer/geometric mean concentration
ELISA	Enzyme-linked immunosorbent assay
EU/mL	ELISA-unit per milliliter
VE	Vaccine efficacy
HR	Hazard Ratio
RR	Risk ratio

1 Introduction

1.1 Global burden of malaria

In 2020, there were an estimated 241 million malaria cases in 85 countries where the disease is endemic. Six sub-Saharan African countries (Nigeria, the Democratic Republic of Congo, Uganda, Mozambique, Angola, and Burkina Faso) accounted for 55% of malaria cases (1). More than 90% of the sub-Saharan African population lives in malaria-endemic areas, where 99,7% of estimated malaria cases are due to *Plasmodium falciparum*, one of six *Plasmodium* parasite species that infect humans (2).

The global burden of malaria is borne disproportionally by children in Sub-Saharan Africa. It is estimated that 627 000 people died of malaria in 2020, and out of these, 77% occurred in children below the age of 5 (1). Children with severe malaria frequently develop severe anemia, cerebral malaria, or respiratory distress (3).

1.1.1 *Plasmodium falciparum*

Plasmodium falciparum is a protozoan parasite that spread to humans through bites from infectious female *Anopheles* mosquitos (4). Mosquitos inject the motile form of the parasite, sporozoite, into the human skin, which then migrates to the liver, where it grows and multiplies over 5-10 days before it enters the bloodstream and starts infecting red blood cells (5). Once inside the red blood cells, merozoites grow into schizonts until they burst the cell, releasing more daughter merozoites into the bloodstream, which infects more red blood cells to perpetuate the erythrocytic cycle. It is during this erythrocytic cycle that the parasites cause the symptoms of malaria disease. Some of the infected red blood cells deviate from this cycle and develop into male and female gametocytes, which are taken up by the female *Anopheles* mosquito in a blood meal from an infected person. The male and female gametocytes fuse to generate zygotes, which are then further developed into oocysts. After growth and multiplications in the sporogonic cycle, the oocyst ruptures to release infectious sporozoites. The sporozoites can then be inoculated into a new human host to restart the cycle (6).

Infection with *P. falciparum* results in symptoms such as severe headaches, fever-like symptoms, chills, joint pain, and red blood cell lysis and dysregulation. The latter can in turn

lead to anemia (2). The severity of the clinical disease spans from asymptomatic infection in those who have developed partial immunity to a severe life-threatening disease on the other side of the spectrum of non-immune individuals (4).

1.1.2 Treatment and prevention

Malaria can be treated with anti-malarial drugs such as chloroquine phosphate and artemisinin-based combination therapies (ACTs). However, the usefulness of drugs is limited by both their lack of availability among the people who need them the most and frequently by the evolution of drug-resistant parasites (7). Not to mention that it is always better to prevent illness than to treat it.

Current preventive interventions for malaria focus on environmental measures and chemoprevention. Specifically, this includes insecticide-treated bed nets and indoor insecticide spray for mosquito control, and seasonal malaria chemoprevention (5, 7). Vector control aims to reduce the initial transmission of parasites from mosquitos to humans, and antimalarial drugs are used to suppress blood-stage infection in humans or prompt treatment of the disease (8).

Preventive interventions have contributed to a decline in malaria mortality rates globally, but nearly 40% of the world´s population is still at risk of getting infected (9). *Plasmodium* parasites and mosquito vectors constantly evolve resistance to insecticides and drugs. A safe, effective and affordable malaria vaccine is needed to complement the preventive interventions and close the gap left by them (10).

1.2 Malaria vaccines and vaccine trials

1.2.1 Malaria vaccines

Vaccines are one of the most efficient and effective health interventions to reduce morbidity and mortality of infectious diseases (7). A highly effective vaccine against malaria has been a leading priority since the 1970s. Still, developing a highly effective vaccine has proven challenging due to the complex biology and life cycle of the *Plasmodium* parasites (2, 11). The current malaria vaccine candidates aim to either limit parasite growth and duration of infection or prevent infection (2). The current malaria vaccine candidates use different technologies and target different stages of the parasite´s life cycle to achieve these aims.

The P. falciparum sporozoite (PfSPZ) vaccine is a whole-cell live attenuated sporozoite vaccine that aims to generate a protective immune response against multiple malaria antigens (7). Despite being the leading whole-parasite vaccine, it has shown mixed efficacy results in phase II trials (12).

The limited development of attenuated whole-cell vaccines due to a difficulty with in-vitro production has made molecular vaccines an appealing alternative approach for a malaria vaccine. AMA1 is a promising molecular vaccine candidate against blood-stage of malaria infection but has shown to be only moderately immunogenic with no overall impact of vaccination on disease in a phase I-II trial in children in Mali (7). Transmission blocking vaccines (TBVs) target the transmission stage (mosquito stage) to prevent infection (13). TBV candidates Pfs25 (post-fertilization antigen) and Pfs230 (pre-fertilization antigen) are examples of TBVs (13, 14). TBVs are now in earlier stages of development, with candidates in phase I trials (12).

Despite the challenges in malaria vaccine development, the RTS,S vaccine candidate has shown promising results in numerous trials. In October 2021, it became the first vaccine to be implemented for the prevention of malaria in children living in regions with moderate to high *P. falciparum* transmission (5). The RTS,S vaccine can be co-administered with other vaccines included in the Expanded Programme of Immunization (EPI) (15). RTS,S is a pre-erythrocytic vaccine targeting the sporozoite stage and the infected hepatocyte (2, 7). It is a fusion of a part of the *P. falciparum* circumsporozoite protein (CSP), found at the surface of the sporozoite and of the infected hepatocyte, with hepatitis B surface antigen (HBsAg). This recombinant protein, in combination with an adjuvant system (AS01 or AS02), generates a cell-mediated response to infected liver cells in which the production of antibodies and T cells that are believed to diminish the capacity of the malaria parasite to infect, survive, and develop in the human liver is induced (7, 16). AS01 and AS02 were initially developed as adult formulations AS02A and AS01B, but pediatric 0,25 ml doses of RTS,S/AS02D (RTS,S/AS02A) and RTS,S/AS01E (RTS,S/AS01B) have been made for compatibility with the standard auto-disable EPI syringes (17).

1.2.1.1 RTS,S/AS01 (Mosquirix)

The RTS,S/AS01 vaccine against *P. falciparum* was developed through a partnership between GlaxoSmithKline Biologicals (GSK) and MVI (10). Adjuvant system AS01 is a liposome formulation containing 25µg MPL (3-*O*-desacyl-4′-monophosphoryl lipid A) and 25µg QS-21 (*Quillaja saponaria* Molina, fraction 21) per 0,5mL (18).

1.2.1.2 RTS,S/AS02

The RTS,S/AS02 vaccine against *P. Falciparum* was developed through a collaboration between GSK Biologicals and the Walter Reed Army Institute of Research (MD, USA) (19). The AS02 adjuvant system contains an oil-in-water emulsion with 25µg MPL (3-*O*-desacyl-4′-monophosphoryl lipid A) and 25µg QS-21 (*Quillaja saponaria* Molina, fraction 21) per 0,5mL (17).

1.2.2 Vaccine trials

Before a vaccine can be employed widely for disease control in the target populations, it needs to be thoroughly evaluated and tested through clinical development programs to assess its immunogenicity, efficacy, and safety. Immunogenicity and safety data are provided through the early phases of clinical trials that identify the primary immunization schedule and optimal dose. The protective efficacy of a vaccine is defined as "the reduction in the chance of developing the disease after vaccination relative to the chance when unvaccinated." (20, p. 11). The protective efficacy is determined in a randomized controlled study, comparing the vaccine-treated group versus a control group that did not receive the same vaccine (20).

Phase I-III vaccine trials primarily provide data on the safety, immunogenicity, and efficacy of the vaccine. Still, as these trials are conducted under controlled conditions, they do not provide all information needed to decide on a widespread everyday use of the vaccines. Further safety assessment and real-life data, such as vaccine introduction challenges and public health impact on mortality and morbidity, are gained through phase IV studies and pilot implementations (21).

For malaria vaccines, controlled human malaria challenge (CHMI) in malaria-naïve adults has been used to assess the protective efficacy of a malaria vaccine candidate in the earlier phases of vaccine development. According to Spring, Polhemus, and Ockenhouse (22), CHMI is done accordingly:

> After completing a vaccine regimen, volunteers are bitten by 5 malaria-infected female *Anopheles* mosquitoes in a controlled environment. Volunteers are then monitored daily for peripheral parasitemia in a hotel setting with 24-hour access to a nurse and physician. If a single verified parasite is detected, effective antimalarials are promptly administered. (22, p. 40).

The vaccines that have shown promising results by this safe evaluation are then evaluated through randomized placebo-controlled trials (RCTs) in different phases and demographical

settings (e.g., diverse populations and study sites). The placebo used in RCTs of malaria vaccines is active vaccines that do not target *P. falciparum*, such as rabies vaccines or EPI vaccines (e.g., yellow fever vaccine and measles vaccine).

Immunogenicity results for RTS,S malaria vaccines come from analyzing blood samples from the participants. Antibodies specific for the *Plasmodium falciparum* circumsporozoite protein tandem repeat epitope are assessed by enzyme-linked immunosorbent assay (ELISA) with plates adsorbed with the recombinant antigen R32LR antigen that contains the sequence $[NVDP(NANP)_{15}]_2LR$ with a standard serum as a reference. An anti-circumsporozoite titer is seropositive if higher than a cut-off value of 0,5 Elisa Unit per milliliter (EU/mL) (23).

1.3 Rationale

A few systematic reviews have investigated different aspects of malaria vaccine candidates. The previous reviews have assessed the safety of new adjuvanted vaccines in children, clinical development, challenges and solutions of implementation of RTS,S/AS01, and reported consistency of efficacy and immunogenicity data from phase I-III trials in malaria-naïve adults and malaria-exposed adults, children, and infants from malaria-endemic settings in sub-Saharan Africa (24-26). Reviews of the literature of suggested mechanisms as reasons for moderate vaccine efficacy and variation in VE between study sites and various reviews synthesizing data of different malaria vaccines and intermittent preventive treatment for malaria in infants have also been conducted (27-31).

Even though the development of an effective malaria vaccine has been a global health priority for decades, there is still no existing vaccine that sufficiently protects children in malaria-endemic areas (32). Some studies suggest an inferior efficacy amongst children aged 6-14 weeks compared to children aged 5-17 months receiving the RTS,S vaccine (10, 18, 33). However, the evidence for this has not yet been synthesized.

This systematic review and meta-analysis aim to assess and compare the performance of RTS,S vaccines at different ages of sub-Saharan African children. This thesis will aim to answer the following research questions:

- What is the evidence for i) safety, ii) immunogenicity, and iii) efficacy of RTS,S?
- Is there evidence that RTS,S performance is affected by the age of the vaccinees?

2 Materials and Methods

2.1 Overview

The Cochrane Handbook for Systematic Reviews of Interventions(34) was followed to the best of my ability during the review work. The search strategies and eligibility criteria were predefined before the initiation of the data collection. Publication of the study protocol was considered, but this thesis does not fit the inclusion criteria for publication through PROSPERO.

2.2 Designing the Search Strategy

The search strategy was designed to access both published and unpublished materials. The search strategy comprised three stages:

1. A limited search of PubMed to identify relevant mesh terms and keywords found in the title, abstract and subject descriptors of pertinent articles for the thesis.
2. Terms identified and synonyms used for these terms in respective databases were then used in an extensive literature search in all the chosen databases.
3. Bibliographies and reference lists of the articles found in stage two were searched for more relevant articles.

The initial search terms were chosen based on the beginning of the PICO model:

Population: malaria naïve or Sub-Saharan African (including all the countries in this region)

Intervention: RTS,S/AS01E, Mosquirix, RTS S, RTSS (all terms used for the same vaccine)

Study design: Clinical Trials, RCTs (all phases)

The finished search strategy for PubMed was used as a template for the search strategies for the other databases, aiming to have as identical as possible search strategies in all databases. The search strategies are not similar because the databases have different mesh terms, spellings, limitations, and filters. Also, some of the databases only comprised trials and did not need trials included in the search strategy.

2.3 Databases, search strategies, and data searches.

Databases searched included PubMed, Ovid Embase, Clinicaltrials.gov, Scopus, and Cochrane Central Register of Controlled Trials (CENTRAL). Table 2.1 shows the included databases with their respective search strategies.

Table 2.1: Databases with their respective search strategies.

Database	Search strategy
PubMed	((RTS,S malaria vaccine) OR (RTSS malaria vaccine) OR (S vaccine, anti-malaria) OR (RTSS AS) OR (RTS,S AS02D) OR (Plasmodium falciparum malaria vaccine 257049) OR (MosQUIRIX) OR (RTS,S AS01E)) AND ((Malaria naive) OR ((Malaria endemic) OR (Sub-Saharan Africa) OR Angola OR Benin OR Botswana OR (Burkina Faso) OR Burundi OR (Cape Verde) OR Cameroon OR (Central African Republic) OR Chad OR Comoros OR (the Democratic Republic of the Congo) OR (Republic of the Congo) OR (Cote d'Ivoire) OR Djibouti OR (Equatorial Guinea) OR Eritrea OR Ethiopia OR Gabon OR Gambia OR Ghana OR Guinea OR (Guinea-Bissau) OR Kenya OR Lesotho OR Liberia OR Madagascar OR Malawi OR Mali OR Mauritania OR Mauritius OR Mozambique OR Namibia OR Niger OR Nigeria OR Rwanda OR (Sao Tome and Principe) OR Senegal OR Seychelles OR (Sierra Leone) OR Somalia OR (South Africa) OR (South Sudan) OR Sudan OR Swaziland OR Tanzania OR Togo OR Uganda OR Zambia OR Zimbabwe)) **|** Clinical Trial, Clinical Trial Protocol, Clinical Trial, Phase I, Clinical Trial, Phase II, Clinical Trial, Phase III, Clinical Trial, Phase IV, Controlled Clinical Trial, Randomized Controlled Trial
OVID Embase	(((mosquirix OR rts s OR "rts s as 01" OR rts s as01 OR rts,s OR rts,s as01).mp.) AND (((Africa south of the sahara OR sub-saharan Africa OR subsaharan africa OR Angola OR Benin OR Botswana OR Burundi OR Cabo Verde OR Cameroon OR Central African Republic OR Chad OR Comoros OR Democratic Republic of the Congo OR Djibouti OR Cote d Ivoire OR Equatorial Guinea OR Eritrea OR Ethiopia OR Gabon OR Gambia OR Ghana OR Guinea OR Guinea-Bissau OR Kenya OR Lesotho OR Liberia OR Madagascar OR Malawi OR Mali OR Mauritania OR Mauritius OR Mozambique OR Namibia OR Niger OR Nigeria OR Rwanda OR (Sao Tome and Principe) OR Senegal OR Seychelles OR Sierra Leone OR Somalia OR South Africa OR South Sudan OR Sudan OR Swaziland OR Togo OR Tanzania OR Uganda OR Zambia OR Zimbabwe OR malaria endemic).mp.) OR malaria naive.mp.)) AND ((Clinical trial) OR (Randomized controlled trial) OR (Randomization) OR (Single blind procedure) OR (Double blind procedure) OR (Crossover procedure) OR (Placebo) OR (Randomi?ed controlled trial$.tw.) OR (Rct.tw.) OR (Random allocation.tw.) OR (Randomly allocated.tw.) OR (Allocated randomly.tw.) OR ((allocated adj2 random).tw.) OR (Single blind$.tw.) OR (Double blind$.tw.))
CENTRAL	((RTS,S malaria vaccine) OR (RTSS malaria vaccine) OR (S vaccine, anti-malaria) OR (RTSS AS) OR (RTS,S AS02D) OR (Plasmodium falciparum malaria vaccine 257049) OR (MosQUIRIX) OR (RTS,S AS01E) OR (mosquirix) OR (rts s) OR (rts s as 01) OR (rts s as01) OR (rts,s) OR (rts,s as01) OR (rts,s/as01))AND (((Malaria endemic) OR (sub-saharan Africa) OR (Africa south of the Sahara) OR (subsaharan Africa) OR Angola OR Benin OR Botswana OR (Burkina Faso) OR Burundi OR (Cape Verde) OR Cameroon OR (Central African Republic) OR Chad OR Comoros OR (the Democratic Republic of the Congo) OR (Republic of the Congo) OR (Cote d'Ivoire) OR Djibouti OR (Equatorial Guinea) OR Eritrea OR Ethiopia OR Gabon OR Gambia OR Ghana OR Guinea OR (Guinea-Bissau) OR Kenya OR Lesotho OR Liberia OR Madagascar OR Malawi OR Mali OR Mauritania OR Mauritius OR Mozambique OR Namibia OR Niger OR Nigeria OR Rwanda OR (Sao Tome and Principe) OR Senegal OR Seychelles OR (Sierra Leone) OR Somalia OR (South Africa) OR (South Sudan) OR Sudan OR Swaziland OR Tanzania OR Togo OR Uganda OR Zambia OR Zimbabwe) OR (Malaria-naïve OR Malaria-naive OR Malaria naïve OR Malaria naïve)) **|** Trials

Database	Search strategy
ClinicalTrials.gov	((RTS,S malaria vaccine) OR (RTSS malaria vaccine) OR (S vaccine, anti-malaria) OR (RTSS AS) OR (RTS,S AS02D) OR (Plasmodium falciparum malaria vaccine 257049) OR (MosQUIRIX) OR (RTS,S AS01E) OR (mosquirix) OR (rts s) OR (rts s as 01) OR (rts s as01) OR (rts,s) OR (rts,s as01) OR (rts,s/as01)) | Recruiting, Not yet recruiting, Available, Active, not recruiting, Completed, Enrolling by invitation, Suspended, Terminated, Withdrawn, Temporarily not available, Approved for marketing, Unknown status, Interventional, Accepts Healthy Volunteers, Early Phase 1, Phase 1, Phase 2, Phase 3, Phase 4
SCOPUS	((RTS,S malaria vaccine) OR (RTSS malaria vaccine) OR (S vaccine, anti-malaria) OR (RTSS AS) OR (RTS,S AS02D) OR (Plasmodium falciparum malaria vaccine 257049) OR (MosQUIRIX) OR (RTS,S AS01E) OR (mosquirix) OR (rts s) OR (rts s as 01) OR (rts s as01) OR (rts,s) OR (rts,s as01) OR (rts,s/as01)) AND (((Malaria endemic) OR (sub-saharan Africa) OR (Africa south of the Sahara) OR (subsaharan Africa) OR Angola OR Benin OR Botswana OR (Burkina Faso) OR Burundi OR (Cape Verde) OR Cameroon OR (Central African Republic) OR Chad OR Comoros OR (the Democratic Republic of the Congo) OR (Republic of the Congo) OR (Cote d'Ivoire) OR Djibouti OR (Equatorial Guinea) OR Eritrea OR Ethiopia OR Gabon OR Gambia OR Ghana OR Guinea OR (Guinea-Bissau) OR Kenya OR Lesotho OR Liberia OR Madagascar OR Malawi OR Mali OR Mauritania OR Mauritius OR Mozambique OR Namibia OR Niger OR Nigeria OR Rwanda OR (Sao Tome and Principe) OR Senegal OR Seychelles OR (Sierra Leone) OR Somalia OR (South Africa) OR (South Sudan) OR Sudan OR Swaziland OR Tanzania OR Togo OR Uganda OR Zambia OR Zimbabwe) OR (Malaria-naïve OR Malaria-naive OR Malaria naïve OR Malaria naïve)) AND ((Clinical trial) OR (Clinical trial phase 1) OR (clinical trial phase 2) OR (clinical trial phase 3) OR (clinical trial phase 4) OR (clinical trial protocol) OR (Randomized controlled trial) OR (RCT))

Everything written behind "\|" are filters

2.3.1 Manual data search

In addition to collecting data from the databases, reference lists and bibliographies of the included articles collected from the databases were searched, and relevant articles not found in the primary data search were included based on their title.

2.3.2 Secondary data search

The data search was repeated to include all new relevant articles that might have been published post the primary data search. The same search strategies were used for the same databases, and newly published articles were judged for inclusion based on their title and abstract.

2.4 Eligibility criteria for studies

Records found in the data search were included if they were written in English and reported malaria vaccine, placebo-controlled trials of RTS,S (phase I-III) in malaria-exposed children.

Records of studies without an active placebo or control group and studies that compared two different versions of RTS,S with each other were excluded. Editorials, cross-sectional studies, cohort studies, trial registries, conference abstracts, reviews, meta-analyses, and other studies that are not vaccine trials were excluded. Studies were also excluded if they were not written in English or not available in full text.

2.5 Abstract Screening and full-text screening

All records found when running the search strategies in the different databases were exported to EndNote 20. Bramer's method(35) was used to remove duplicates in EndNote.

The abstract screening was logged in an excel document, and records were excluded or included for full-text screening based on their title and abstract. Reasons for exclusion were that the records did not report RTS,S vaccine trials, trials examining irrelevant outcomes (e.g. antibody classes or CD4+ cell responses), conference abstracts, trial registries with no published results, written in another language than English, or not available in full text.

The references included after abstract screening were then screened in a full-text screening. This was also logged in an excel document. References were excluded if they were not articles (trial registries), follow-up studies, or not comparable because they used different

participants (e.g., adult populations or participants with HIV) or compared RTS,S with seasonal chemoprevention or one RTS,S version with another.

2.6 Outcome measures

The malaria vaccine trials report safety data and data on how well the vaccine performs in the study population, immunogenicity, and efficacy results. In this review, the outcome measures of interest are serious adverse events (SAEs), antibody titers of anti-circumsporozoite (anti-CS) antibodies, and vaccine efficacy (VE) results.

Adverse events in vaccine trials are defined as "any undesirable experience associated with the use of a medical product in a patient."(36) SAEs are adverse events that, e.g., result in death, are life-threatening, require initial or prolonged hospitalization or result in disability or permanent damage to the patient.

Immunogenicity results include seropositivity rates and geometric mean titers (GMTs) or geometric mean concentrations (GMCs) for anti-CS antibodies at baseline vs. 1-month post the 3rd dose of the placebo vaccine and RTS,S vaccine was reported for most vaccine trials.

Vaccine efficacy for the prevention of clinical malaria cases is calculated as a 1-hazard ratio (1-HR). As not all studies report VE, and it is not possible to calculate vaccine efficacy without access to individual participant data, it was decided that VE results would not be a part of a meta-analysis in this review.

2.7 Data extraction

The following information was extracted from the included studies and entered an excel document:

- Study characteristics: Author name(s), year of publication, study location (country), study period, study design, study objectives (primary, secondary), and key findings
- Study population: age, sex (% male), the total number of participants, and number of participants in each study group
- Description of treatment (RTS,S + adjuvant system, dose-volume + concentration)
- Description of the comparator (active placebo vaccine(s), number of doses + dose volume)

- Vaccination schedule
- Outcome measures: number of SAEs, anti-CS antibody GMT results, VE results if reported

2.8 Quality assessment of included studies

The studies included in the review were quality assessed using Cochrane Collaborations´ Risk of Bias Tool version 2 (RoB2)(37) Studies evaluated as having a high risk of bias were excluded from the meta-analysis. The studies were quality assessed for bias in 5 domains:

- Domain 1: Bias arising from the randomization process.
- Domain 2: Bias due to deviations from the intended interventions.
- Domain 3: Bias due to missing outcome data.
- Domain 4: Bias in the measurement of the outcome.
- Domain 5: Bias in the selection of the reported result.

2.9 Data analysis

Fixed effects models were used to estimate weighted mean differences in GMTs and risk ratios (relative risk, RR) of the occurrence of SAEs in RTS,S-vaccine groups compared to placebo groups. The studies were sub-grouped based on the age of the participants. The estimated effect sizes (weighted mean difference in GMTs and RR of SAEs) were considered statistically significant if the 95% confidence interval (CI) did not include 1 (SAEs) or 0 (GMTs).

Heterogeneity between the studies in each subgroup and overall heterogeneity was analyzed using Cochran´s Q and Higgins and Thompson's I^2 statistic. The Q-test told if there were more variations between studies than expected from sampling error alone. Heterogeneity was considered statistically significant if the p-value was less than 0,05 (38). The I^2 index shows how much of the total variation cannot be explained by random error and was interpreted after this "rule of thumb" (39):

I^2= 25%: low heterogeneity

I^2 = 50%: moderate heterogeneity.

I^2 = 75%: substantial heterogeneity.

Small-study effects and publication bias were assessed and visualized by funnel plots and additional Egger´s regression test and Begg´s rank test in the case of asymmetric funnel plots. Publication bias and small-study effects were considered statistically significant with asymmetric funnel plots and p-values ≤ 0,05 in the Egger test and p-values>0,1 in Begg´s test (40).

Sensitivity analyses were done for all outcomes to check the robustness of the assumptions and choices made prior to the meta-analysis. All statistical analyses were conducted in STATA version 17.

2.10 Ethical considerations

As there is no original research in this review, and the data used in this study is secondary, based on publicly available articles, application for ethical approval is not required and was not sought.

3 Results

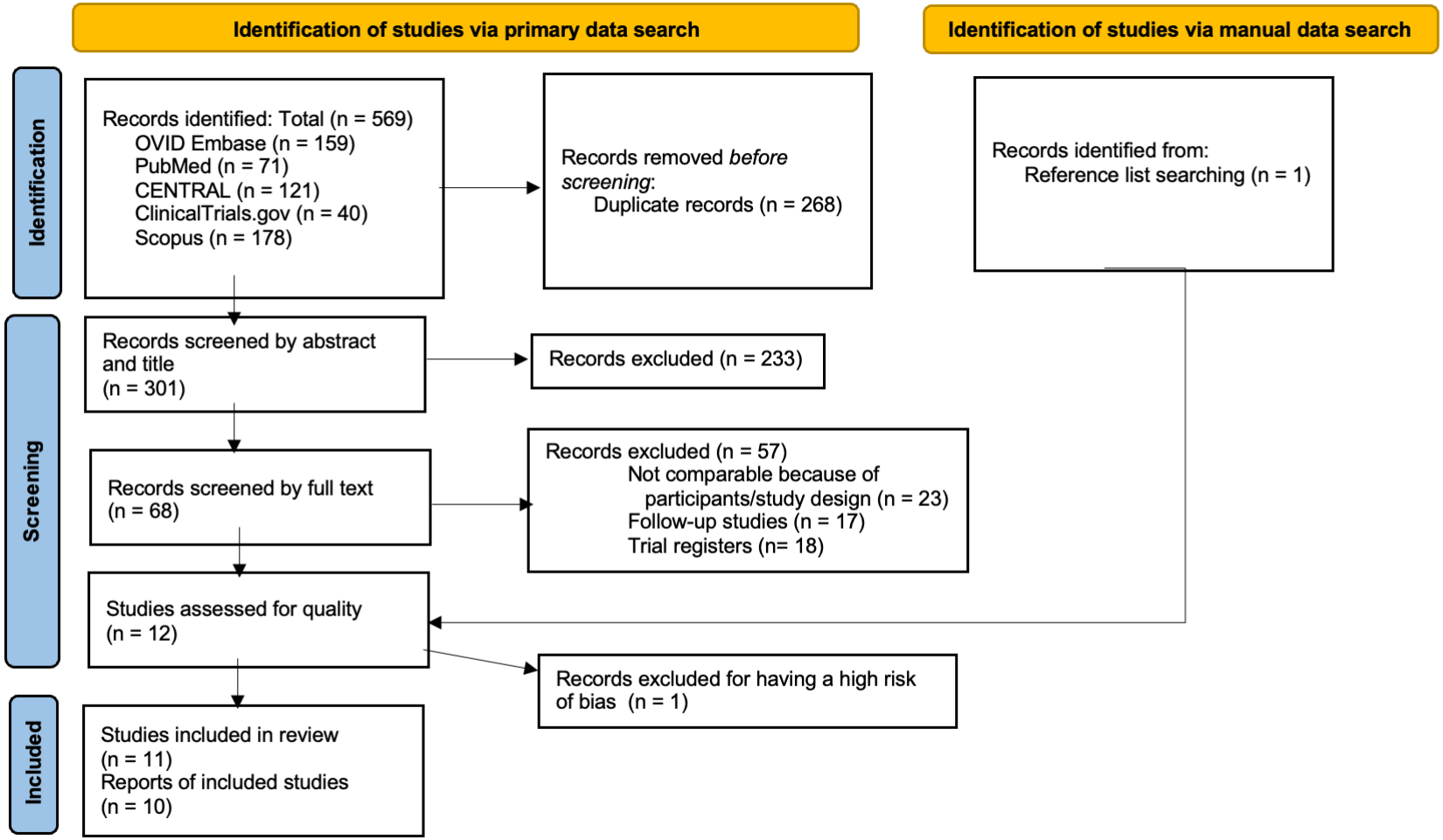
3.1 Study selection and characteristics

3.1.1 Overview of data selection

The primary data search was done on the 16th of January 2022, with 569 records identified. The secondary data search was done 19th of April 2022, and no new relevant records were found and included in the review. A manual data search of the reference lists of the included records identified during the primary data search resulted in the inclusion of 1 relevant record.

After removing duplicates, 301 records were screened based on their title and abstract. In total, 233 papers were excluded for not meeting the eligibility criteria, and the remaining 68 records were screened based on the full-text articles. Another 57 records did not meet eligibility criteria and were excluded from the review. Twelve records were included in the quality assessment, and 11 studies, reported in 10 articles, were included in the meta-analysis.

Figure 3.1 shows the number of records found, screened, and excluded at the different steps during the review work.



From: Page MJ, McKenzie JE, Bossuyt PM, Boutron I, Hoffmann TC, Mulrow CD, et al. The PRISMA 2020 statement: an updated guideline for reporting systematic reviews. BMJ 2021;372:n71. doi: 10.1136/bmj.n71.

Figure 3.1: PRISMA flow-chart of studies identified, excluded, and included at different stages during the review work. 12 studies were quality assessed, and 1 study was excluded from the meta-analysis for having a high risk of bias. 11 studies, reported in 10 articles were included in the meta-analysis.

3.2 Characteristics of the included studies

The essential characteristics of the included studies and their participants are presented in Appendix 1. All ten articles describing the 11 studies were published between 2004 and 2020 and are written in English. They included 19838 participants with age at screening (baseline) ranging from 0 days to 11 years of age and a percentage of males ranging from 32% to 83%.

All the studies were conducted in one or more of the following sub-Saharan African countries: Burkina Faso, Ghana, Gabon, Kenya, Tanzania, Malawi, Mozambique, and The Gambia.

Five studies(17, 18, 23, 41, 42) assessed the performance of RTS,S/AS01, while six studies(16, 33, 43-45) assessed the performance of RTS,S/AS02 in children in different age groups. All participants received three doses of RTS,S at a 0, 1, 2-month schedule (or close to a 0, 1, 2-month schedule) or various amounts of an active placebo/control vaccine. The most used placebo vaccines were Hepatitis B, Rabies, and EPI vaccines (e.g., yellow fever and measles vaccines). The placebo used in each study was chosen based on which vaccines were already to be given to children of the same age as the participants (e.g., in national immunization programs) and differed between age groups and study locations.

All the included studies reported safety and immunogenicity data. All studies reported SAEs in both the RTS,S and control groups and any adverse events experienced by the participants in both groups. The studies report all participants' mean antibody concentrations/titers as the immunogenicity results. Eight studies(17, 18, 23, 33, 41-44) reported immunogenicity as GMT/GMC with EU/mL as a unit, while three studies(16, 45) used μg/mL as the unit. All studies reported seronegative anti-CS antibodies at baseline in both the RTS,S and placebo groups.

Five studies(33, 41-44) also reported data on vaccine efficacy, as shown in table 3.1. RTS,S/AS01 has a reported efficacy of 55% (55% (95% CI: 31 to 70; p<0,001(41)), 55,8% (95% CI: 51,3 to 59,8; p<0,001(42)) against the first or only episode of clinical malaria in children aged 5-17 months. RTS,S/AS02A has a reported efficacy of 26,9% (95% CI:37,1 to 77,3; p=0,009(33)) against new malaria infections in children aged 1-4 years. RTS,S/AS02D has a reported efficacy of around 60% (60,6% (95% CI: 10,4 to 82,6; p=0,03(43)), 62,2% (95% CI: 37,1 to 77,3; p=0,0002(44))) against infection or parasitemia in children aged 0-20 weeks.

Table 3.1: Vaccine efficacy results from the included studies

Author (year) (Reference)	RTS,S vaccine	Placebo vaccine	Crude vaccine efficacy results	
			Value (95% CI)	p-value
Abdulla (2008) (43)	RTS,S/AS02D	Hepatitis B vaccine (engerix B)	60.6% (10.4, 82.6)	0.03
Alonso (2004) (33)	RTS,S/AS02A	Pneumococcal conjugate and *Haemophilus influenzae* type b vaccine or hepatitis B vaccine	26.9% (7.4, 42.2)	0.009
Aponte (2007) (44)	RTS,S/AS02D	Hepatitis B vaccine (engerix B)	62.2% (27.1, 77.3)	0.0002
Bejon (2008) (41)	RTS,S/AS01E	Rabies vaccine (Sanofi-Pasteur)	55% (31, 70)	<0.001
RTS,S Clinical Trials Partnership (2011) (42)	RTS,S/AS01	Rabies vaccine (VeroRab)	55.8% (51.3, 59.8)	<0.001

3.3 Quality assessment

12 records reporting 11 studies were assessed for bias in a quality assessment according to the Cochrane Handbook for Systematic Reviews of Interventions(34). The RoB2 tool(37) evaluated and classified the five domains as showing “some concerns” or being at a “low” or “high” risk of bias. Appendix 2 offers the full quality assessment for each study.

3.3.1 Bias arising from the randomization process

Eight(16, 17, 23, 33, 41, 42, 44) of the 12 quality assessed studies were classified as low risk of bias because of their well-described randomization and concealed allocation sequences. There were no baseline differences between the RTS,S and placebo groups. Three(18, 43, 45) studies showed some concerns because randomization and allocation sequence concealment was not indicated. One study(46) was ranked as high risk of bias because the allocation sequence was not concealed, and no information on randomization was provided.

3.3.2 Bias due to deviations from the intended interventions

Nine studies(16, 17, 33, 41-45) were classified as low risk of bias because both participants (and their parents), researchers, and people delivering the intervention were blinded to the participants' assigned intervention. Three studies(18, 23, 46) were open trials, where the researchers were aware of the vaccination schedule but not the given intervention of the

participants. The participants' parents were not blinded to the assignment. These three studies were classified as showing some concerns.

3.3.3 Bias due to missing outcome data

All studies(16-18, 33, 41-46) were classified as low risk of bias because outcome data were available for almost all participants. The missing outcome data were well-presented and similar in both placebo and RTS,S groups.

3.3.4 Bias in measurement of the outcome

All studies used appropriate methods of measuring the outcome in both RTS,S and placebo groups and were classified as low risk of bias(16-18, 33, 41-45) or showing some concerns(23, 46) based on whether all of the outcome assessors were aware of the participants assigned intervention.

3.3.5 Bias in selection of the reported result

Nine studies(17, 18, 23, 33, 41-44, 46) were classified as low risk of bias because the outcome measures and analyses followed a pre-specified analysis plan. Three studies(16, 45) were classified as showing some concerns because there was no information on whether the analyses were performed according to the protocol or not.

3.3.6 Overall risk of bias

Figure 3.2 shows the overall result of the quality assessment for all studies. Only one study(46) had an overall high risk of bias. It was excluded from the meta-analyses. 6 studies showed some concerns(16, 18, 23, 43, 45). The remaining five studies(17, 33, 41, 42, 44) were assessed to have an overall low risk of bias.

Study ID	Experimental	Comparator	Outcome	D1	D2	D3	D4	D5	Overall
Abdulla (2008)	RTS,S/AS02D	Hepatitis B (Engerix)	SAEs, GMTs						
Agandji (2010)	RTS,S/AS01E	EPI-vaccines only	SAEs, GMTs						
Alonso (2004)	RTS,S/AS02A	Pneumococcal conjungate or hepatitis B	SAEs, GMTs						
Aponte (2007)	RTS,S/AS02D	Hepatitis B (Engerix)	SAEs, GMTs						
Asante (2020)	RTS,S/AS01	Yellow Fever and MR vaccines only	SAEs, GMTs						
Bejon (2008)	RTS,S/AS01E	Rabies vaccine	SAEs, GMTs						
Bojang (2005) A	RTS,S/AS02A	Rabies vaccine	SAEs						
Bojang (2005) B	RTS,S/AS02A	Rabies vaccine	SAEs						
Macete (2007)	RTS,S/AS02A	Hepatitis B (Engerix)	SAEs						
Owusu-Agyei (2009)	RTS,S/AS01E	Rabies vaccine	SAEs, GMTs						
RTS,S Clinical Trial Partnership (2011)	RTS,S/AS01	Meningococcal conjugate or rabies vaccine	SAEs, GMTs						
Witte (2020)	RTS,S/AS01E	Hepatitis B (Engerix)	SAEs, GMTs						

Figure 3.2: Risk of bias summary of the quality assessed studies. The risk of bias in each domain and the overall risk of bias are classified as low risk of bias (green), showing some concerns (yellow), or having a high risk of bias (red).

3.4 Meta-analyses

A total of 10 articles reporting 11 studies were included in the meta-analyses. The studies were categorized as RTS,S/AS01 or RTS,S/AS02-studies and then sub-grouped into "0-4 months" or "5 months and older" based on the age of the study participants. For each outcome measure, two meta-analyses were done: one for RTS,S/AS01 studies and one for RTS,S/AS02-studies.

3.4.1 Meta-analyses of SAEs

Eleven studies were included in the meta-analysis of SAEs. One of the studies(42) reported results for two different age groups (6-12 weeks and 5-17 months) with corresponding placebo groups and is therefore presented in both subgroups in the RTS,S/AS01 analysis.

For both RTS,S/AS01 and RTS,S/AS02, the risk of having an SAE was higher among the participants aged 0-4 months than participants older than five months. The overall relative risk of having an SAE after vaccination was statistically significantly reduced by 15% or 24% when vaccinated with RTS,S/AS01 or RTS,S/AS02 compared to immunization with a placebo vaccine. The estimated risk ratios of SAEs in the RTS,S/AS01 and RTS,S/AS02 groups vs. the placebo groups (stratified based on the age of the participants) are presented in figures 3.3 and 3.4, respectively.

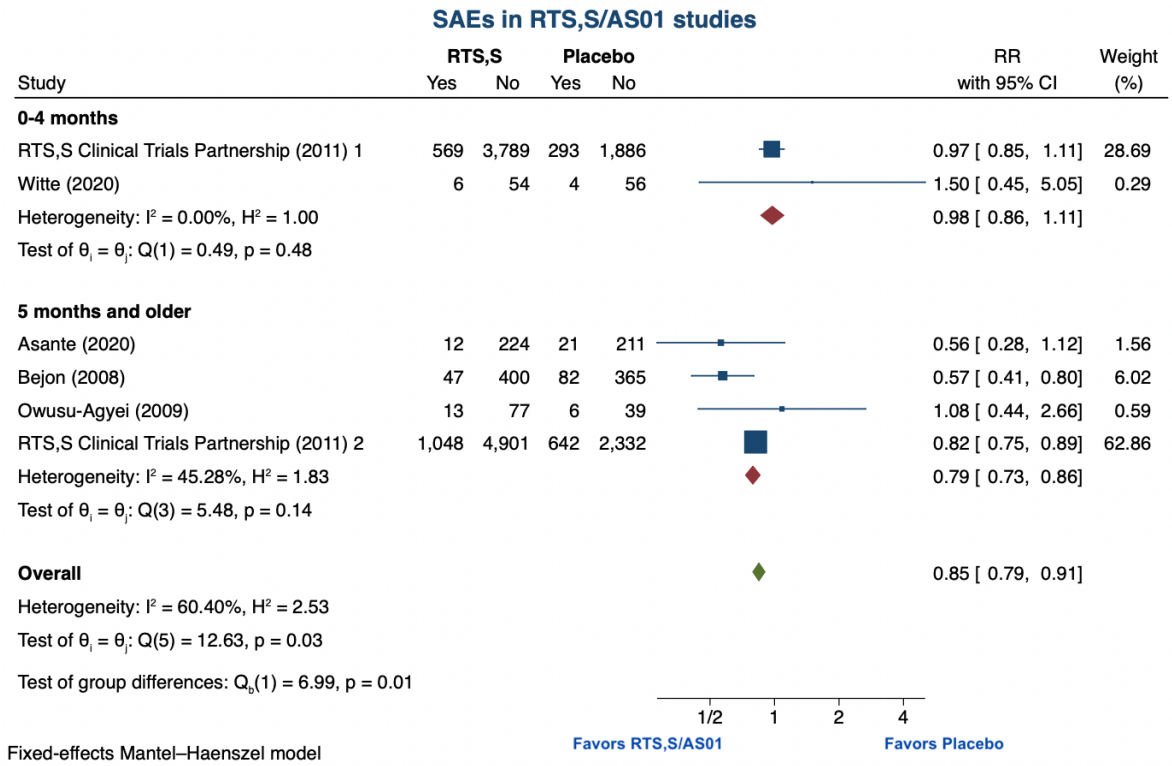


Figure 3.3: Forest plot depicting the overall estimated risk ratios of SAEs in the RTS,S/AS01 group compared to the placebo group.

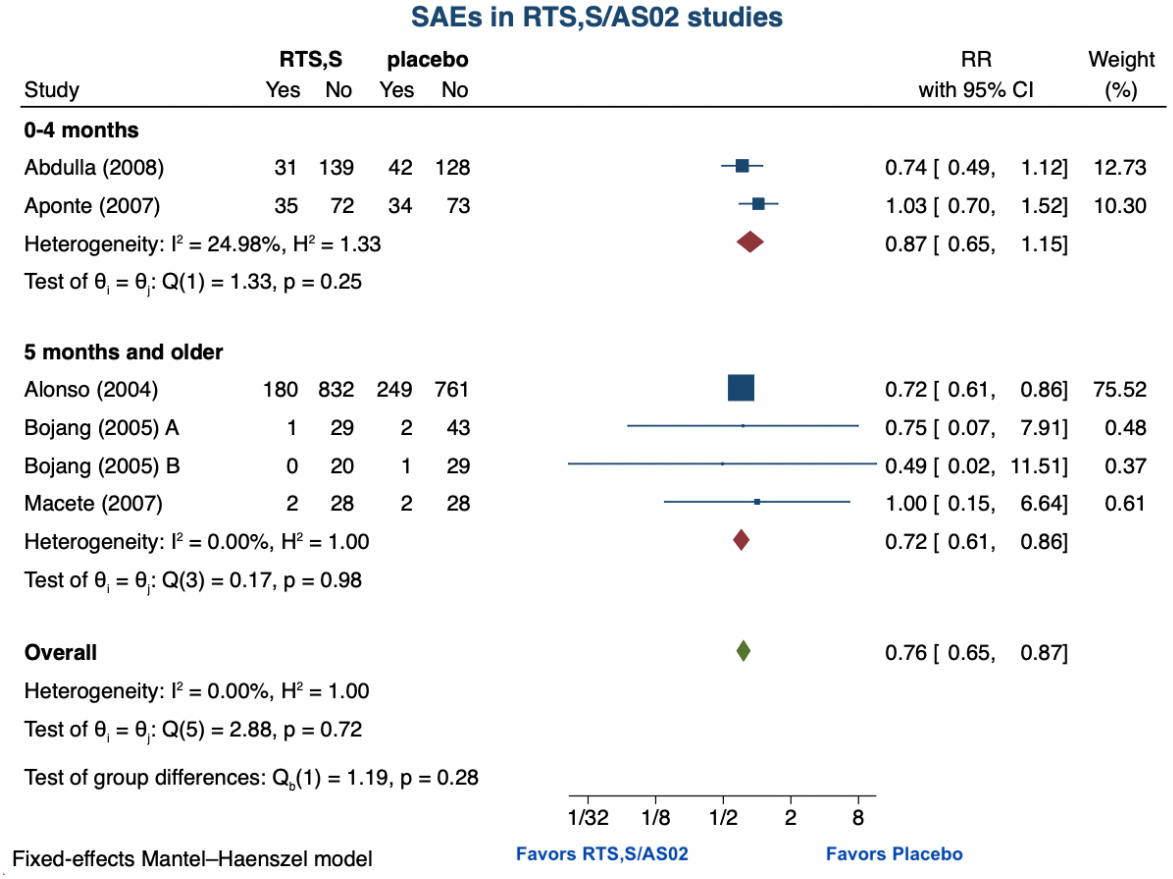


Figure 3.4: Forest plot depicting the overall estimated risk ratios of SAEs in the RTS,S/AS02 group compared to the placebo group.

3.4.2 Meta-analyses of anti-CS GMTs

Nine studies were included in the meta-analysis of anti-CS GMT. Three RTS,S/AS02 studies(16, 45) reported GMTs in another unit (µg/mL instead of EU/mL) and were excluded from the meta-analysis. Two of the included studies(33, 42) reported results for two different vaccine cohorts with corresponding placebo groups and are therefore presented twice in the RTS,S/AS01 analysis.

On average, participants vaccinated with RTS,S/AS01 and RTS,S/AS02 increased the anti-CS antibody GMT by 315,3 EU/mL (95% CI 330,97 to 329,66) and 127,92 EU/mL (95% CI 116,34 to 139,49), respectively, compared to participants vaccinated with a placebo vaccine. For both RTS,S/AS01 and RTS,S/AS02, the mean difference in GMT is higher in the participants aged five months and older than in those 0-4 months of age. The estimated mean difference in anti-CS antibody GMTs between the RTS,S/AS01 and RTS,S/AS02 groups and the placebo groups (stratified based on the age of the participants) are presented in figures 3.5 and 3.6, respectively.

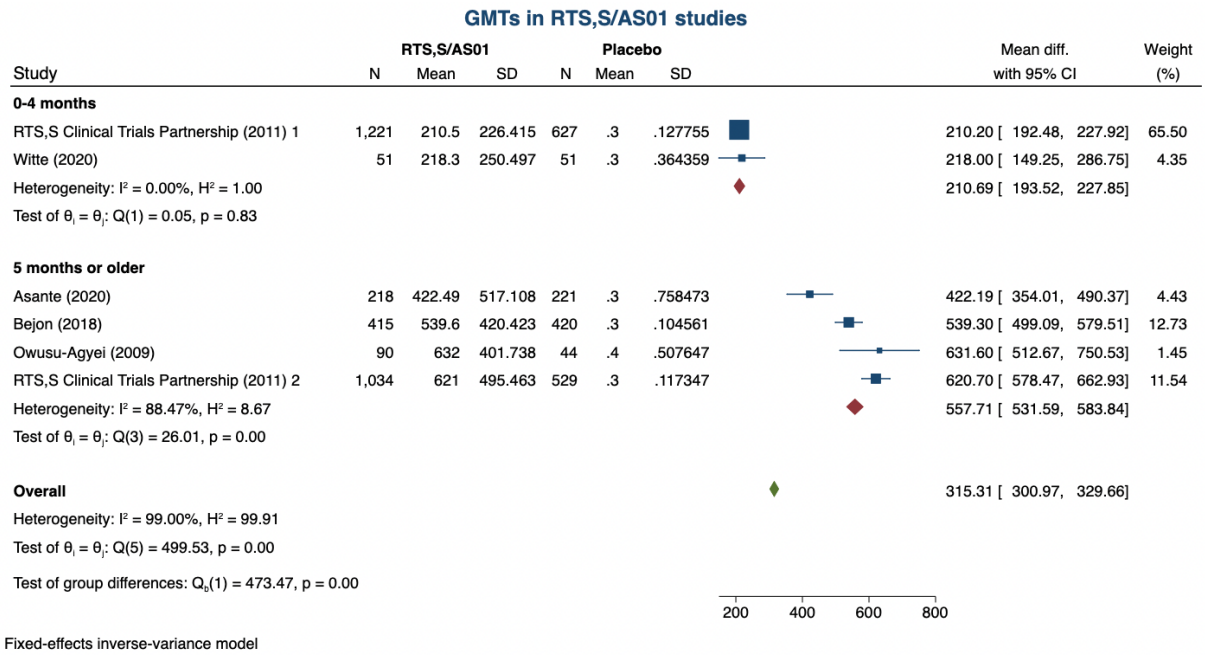


Figure 3.5: Forest plot depicting the weighted mean differences in GMTs between RTS,S/AS01, and placebo groups.

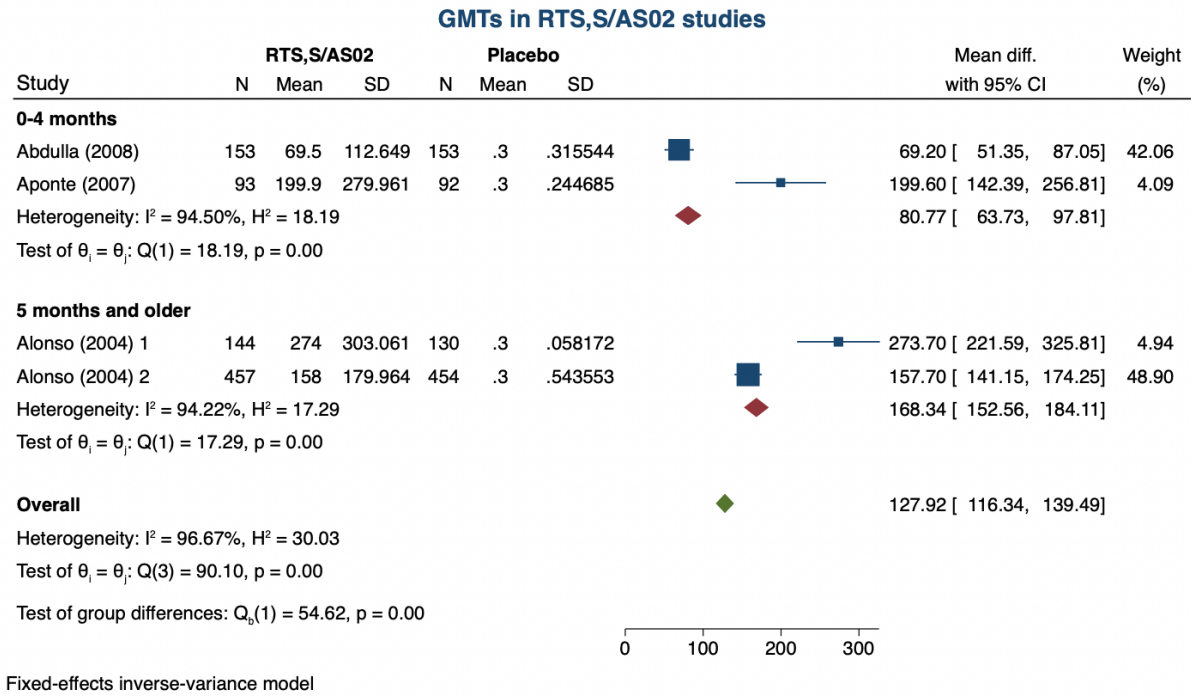


Figure 3.6: Forest plot depicting the weighted mean differences in GMTs between RTS,S/AS02, and placebo groups.

3.4.3 Analysis of publication bias

Funnel plots were produced for all meta-analyses to assess whether there were any small-study effects or publication bias in the analysis. The funnel plots for SAEs in the RTS,S/AS01 and RTS,S/AS02 groups are shown in figures 3.7 and 3.8, respectively. Both funnel plots were symmetric, with the same number of studies on the left and right sides of the funnel plot, indicating no publication bias detected.

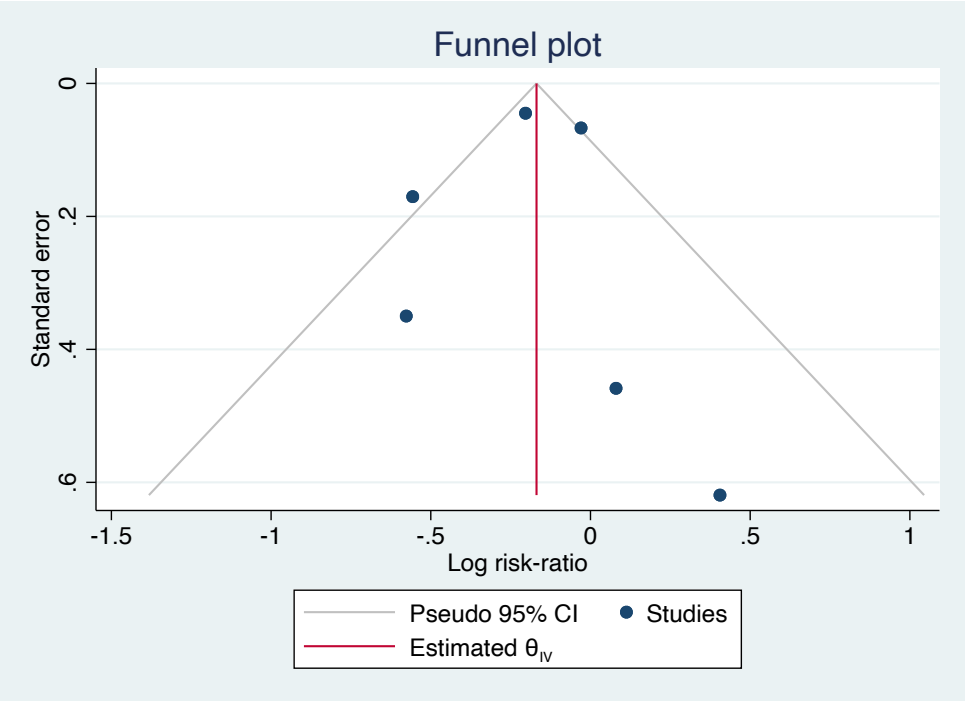


Figure 3.7: Funnel plot of SAEs in RTS,S/AS01 studies.

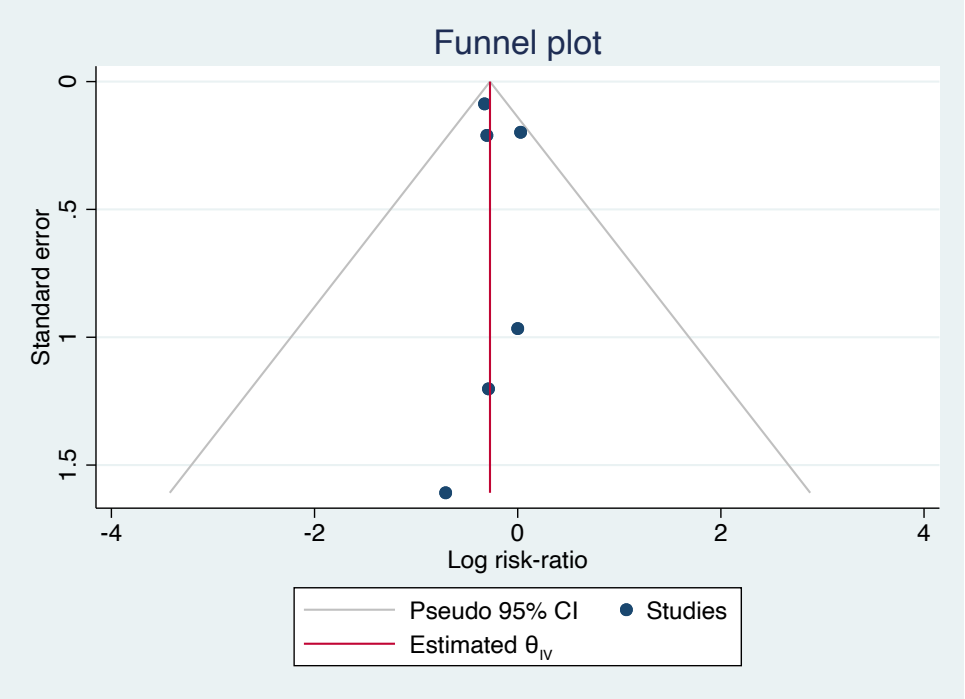


Figure 3.8: Funnel plot of SAEs in RTS,S/AS02 studies.

The funnel plots for GMTs in the RTS,S/AS01 and RTS,S/AS02 groups are shown in figures 3.9 and 3.10, respectively. They both show asymmetry with multiple outlier studies. Results of Egger´s regression tests and Begg´s rank tests showed no significant small-study effect or

publication bias, with p-values>0,05 as results for both tests for both RTS,S/AS01 and RTS,S/AS02-studies. The output of the Egger test and Begg's test for small-study effects for RTS,S/AS01 and RTS,S/AS02 studies are presented in appendices 3 and 4, respectively.

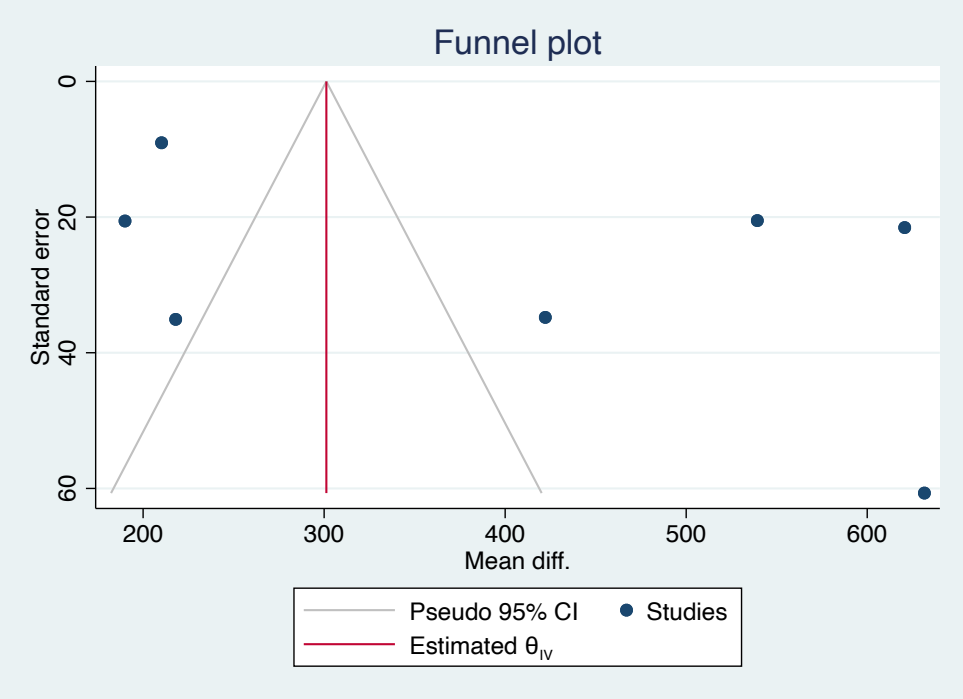


Figure 3.9: Funnel plot of GMTs in RTS,S/AS01 studies.

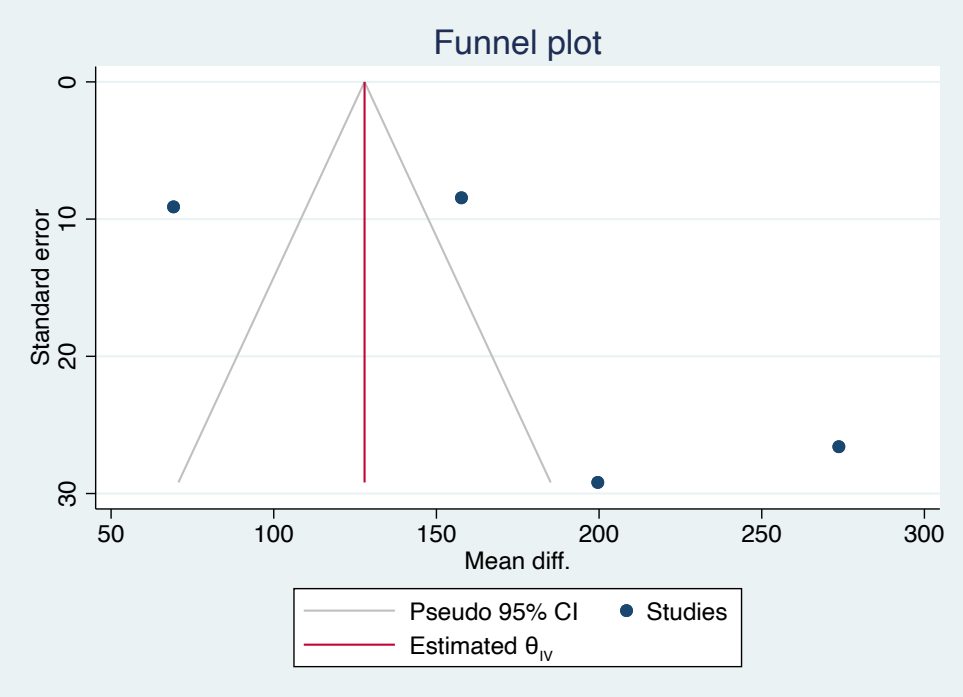


Figure 3.10: Funnel plot of GMTs in RTS,S/AS02 studies.

3.4.4 Sensitivity analyses

Two sensitivity analyses were conducted for the meta-analyses of the SAEs. The first sensitivity analysis included the study with a high risk of bias(43) in the analysis of the RTS,S/AS01-studies. The overall and subgroup RR results and heterogeneity results were similar to the meta-analysis in which the study was excluded. The other sensitivity analysis done for SAEs included all studies without differentiation between the RTS,S/AS01, and RTS,S/AS02 vaccine formulations, but still subgrouping the studies by age. The results were similar to the RTS,S/AS01-results, with a low overall heterogeneity (I^2=36,3%) and no heterogeneity in the subgroups. The overall relative risk of an SAE was 0,83 (95% CI 0,79 to 0,89) in the sensitivity analysis, compared to 0,84 (95% CI 0,79 to 0,90) for RTS,S/AS01 and 0,76 (95% CI 0,66 to 0,88) for RTS,S/AS02.

Three sensitivity analyses were conducted for the meta-analyses of GMTs. The first sensitivity analysis included the study with a high risk of bias(43) in the analysis of the RTS,S/AS01-studies. It resulted in a lower overall mean difference estimate but a higher mean difference in anti-CS GMTs in the 0-4 months age group. Another sensitivity analysis on the RTS,S/AS01 studies evaluated the results when the estimated GMT-value and confidence intervals for GMTs in placebo groups were changed from 0,3 [0.29, 0,31] to 0,3 [0,26, 0,34], which produced similar results in both analyses. The final sensitivity analysis was performed in all studies, without differentiation between the RTS,S/AS01 and RTS,S/AS02 formulations (including 3 RTS,S/AS02-studies that used another unit than the rest of the studies(16, 45)), but still subgrouping the studies by age. The analysis resulted in an overall lower mean difference in GMT (175,69 EU/mL, 95% CI[167.82, 183.56]).

All forest plots for the sensitivity analyses are presented in appendices 5 to 9.

4 Discussion

4.1 Discussion of methods

4.1.1 Search strategy and databases

The search strategy was thoroughly designed to access both published and unpublished materials, with a broad spectrum of studies. To broaden the search, it was decided to include both malaria naïve and malaria exposed individuals and all possible spellings of the RTS,S vaccine. It was decided to limit the search to clinical trials and RCTs of all phases to exclude irrelevant study types when searching studies.

Although no studies of malaria naïve and malaria exposed adults were included in the review, they were included in the search strategy due to uncertainty regarding the number of relevant studies to be found in the data search. A broader data search identifies more results, including more irrelevant studies, but a narrow search might, on the other hand, not identify all relevant studies. Even though the screening process is more comprehensive with more results, a broader search was thought to be the best alternative.

To ensure that all relevant articles, both published and unpublished materials, were found, it was decided to use five different databases where RTS,S vaccine trials might occur. Databases included were PubMed, OVID Embase, CENTRAL, ClinicalTrials.gov (beta version), and SCOPUS. The beta version of ClinicalTrials.gov was used because the newer page will be available in the future for anyone wanting to repeat the data search. All results found in the data search of the beta version of ClinicalTrials.gov were available on the old page when writing this thesis, but it cannot be guaranteed that this will be the case in the future.

The WHO International Clinical Trials Registry Platform (ICTRP) was also supposed to be included. However, due to technical difficulties with this database and the fact that CENTRAL has results from both ClinicalTrials.gov and ICTRP, ICTRP was excluded as a database. ICTRP and ClinicalTrials.gov are similar and likely to include the same trial registers, thus overlapping results, which also justifies excluding ICTRP as a database.

4.1.2 Data analysis

The studies included in the review were quality assessed using Cochrane Collaborations´ Risk of Bias Tool version 2 (RoB2)(37) to limit the risk of a biased meta-analysis result. Studies evaluated as having a high risk of bias were excluded from the meta-analysis. These studies might influence the results, potentially showing a better vaccine effect. The studies graded as «some concerns» and «low risk of bias» were included in the meta-analysis, allowing for a more precise pooled result of an effect. The more studies included, the narrower the confidence interval for the pooled effect will be.

Data reported in the studies were used as the input for the meta-analyses. The number of SAEs in the RTS,S and placebo groups are reported in the studies, and the number of no SAEs was calculated for both groups in all studies (total number of participants, n - number of SAEs).

Vaccine efficacy results of the included studies were not pooled by a meta-analysis because only a few studies reported vaccine efficacy. Estimating the vaccine efficacy for the rest of the studies is impossible without the individual participant data available.

The input data for the meta-analyses of the mean difference in GMTs for RTS,S compared to placebo is a mean (GMT) and a standard deviation (SD) of the mean. However, the studies only reported a GMT and the corresponding 95% Confidence interval (CI). Therefore, an SD of the reported GMTs was estimated from the reported 95% CIs of the GMTs, adding slight uncertainty to the meta-analysis. One study(23) did not report a GMT 1 month after 3rd dose for the placebo vaccine. Therefore, an estimated value of 0,3 EU/mL (95% CI 0,2 to 0,4) was given, which seemed reasonable compared to the reported placebo GMTs. Too narrow confidence intervals of the GMTs for the placebo vaccine due to only one decimal reported giving an SD of 0, which is not compatible with meta-data. For these studies an estimated wider interval was imputed, by adding decimals ([0.3, 0.3] changed to [0.29, 0.31]).

Heterogeneity analyses are an essential part of meta-analyses because they assesses the variability across the studies included in the meta-analysis and can help make sensible decisions about pooling the data or making particular comparisons (47). Fixed-effects model meta-analyses were used to pool outcome data in this review because of an assumption that the included studies were similar enough to meta-analyze. All included studies assess the performance of RTS,S-vaccines, compared to active placebo vaccines, through randomized

controlled trials in sub-Saharan African children. By excluding studies with a high risk of bias and only pooling outcome data reported in the same unit and found by similar methods, it was assumed to be no clinical or methodological differences between the studies that could affect the results of the meta-analyses. Statistical heterogeneity was assessed by Cochran´s Q and Higgins and Thompson's I^2 statistic in the meta-analyses. Heterogeneity was also explored through subgroup analysis.

The meta-analyses were stratified based on the age of the participants in the included studies into two subgroups per analysis: "0-4 months" of age and "5 months and older", to make comparisons between subgroups in the data, as well as the overall pooled effect estimate. The reason for stratifying by the age of the participants was that some studies had shown different effects of the RTS,S vaccine in neonatal/infants compared to older children (18, 33). Therefore, age as a potential effect modifier was considered in the subgroup analyses.

Funnel plots, and additional statistical tests in case of asymmetrical funnel plots, were made to visualize any publication bias (caused by missing studies) or small-study effects related to the meta-analysis. Assessing this bias is essential, as the mean effect computed by the meta-analysis will reflect the publication bias if the included studies are a biased sample of all relevant studies.

Sensitivity analyses were done for all outcomes to assess the robustness of the findings when different assumptions and choices were made, and thus check whether the assumptions and choices made prior to the meta-analyses were appropriate. In the sensitivity analyses, the meta-analyses were repeated, but with slight changes such as including all excluded studies or using different estimated values in calculating the SD of anti-CS GMTs.

4.2 Discussion of results

4.2.1 SAEs

The meta-analyses of SAEs in the RTS,S vaccine groups compared to the placebo groups showed that the participants overall had a lower risk of having SAEs after vaccination with RTS,S/AS01, or RTS,S/AS02 compared to vaccination with a placebo vaccine. This could be because the placebo vaccines used in the studies are active vaccines that might have side effects that cause adverse events for the participants. However, most of the included studies

stated that the cases of SAEs were not causally related to vaccination (16-18, 23, 33, 41, 44, 45).

With an overall RR of 0,76 (95% CI 0,65 to 0,87), the RTS,S/AS02 vaccinated group had the lowest risk of having SAEs after vaccination compared to vaccination with placebo vaccine (RTS,S/AS01: RR 0,85 (95% CI 0,79 to 0,91)), although there is no statistically significant difference in RR between RTS,S/AS02 and RTS,S/AS01 because the point estimate for RTS,S/AS01 is overlapped by the confidence interval for RTS,S/AS02.

A visual inspection of the forest plots for both the RTS,S/AS01 and RTS,S/AS02 studies shows overlapping confidence intervals for all the studies, in all subgroups, indicating little heterogeneity between the SAE results of the studies. Statistical heterogeneity analyses identified no overall heterogeneity for the RTS,S/AS02 studies but moderate overall heterogeneity for the RTS,S/AS01 studies (I^2=60,40%, p=0,03). When exploring subgroups, the heterogeneity seems to come from the "5 months and older" group (I^2=45,28%, p=0,04), although there was no statistically significant heterogeneity for either RTS,S/AS01 subgroup. The overall heterogeneity was not viewed as an important impact on the relative risks of having an SAE after vaccination with a RTS,S-vaccine compared to a placebo vaccine.

The sensitivity analysis for the SAEs in the RTS,S/AS01 vaccine groups compared to the placebo groups showed similar results as the primary meta-analysis. This indicates that the premade choice of excluding the study with a high risk of bias from the meta-analyses did not matter that much for the analysis. Still, it may have given a more precise estimate of the pooled relative risk of having SAEs in the RTS,S vaccine groups compared to the placebo groups.

4.2.2 Anti-CS GMTs

A visual inspection of the forest plots for GMTs in the RTS,S/AS01 studies show overlapping confidence intervals in both subgroups, but with an outlier study in the "5 months and older" group. The statistical heterogeneity analyses show no heterogeneity in the "0-4 months" group but substantial heterogeneity in the "5 months and older" group (I^2=88,47%, p<0,05) and a considerable statistically significant overall heterogeneity (I^2=99,0%, p<0,05) in the RTS,S/AS01 studies. For the RTS,S/AS02 studies, the visual inspection showed no overlap in confidence interval in either of the subgroups and a statistically significant overall heterogeneity (I^2=96,67%, p<0,05). Pooling of the RTS,S/AS01 and RTS,S/AS02 studies may

produce misleading results because of the high heterogeneity (considerable variations in estimated mean differences for the studies, even when the same method is used to measure the anti-CS GMTs in all studies). It is therefore not possible to make meaningful conclusions on the results of the meta-analyses of anti-CS GMTs in the RTS,S vaccine groups compared to the placebo groups.

However, the trends visualized in the meta-analyses can be commented on. The meta-analyses of anti-CS GMTs show that participants vaccinated with RTS,S/AS01 and RTS,S/AS02 increased the anti-CS antibody GMT by 315,3 EU/mL (95% CI 330,97 to 329,66) and 127,92 EU/mL (95% CI 116,34 to 139,49), respectively, compared to participants vaccinated with a placebo vaccine. For both RTS,S/AS01 and RTS,S/AS02, there is a trend of a higher pooled mean difference in anti-CS GMTs in the participants aged five months and older, with mean differences in anti-CS GMTs ranging from 422,2-620,7EU/mL and 157,7-273,7EU/mL in RTS,S/AS01 and RTS,SAS02 respectively, than in participants 0-4 months of age (mean differences in mean anti-CS GMTs ranging from 210,2-218,0 EU/mL in RTS,S/AS01 studies and 69,2-199,6EU/mL in RTS,S/AS02 studies). Although there has not been an established correlation of protection, an association between higher anti-CS titers and decreased risk of *Plasmodium falciparum* malaria infection has been suggested (18, 33, 43, 44). It cannot be concluded that RTS,S/AS01 and RTS,S/AS02 have efficacy against infection based on antibody titer results, but positive mean differences in anti-CS GMTs in RTS,S vaccine groups compared to placebo groups in all studies may suggest higher protection of infection in participants receiving RTS,S/AS01 or RTS,S/AS02 vaccines compared to participants receiving placebo vaccines, as these participants have a higher mean anti-CS antibody titer.

Random-effects model meta-analyses could have been conducted to assess mean differences in anti-CS GMTs, as the random-effects model includes consideration of heterogeneity in the effect estimate (47). However, the random-effects model does not remove the heterogeneity, which is likely due to the variations in estimated anti-CS GMTs in RTS,S vaccine groups compared to the placebo groups in the studies. Also, decisions for including random-effects model meta-analyses as sensitivity analyses should have been made prior to the meta-analyses to avoid risk of reporting bias.

4.3 Strengths and limitations

This systematic review included RCTs, which give the highest level of evidence because of their unbiased study design. The included RCTs have a low risk of systematic errors and confounding factors. Participants are balanced in age, demographic characteristics, and other factors that may cause confounding between the comparison groups. The RCTs included in this systematic review and meta-analyses are identified through thorough search strategies and are quality assessed by Cochrane guidelines. Only one of the studies was classified as having a high risk of bias and was excluded from the meta-analyses, allowing a potentially more correct result. No publication bias or small-study effects were found in the data analyses which ensured that an unbiased sample of studies was included in this systematic review and meta-analysis.

A significant limitation of this thesis is that it is written, and all the work is done, by one author, only. The Cochrane handbook of systematic reviews of interventions(34) states:

> Systematic reviews should be undertaken by a team. Working as a team not only spreads the effort but ensures that tasks such as the selection of studies for eligibility, data extraction, and rating the certainty of the evidence will be performed by at least two people independently, minimizing the likelihood of errors. (33, chapter 1.3)

In Cochrane reviews, all stages are duplicated to ensure that one author isn´t missing anything in the data extraction or deciding which papers should be excluded or included in the review with a biased point of view. There is an element of subjectivity in the eligibility criteria set for included studies in this review. However, all these decisions were prespecified in the protocol, making them transparent and reducing the risk of a biased review.

Another limitation of this review is that considerable heterogeneity was observed across the studies included in the meta-analyses of anti-CS GMTs, both overall and in subgroup analyses. Few studies reporting vaccine efficacy made it impossible to synthesize these valuable results in a meta-analysis. The thesis work had to be restricted due to time constraints, and it was not possible to assess all variables that would have been clinically meaningful to determine in a systematic review and meta-analysis, e.g., the performance of the RTS,S vaccine in malaria naïve and malaria exposed adult populations as well as in the sub-Saharan African children that were assessed in this review.

5 Conclusion

Vaccination of sub-Saharan African children with three doses of RTS,S/AS01 or RTS,S/AS02 at a 0,1,2-month schedule has been evaluated in several RCTs. When synthesizing these studies together, it was shown that the RTS,S vaccines had a lower risk of SAEs after vaccination compared to the active placebo vaccines, suggesting that the RTS,S vaccines are safe and well-tolerated. The pooled mean differences of anti-CS GMTs in the RTS,S-vaccine groups compared to the placebo groups did not provide clinically meaningful conclusions because of the considerable heterogeneity between the estimated effect sizes in the studies. Not all included studies reported vaccine efficacy results. As individual participant data are necessary to estimate HRs, and thereby vaccine efficacy, VE results were not pooled in a meta-analysis. Further investigation is needed to conclude whether the performance of the RTS,S vaccines is affected by the age of the vaccinees.

As the RTS,S/AS01 vaccine has been approved and is to be implemented in the Expanded Program of Immunization in sub-Saharan African children, further research should address this vaccine. A systematic review synthesizing and comparing follow-up data from phase IV studies and pilot implementations would be an interesting addition to what is already known about this vaccine's long-term immunogenicity, safety, and protective efficacy in sub-Saharan African children.

6 References

1. World Health Organization. World Malaria Report 2021. Geneva; 2021. Contract No.: CC BY-NC-SA 3.0 IGO.
2. Palacpac NMQ, Horii T. Malaria vaccines: facing unknowns. F1000Res. 2020;9:F1000 Faculty Rev-296.
3. World Health Organization. World Malaria Report 2020. 2020.
4. World Health Organization. Malaria 2022 [Available from: https://www.who.int/news-room/fact-sheets/detail/malaria.
5. World Health Organization. Full Evidence Report on the RTS,S/AS01 Malaria Vaccine. SAGE Yellow Book (October 2021); 2021 September, 2021.
6. Prevention CfDCa. About Malaria: Biology: Lifecycle 2020 [Available from: https://www.cdc.gov/malaria/about/biology/index.html.
7. Bairwa M, Rajput M, Khanna P, Rohilla R, Verma R, Chawla S. Malaria vaccine: A bright prospect for elimination of malaria. Human Vaccines and Immunotherapeutics. 2012;8(6):819-22.
8. Owusu-Ofori K, O'Shirley O, Bates I. Global challenges of managing Malaria risk. Vox Sang. 2016;111(Supplement 1):15.
9. Escalante A, Pacheco M. Malaria Molecular Epidemiology: an Evolutionary Genetics Perspective. Microbiology Spectrum. 2019;7(4).
10. Aggarwal A, Garg N. Newer Vaccines against Mosquito-borne Diseases. Indian J Pediatr. 2018;85(2):117-23.
11. Rosenthal P, John C, Rabinovich N. Malaria: How Are We Doing and How Can We Do Better? The American journal of tropical medicine and hygiene. 2019;100(2):239-41.
12. Feachem RG, Chen I, Akbari o, Bertozzi-Villa A, Bhatt S. Malaria Eradication Within a Generation: Ambitious, Achievable, and Necessary. The Lancet. 2019;394(10203):1056-112.
13. Coelho CH, Doritchamou JYA, Zaidi I, Duffy PE. Advances in malaria vaccine development: report from the 2017 malaria vaccine symposium. NPJ Vaccines. 2017;2:34.
14. Muthui M, Kamau A, Bousema T, Blagborough AM, Bejon P, Kapulu M. Immune Responses to Gametocyte Antigens in a Malaria Endemic Population - The African falciparum Context: A Systematic Review and Meta-Analysis. Front Immunol. 2019;10.

15. Cohen J, Nussenzweig V, Nussenzweig R, Vekemans J, Leach A. From the circumsporozoite protein to the RTS,S/AS candidate vaccine. Human Vaccines. 2010;6(1).
16. Bojang KA, Olodude F, Pinder M, Ofori-Anyinam O, Vigneron L, Fitzpatrick S, et al. Safety and immunogenicty of RTS,S/AS02A candidate malaria vaccine in Gambian children. Vaccine. 2005;23(32):4148-57.
17. Owusu-Agyei S, Ansong D, Asante K, Owusu SK, Owusu R, Brobby NAW, et al. Randomized controlled trial of RTS,S/AS02<inf>D</inf> and RTS,S/AS01<inf>E</inf> malaria candidate vaccines given according to different schedules in Ghanaian children. PLoS One. 2009;4(10) (no pagination).
18. Witte D, Cunliffe NA, Turner AM, Ngulube E, Ofori-Anyinam O, Vekemans J, et al. Safety and Immunogenicity of Seven Dosing Regimens of the Candidate RTS,S/AS01^E Malaria Vaccine Integrated within an Expanded Program on Immunization Regimen: A Phase II, Single-Center, Open, Controlled Trial in Infants in Malawi. Pediatr Infect Dis J. 2020:483-91.
19. Bojang KA. RTS,S/AS02A for malaria. Expert Review of Vaccines. 2006;5(5):611-5.
20. EMEA, CHMP, VWP. Guideline on Clinical Evaluation of New Vaccines. Guidelines. ema.europe.eu: Committee for Medicinal Products for Human Use (CHMP), Agency EM; 2006 October 18, 2006.
21. Asante KP, Malm K, Darko DM, Koram KA. Re: Musings on malaria morbidity and mortality after the new Mosquirix(®) vaccine. Ghana Med J. 2019;53(3):252-3.
22. Spring M, Polhemus M, Ockenhouse C. Controlled human malaria infection. J Infect Dis. 2014;209(SUPPL. 2):S40-S5.
23. Asante KP, Ansong D, Kaali S, Adjei S, Lievens M, Nana Badu L, et al. Immunogenicity and safety of the RTS,S/AS01 malaria vaccine co-administered with measles, rubella and yellow fever vaccines in Ghanaian children: a phase IIIb, multi-center, non-inferiority, randomized, open, controlled trial. Vaccine. 2020;38(18):3411-21.
24. Stassijns J, Bollaerts K, Baay M, Verstraeten T. A systematic review and meta-analysis on the safety of newly adjuvanted vaccines among children. Vaccine. 2016;34(6):714-22.
25. Agnandji ST, Fernandes JF, Bache EB, Ramharter M. Clinical development of RTS,S/AS malaria vaccine: A systematic review of clinical Phase I-III trials. Future Microbiol. 2015;10(10):1553-78.

26. Dimala CA, Kika BT, Kadia BM, Blencowe H. Current challenges and proposed solutions to the effective implementation of the RTS, S/AS01 Malaria Vaccine Program in sub-Saharan Africa: A systematic review. PLoS One. 2019;13(12):e0209744.
27. Bell GJ, Agnandji ST, Asante KP, Ghansah A, Kamthunzi P, Emch M, et al. Impacts of Ecology, Parasite Antigenic Variation, and Human Genetics on RTS,S/AS01e Malaria Vaccine Efficacy. Curr Epidemiol Rep. 2021:1-10.
28. Graves PM, Gelband H. Vaccines for preventing malaria (blood-stage). Cochrane Database Syst Rev. 2006(4).
29. Graves PM, Gelband H. Vaccines for preventing malaria (pre-erythrocytic). Cochrane Database Syst Rev. 2006(4).
30. Graves PM, Gelband H. Vaccines for preventing malaria (SPf66). Cochrane Database Syst Rev. 2006(2).
31. Esu EB, Oringanje C, Meremikwu MM. Intermittent preventive treatment for malaria in infants. Cochrane Database Syst Rev. 2021(7).
32. Stanisic DI, McCall MBB. Correlates of malaria vaccine efficacy. Expert Review of Vaccines. 2021;20(2):143-61.
33. Alonso PL, Sacarlal J, Aponte JJ, Leach A, Macete E, Milman J, et al. Efficacy of the RTS,S/AS02A vaccine against Plasmodium falciparum infection and disease in young African children: randomised controlled trial. Lancet (london, england). 2004;364(9443):1411-20.
34. Higgins J, Thomas J, Chandler J, Cumpston M, Li T, Page M, et al. Cochrane Handbook for Systematic Reviews of Interventions. Cochrane2022. Available from: https://training.cochrane.org/handbook.
35. Bramer WM, Giustini D, de Jonge GB, Holland L, Bekhuis T. De-duplication of database search results for systematic reviews in EndNote. Journal of the Medical Library Association : JMLA. 2016;104(3):240-3.
36. U.S Food & Drug Administration. What is a Serious Adverse Event? fda.gov2016 [updated 02.01.2016. Available from: https://www.fda.gov/safety/reporting-serious-problems-fda/what-serious-adverse-event.
37. Sterne J, Savović J, Page M, Elbers R, Blencowe N, Boutron I, et al. RoB 2: a revised tool for assessing risk of bias in randomised trials. BMJ. 2019;366(14898).
38. Hoaglin DC. Misunderstandings about Q and 'Cochran's Q Test' in Meta-Analysis. Stat Med. 2016;35(4):485-95.

39. Higgins J, Thompson SG. Quantifying Heterogeneity in a Meta-Analysis. Stat Med. 2002;21(11):1539-58.
40. Begg CB, Mazumdar M. Operating characteristics of a rank correlation test for publication bias. Biometrics. 1994;50(4):1088-101.
41. Bejon P, Lusingu J, Olotu A, Leach A, Lievens M, Vekemans J, et al. Efficacy of RTS,S/AS01E vaccine against malaria in children 5 to 17 months of age. N Engl J Med. 2008;359(24):2521-32.
42. RTSS Clinical Trials Partnership. First Results of Phase 3 Trial of RTS,S/AS01 Malaria Vaccine in African Children. N Engl J Med. 2011;365(20):1863-75.
43. Abdulla S, Oberholzer R, Juma O, Kubhoja S, Machera F, Membi C, et al. Safety and immunogenicity of RTS,S/AS02D malaria vaccine in infants. N Engl J Med. 2008;359(24):2533-44.
44. Aponte JJ, Aide P, Renom M, Mandomando I, Bassat Q, Sacarlal J, et al. Safety of the RTS,S/AS02D candidate malaria vaccine in infants living in a highly endemic area of Mozambique: a double blind randomised controlled phase I/IIb trial. Lancet. 2007;370(9598):1543-51.
45. Macete E, Aponte JJ, Guinovart C, Sacarlal J, Ofori-Anyinam O, Mandomando I, et al. Safety and immunogenicity of the RTS,S/AS02A candidate malaria vaccine in children aged 1-4 in Mozambique. Trop Med Int Health. 2007;12(1):37-46.
46. Agnandji ST, Asante KP, Lyimo J, Vekemans J, Soulanoudjingar SS, Owusu R, et al. Evaluation of the safety and immunogenicity of the RTS,S/AS01E malaria candidate vaccine when integrated in the expanded program of immunization. J Infect Dis. 2010;202(7):1076-87.
47. Ryan R. Heterogeneity and subgroup analyses in Cochrane Consumers and Communication Group reviews: planning the analysis at protocol stage cccrg.cochrane.org2016 [Available from: https://cccrg.cochrane.org/sites/cccrg.cochrane.org/files/public/uploads/heterogeneity_subgroup_analyses_revising_december_1st_2016.pdf.
48. Bejon P, Cook J, Bergmann-Leitner E, Olotu A, Lusingu J, Mwacharo J, et al. Effect of the pre-erythrocytic candidate malaria vaccine RTS,S/AS01E on blood stage immunity in young children. J Infect Dis. 2011;204(1):9-18.

7 Appendices

Appendix 1: Characteristics of included studies

Appendix 2: Quality assessment

Appendix 3: Output of Egger test and Begg's test for small-study effects for RTS,S/AS01 studies

Appendix 4: Output of Egger test and Begg's test for small-study effects for RTS,S/AS02 studies

Appendix 5: Sensitivity analysis of SAEs in all RTS,S/AS01 studies

Appendix 6: Sensitivity analysis of SAEs in all studies

Appendix 7: Sensitivity analysis of GMTs in all RTS,S/AS01 studies

Appendix 8: Sensitivity analysis of GMTs in included RTS,S/AS01-studies with a different 95% CI estimate

Appendix 9: Sensitivity analysis of GMTs in all studies

Appendix 1: characteristics of included studies

Author (year), study location	Vaccination schedule, number of doses, and vaccine and placebo information	Total number of participants, mean age at screening, %male	GMTs (EU/mL) at baseline/screening (95% CI)		Crude Vaccine Efficacy Results		Author´s conclusions
			Vaccine	Placebo	Value (95% CI)	p-value	
Abdulla (2008), Tanzania	3 doses of RTS,S/AS02D (0.5mL, 25μg RTS,S) or Hepatitis B (Engerix-B) at 8, 12 and 16 weeks of age	340 participants, mean age: 7,9 weeks male: 82%	0.3 (0.3, 0.4)	0.4 (0.3, 0.4)	60.6% (10.4, 82.6)	0.03	Promising safety profile, did not interfere with EPI vaccine, reduced incidence of malaria infection
Alonso (2004), Mozambique	3 doses of RTS,S/AS02A (0,25mL), or 2 doses of pneumococcal conjungate vaccine + 1 dose of haemophilus influenzae type b vaccine (children <24 months) / 3 doses paediatric hepatitis B vaccine (children > 24 months) at a 0, 1, 2 month schedule	2022 participants, mean age: 35,8 months	0.3 (0.3, 0.3)	0.3 (0.3, 0.3)	26.9% (7.4, 42.2)	0.009	RTS,S/AS0A was safe, well tolerated and immunogenic
Aponte (2007), Mozambique	3 doses of RTS,S/AS02D (0.5mL) or Hepatitis B (Engerix-B) at 10, 14 and 18 weeks of age	214 participants, mean age: 8,3 weeks, male: 44,5%	0.4 (0.3, 0.5)	0.4 (0.3, 0.4)	62.2% (37.1, 77.3)	0.0002	The vaccine was safe, well tolerated and immunogenic in young infants
Asante (2020), Ghana	3 doses of RTS,S/AS01 (0.5 mL) at 6, 7.5 and 9 months of age or Yellow Fever (Stamaril) and MR (MR-VAC) vaccines at 9 months of age	468 Participants, mean age: 6,3 months, male: 50,5%	0.96 (0.94, 0.98)	-	-	-	RTS,S/AS01 can be co-administered with Yellow fever and measles vaccination at 9 months of age during EPI visits.

Author (year), study location	Vaccination schedule, number of doses, and vaccine and placebo information	Total number of participants, mean age at screening, %male	GMTs (EU/mL) at baseline/screening (95% CI)		Crude Vaccine Efficacy Results		Author´s conclusions
			Vaccine	Placebo	Value (95% CI)	p-value	
Bejon (2008), Kenya, Tanzania	3 doses of RTS,S/AS01E or Rabies vaccine BP(Sanofi-Pasteur) at a 0, 1, 2 months schedule	894 participants, mean age: 11,4 months, male: 50%	0.3 (0.3, 0.3)	0.3 (0.3, 0.3)	55% (31, 70)	<0.001	RTS,S/AS01E shows promise as a candidate malaria vaccine
Bojang (2005) A, Gambia	3 doses of RTS,S/AS02A (25µg RTS,S) or Rabies vaccine (Mérieux HDCV, 0,5mL) at a 0, 1 and 3 month schedule	75 participants, mean age: 2,8 years, male: 48,9%	≤0.5µg/mL	-	-	-	The RTS,S/AS02A vaccine was safe at all dose levels in both age groups
Bojang (2005) B, Gambia		50 participants, mean age: 8,0 years, male: 54,4%	0.6-1.0 µg/mL	-	-	-	
Macete (2007), Mozambique	3 doses of RTS,S/AS02A (0,25mL) or Hepatitis B (Engerix B, 0.5 mL) at 0, 1, 2 month schedule	60 participants, mean age: 2,6 years, male: 83%	-	-	-	-	RTS,S/AS02A was found to be safe and well tolerated, and highly immunogenic for anti-CS protein antibody response
Owusu-Agyei (2009), Ghana	3 doses of RTS,S/AS01E or Rabies vaccine (Rabipur) at a 0, 1, 2 month schedule	135 participants, mean age: 10,9 months, male: 48%	0.3 (0.3, 0.4)	0.3 (0.3, 0.4)	-	-	RTS,S/AS02D and RTS,S/AS01E were well tolerated, but RTS,S/AS01E gave greater anti-CS responses than RTS,S/AS02D with 3 doses

Author (year), study location	Vaccination schedule, number of doses, and vaccine and placebo information	Total number of participants, mean age at screening, %male	GMTs (EU/mL) at baseline/screening (95% CI)		Crude Vaccine Efficacy Results		Author´s conclusions
			Vaccine	Placebo	Value (95% CI)	p-value	
RTS,S Clinical Trials Partnership (2011), Burkina Faso, Ghana, Gabon, Kenya, Tanzania, Malawi, Mozambique	3 doses of RTS,S/AS01 or meningococcal serogroup C conjugate vaccine (Menjugate, Novartis) at a 0, 1, 2 month schedule	6537 participants, mean age: 1,2 months, male: 50,4%	0.4 (0.4, 0.4)	0.4 (0.4, 0.5)	-	-	The RTS,S/AS01 vaccine provided protection against both clinical and severe malaria in African children
	3 doses of RTS,S/AS01 or Rabies vaccine (VeroRab) at a 0, 1, 2 month schedule	8923 participants, mean age: 10,6 months, male: 49,8%	0.3 (0.3, 0.3)	0.3 (0.3, 0.3)	55.8% (51.3, 59.8)	<0.001	
Witte (2020), Malawi	3 doses of RTS,S/AS01E (0.5 mL) at a 0, 1, 2 month (6, 10, 14 weeks of age) schedule or 1 dose of Hepatitis B (engerix-B) vaccine at month 0	120 participants, mean age: 0,4 days, male: 32%	0.4 (0.3, 0.5)	0.5 (0.4, 0.7)	-	-	Initiation of RTS,S/AS01E vaccination above 6 weeks of age tended to improve anti-CS antibody responses.

Appendix 2: Quality assessment of all studies

Study ID	Abdulla (2008)	**Review ID**	(43)	**Assessor**	SMD
Experimental	RTS,S/AS02D	**Comparator**	Hepatitis B (Engerix)	**Outcome**	# of SAEs, GMTs

Risk of Bias assessment: Some concerns

Bias domain	**Questions to consider**	**Assessor´s judgement**	**Support for judgement**
Bias arising from the randomization process	Was the allocation sequence random? Was the allocation sequence concealed until participants were enrolled and assigned to interventions? Did baseline differences between intervention groups suggest a problem with the randomization process?	***Some concerns***	No information on randomization methods and allocation sequence, only a statement that the study is randomized and double-blind. "The demographic profiles of [the experimental and comparator groups] were balanced in terms of sex, age and distance from health center."
Bias due to deviations from the intended interventions	Were participants aware of their assigned intervention during the trial? Were carers and people delivering the interventions aware of participants' assigned intervention during the trial? Was an appropriate analysis used to estimate the effect of assignment to intervention?	***Low risk of bias***	"observers who were unaware of the study-group assignments determined that none of the [SAEs] were related to vaccination" Appropriate and well-described analysis.
Bias due to missing outcome data	Were data for this outcome available for all, or nearly all, participants randomized?	***Low risk of bias***	Outcome data for all outcomes from almost all participants, described why some are not included in the different analyses.
Bias in measurement of the outcome	Was the method of measuring the outcome inappropriate? Could measurement or ascertainment of the outcome have differed between intervention groups? Were outcome assessors aware of the intervention received by study participants?	***Low risk of bias***	"observers who were unaware of the study-group assignments determined […]" Appropriate methods of measuring outcomes, similar for both groups.
Bias in selection of the reported result	Were the data that produced this result analyzed in accordance with a pre-specified analysis plan that was finalized before unblinded outcome data were available for analysis? Is the numerical results being assessed likely to have been selected, on the basis of the results, from… .. multiple eligible outcome measurements (e.g. scales, definitions, time points) within the outcome domain? ... multiple eligible analyses of the data?	***Low risk of bias***	Well-described, per-protocol analyses at baseline and 1 month after 3rd dose. "The [statistical] analysis was based on a prospectively defined report and analysis plan"

Study ID	Agandji (2010)	**Review ID**	(46)	**Assessor**	SMD
Experimental	RTS,S/AS01E	**Comparator**	EPI-vaccines only	**Outcome**	# of SAEs, GMTs

Risk of Bias assessment: High risk of bias

Bias domain	**Questions to consider**	**Assessor´s judgement**	**Support for judgement**
Bias arising from the randomization process	Was the allocation sequence random? Was the allocation sequence concealed until participants were enrolled and assigned to interventions? Did baseline differences between intervention groups suggest a problem with the randomization process?	***High risk of bias***	No information on randomization methods and allocation sequence, only a statement that the study is randomized. "Age, sex and weight were balanced between groups"
Bias due to deviations from the intended interventions	Were participants aware of their assigned intervention during the trial? Were carers and people delivering the interventions aware of participants' assigned intervention during the trial? Was an appropriate analysis used to estimate the effect of assignment to intervention?	***Some concerns***	Open trial (participants and researchers know which treatment is being assigned). Appropriate and well-described analysis.
Bias due to missing outcome data	Were data for this outcome available for all, or nearly all, participants randomized?	***Low risk of bias***	Outcome data for all outcomes from almost all participants, similar numbers in the different groups.
Bias in measurement of the outcome	Was the method of measuring the outcome inappropriate? Could measurement or ascertainment of the outcome have differed between intervention groups? Were outcome assessors aware of the intervention received by study participants?	***Some concerns***	Appropriate and well-described methods of measuring outcomes, quite similar for all groups. Open trial, outcome assessors may be aware of the intervention received by study participants.
Bias in selection of the reported result	Were the data that produced this result analyzed in accordance with a pre-specified analysis plan that was finalized before unblinded outcome data were available for analysis? Is the numerical results being assessed likely to have been selected, on the basis of the results, from… .. multiple eligible outcome measurements (e.g. scales, definitions, time points) within the outcome domain? ... multiple eligible analyses of the data?	***Low risk of bias***	Well-described, per-protocol analyses at baseline, 1 month after 3rd dose and at later time points. "[…] prospectively registered at ClinicalTrials.gov." "The independent data monitoring committee […] reviewed the ethical, **quality** and safety aspects of the study conduct"

Study ID	Alonso (2004)	**Review ID**	(33)	**Assessor**	SMD
Experimental	RTS,S/AS02A	**Comparator**	Pneumococcal conjungate + haemophilus influenzae (b) (>24 months) or hepatitis B	**Outcome**	# of SAEs, GMTs

Risk of Bias assessment: Low risk of bias

Bias domain	Questions to consider	Assessor´s judgement	Support for judgement
Bias arising from the randomization process	Was the allocation sequence random? Was the allocation sequence concealed until participants were enrolled and assigned to interventions? Did baseline differences between intervention groups suggest a problem with the randomization process?	***Low risk of bias***	"We randomly allocated children […] Block randomization was done with SAS software version 8 (1/1 ratio, block size 6). The code was released to the investigators after completion of follow-up"
Bias due to deviations from the intended interventions	Were participants aware of their assigned intervention during the trial? Were carers and people delivering the interventions aware of participants' assigned intervention during the trial? Was an appropriate analysis used to estimate the effect of assignment to intervention?	***Low risk of bias***	"A vaccination team prepared the vaccine and masked the contents of the syringe with opaque tape before vaccination. This team was not involved in any other study procedures" Appropriate and well-described analysis.
Bias due to missing outcome data	Were data for this outcome available for all, or nearly all, participants randomized?	***Low risk of bias***	Outcome data for all outcomes from almost all participants, similar numbers in the different groups in both cohorts.
Bias in measurement of the outcome	Was the method of measuring the outcome inappropriate? Could measurement or ascertainment of the outcome have differed between intervention groups? Were outcome assessors aware of the intervention received by study participants?	***Low risk of bias***	The intervention groups were comparable, although "indicators suggest that malaria transmission was higher in the study area of cohort 2 than cohort 1". Outcome measurements were appropriate.
Bias in selection of the reported result	Were the data that produced this result analyzed in accordance with a pre-specified analysis plan that was finalized before unblinded outcome data were available for analysis? Is the numerical results being assessed likely to have been selected, on the basis of the results, from… .. multiple eligible outcome measurements (e.g. scales, definitions, time points) within the outcome domain? ... multiple eligible analyses of the data?	***Low risk of bias***	"Analysis of this trial strictly adhered to a detailed report and analysis plan established before unmasking"

Study ID	Aponte (2007)	**Review ID**	(44)	**Assessor**	SMD
Experimental	RTS,S/AS02D	**Comparator**	Hepatitis B (engerix)	**Outcome**	# of SAEs, GMTs

Risk of Bias Assessment: low risk of bias

Bias domain	**Questions to consider**	**Assessor´s judgement**	**Support for judgement**
Bias arising from the randomization process	Was the allocation sequence random? Was the allocation sequence concealed until participants were enrolled and assigned to interventions? Did baseline differences between intervention groups suggest a problem with the randomization process?	***Low risk of bias***	"Block (1:1 ratio, block size of 2) randomization was done at GSK Biologicals (SAS version 8). The code was released once databases had been monitored, checked for inconsistencies, and locked"
Bias due to deviations from the intended interventions	Were participants aware of their assigned intervention during the trial? Were carers and people delivering the interventions aware of participants' assigned intervention during the trial? Was an appropriate analysis used to estimate the effect of assignment to intervention?	***Low risk of bias***	"The vaccination team was not blinded but was not involved in any other study procedures. No other members of the trial team were aware of the which study vaccine any child received". Vaccines were masked for mothers.
Bias due to missing outcome data	Were data for this outcome available for all, or nearly all, participants randomized?	***Low risk of bias***	Intention-to-treat safety analysis including all children who received at least one dose. Outcome data numbers similar in both groups.
Bias in measurement of the outcome	Was the method of measuring the outcome inappropriate? Could measurement or ascertainment of the outcome have differed between intervention groups? Were outcome assessors aware of the intervention received by study participants?	***Low risk of bias***	Appropriate and well-described methods of measuring outcomes, similar for both groups.
Bias in selection of the reported result	Were the data that produced this result analyzed in accordance with a pre-specified analysis plan that was finalized before unblinded outcome data were available for analysis? Is the numerical results being assessed likely to have been selected, on the basis of the results, from… .. multiple eligible outcome measurements (e.g. scales, definitions, time points) within the outcome domain? ... multiple eligible analyses of the data? points) within the outcome domain? ... multiple eligible analyses of the data?	***Low risk of bias***	Well-described, per-protocol analyses at baseline, 1 month after 3rd dose and at later time points. "The analysis was based on a prospectively-defined report and analysis plan"

Study ID	Asante (2020)	Review ID	(23)	Assessor	SMD
Experimental	RTS,S/AS01	Comparator	Yellow Fever and MR vaccines only	Outcome	# of SAEs, GMTs

Risk of Bias Assessment: Some concerns

Bias domain	Questions to consider	Assessor´s judgement	Support for judgement
Bias arising from the randomization process	Was the allocation sequence random? Was the allocation sequence concealed until participants were enrolled and assigned to interventions? Did baseline differences between intervention groups suggest a problem with the randomization process?	***Low risk of bias***	"Participant allocation to a study group was performed using a centralized randomization system". "The randomization of supplies within blocks was performed with SAS". "The demographic charachteristics of the children included in the three study groups were similar"
Bias due to deviations from the intended interventions	Were participants aware of their assigned intervention during the trial? Were carers and people delivering the interventions aware of participants' assigned intervention during the trial? Was an appropriate analysis used to estimate the effect of assignment to intervention?	***Some concerns***	Open trial (participants and researchers know which treatment is being assigned). Appropriate and well-described analysis of estimated effect of assignment to intervention.
Bias due to missing outcome data	Were data for this outcome available for all, or nearly all, participants randomized?	***Low risk of bias***	Outcome data for all outcomes from almost all participants, similar numbers in the different groups.
Bias in measurement of the outcome	Was the method of measuring the outcome inappropriate? Could measurement or ascertainment of the outcome have differed between intervention groups? Were outcome assessors aware of the intervention received by study participants?	***Some concerns***	Appropriate and well-described methods of measuring outcomes, quite similar for all groups. Open trial, outcome assessors may be aware of the intervention received by study participants.
Bias in selection of the reported result	Were the data that produced this result analyzed in accordance with a pre- specified analysis plan that was finalized before unblinded outcome data were available for analysis? Is the numerical results being assessed likely to have been selected, on the basis of the results, from… .. multiple eligible outcome measurements (e.g. scales, definitions, time points) within the outcome domain? ... multiple eligible analyses of the data?	***Low risk of bias***	"Safety was assessed in the exposed set, which included all children who received at least one study treatment (study vaccine or Vitamin A). Immunogenicity results are presented for the per-protocol set for immunogenicity, which included all evaluable children meeting all eligibility criteria, complying with the protocol, and with no elimination criteria during the study"

Study ID	Bejon (2008)	**Review ID**	(48)	**Assessor**	SMD
Experimental	RTS,S/AS01E	**Comparator**	Rabies vaccine	**Outcome**	# of SAEs, GMTs

Risk of Bias assessment: low risk of bias

Bias domain	**Questions to consider**	**Assessor´s judgement**	**Support for judgement**
Bias arising from the randomization process	Was the allocation sequence random? Was the allocation sequence concealed until participants were enrolled and assigned to interventions? Did baseline differences between intervention groups suggest a problem with the randomization process?	***Low risk of bias***	"The database was managed by the sponsor and opened to the principal investigators at the time of unblinding" Appendix describing randomization and allocation. "The demographic characteristics of the participants were balanced between the two vaccine groups"
Bias due to deviations from the intended interventions	Were participants aware of their assigned intervention during the trial? Were carers and people delivering the interventions aware of participants' assigned intervention during the trial? Was an appropriate analysis used to estimate the effect of assignment to intervention?	***Low risk of bias***	"Boxes containing the assigned vaccine were opened out of sight of the investigators who evaluated the study end points, the study subjects and their parents. The syringe used to draw up the vaccine was masked"
Bias due to missing outcome data	Were data for this outcome available for all, or nearly all, participants randomized?	***Low risk of bias***	Outcome data for all outcomes from almost all participants, similar numbers in the different groups.
Bias in measurement of the outcome	Was the method of measuring the outcome inappropriate? Could measurement or ascertainment of the outcome have differed between intervention groups? Were outcome assessors aware of the intervention received by study participants?	***Low risk of bias***	Appropriate and well-described methods of measuring outcomes, similar for both groups.
Bias in selection of the reported result	Were the data that produced this result analyzed in accordance with a pre- specified analysis plan that was finalized before unblinded outcome data were available for analysis? Is the numerical results being assessed likely to have been selected, on the basis of the results, from… .. multiple eligible outcome measurements (e.g. scales, definitions, time points) within the outcome domain? ... multiple eligible analyses of the data?	***Low risk of bias***	"Analysis was performed in parallel by an industry author who is an employee of the sponsor and an academic author" "An analysis plan was agreed on by the data and safety monitoring board, sponsor, and investigators before the unblinding"

Study ID	Bojang (2005) A	Review ID	(16)	Assessor	SMD
Experimental	RTS,S/AS02A	Comparator	Rabies vaccine	Outcome	# of SAEs

Risk of Bias assessment: some concerns

Bias domain	Questions to consider	Assessor´s judgement	Support for judgement
Bias arising from the randomization process	Was the allocation sequence random? Was the allocation sequence concealed until participants were enrolled and assigned to interventions? Did baseline differences between intervention groups suggest a problem with the randomization process?	***Low risk of bias***	"[…] the screening log for all eligible subject was sent to a statistician not part of the clinical team evaluating the vaccine, from which subjects were randomly selected, sorted by descending age and pasted to the ascending study subject ID-numbers".
Bias due to deviations from the intended interventions	Were participants aware of their assigned intervention during the trial? Were carers and people delivering the interventions aware of participants' assigned intervention during the trial? Was an appropriate analysis used to estimate the effect of assignment to intervention?	***Low risk of bias***	"[…] trained field workers who were blinded to treatment allocation". The vaccines were "masked with tape and administered […] by a team of two nurses who played no part in the evaluation of safety and immunogenetic parameters"
Bias due to missing outcome data	Were data for this outcome available for all, or nearly all, participants randomized?	***Low risk of bias***	Outcome data for all outcomes from almost all participants, similar numbers in the different groups.
Bias in measurement of the outcome	Was the method of measuring the outcome inappropriate? Could measurement or ascertainment of the outcome have differed between intervention groups? Were outcome assessors aware of the intervention received by study participants?	***Low risk of bias***	Appropriate and well-described methods of measuring outcomes, similar for all groups. Outcome assessors were blinded to allocation of the subjects.
Bias in selection of the reported result	Were the data that produced this result analyzed in accordance with a pre- specified analysis plan that was finalized before unblinded outcome data were available for analysis? Is the numerical results being assessed likely to have been selected, on the basis of the results, from… .. multiple eligible outcome measurements (e.g. scales, definitions, time points) within the outcome domain? ... multiple eligible analyses of the data?	***Some concerns***	"Analysis of safety, reactogenicity and immunogenicity was conducted on both the Intention-to-treat cohort and the according-to-protocol cohorts"

Study ID	Bojang (2005) B	Review ID	(16)	Assessor	SMD
Experimental	RTS,S/AS02A	Comparator	Rabies vaccine	Outcome	# of SAEs

Risk of Bias assessment: some concerns

Bias domain	Questions to consider	Assessor´s judgement	Support for judgement
Bias arising from the randomization process	Was the allocation sequence random? Was the allocation sequence concealed until participants were enrolled and assigned to interventions? Did baseline differences between intervention groups suggest a problem with the randomization process?	***Low risk of bias***	"[…] the screening log for all eligible subject was sent to a statistician not part of the clinical team evaluating the vaccine, from which subjects were randomly selected, sorted by descending age and pasted to the ascending study subject ID-numbers".
Bias due to deviations from the intended interventions	Were participants aware of their assigned intervention during the trial? Were carers and people delivering the interventions aware of participants' assigned intervention during the trial? Was an appropriate analysis used to estimate the effect of assignment to intervention?	***Low risk of bias***	"[…] trained field workers who were blinded to treatment allocation". The vaccines were "masked with tape and administered […] by a team of two nurses who played no part in the evaluation of safety and immunogenetic parameters"
Bias due to missing outcome data	Were data for this outcome available for all, or nearly all, participants randomized?	***Low risk of bias***	Outcome data for all outcomes from almost all participants, similar numbers in the different groups.
Bias in measurement of the outcome	Was the method of measuring the outcome inappropriate? Could measurement or ascertainment of the outcome have differed between intervention groups? Were outcome assessors aware of the intervention received by study participants?	***Low risk of bias***	Appropriate and well-described methods of measuring outcomes, similar for all groups. Outcome assessors were blinded to allocation of the subjects.
Bias in selection of the reported result	Were the data that produced this result analyzed in accordance with a pre- specified analysis plan that was finalized before unblinded outcome data were available for analysis? Is the numerical results being assessed likely to have been selected, on the basis of the results, from… .. multiple eligible outcome measurements (e.g. scales, definitions, time points) within the outcome domain? ... multiple eligible analyses of the data?	***Some concerns***	"Analysis of safety, reactogenicity and immunogenicity was conducted on both the Intention-to-treat cohort and the according-to-protocol cohorts"

Study ID	Macete (2007)	**Review ID**	(45)	**Assessor**	SMD
Experimental	RTS,S/AS02A	**Comparator**	Hepatitis B (engerix)	**Outcome**	# of SAEs

Risk of Bias assessment: some concerns

Bias domain	**Questions to consider**	**Assessor´s judgement**	**Support for judgement**
Bias arising from the randomization process	Was the allocation sequence random? Was the allocation sequence concealed until participants were enrolled and assigned to interventions? Did baseline differences between intervention groups suggest a problem with the randomization process?	***Some concerns***	No information about randomization and allocation sequence, other than that the study was randomized. "The study groups at enrolment had similar baseline characteristics except for an imbalance in the sex ratio"
Bias due to deviations from the intended interventions	Were participants aware of their assigned intervention during the trial? Were carers and people delivering the interventions aware of participants' assigned intervention during the trial? Was an appropriate analysis used to estimate the effect of assignment to intervention?	***Low risk of bias***	"[…] the vaccination team was not involved in any other study procedures. All staff who participated in the evaluation phase of the trial remained blinded to vaccine assignment of each child" Vaccines were masked for participants and their parents.
Bias due to missing outcome data	Were data for this outcome available for all, or nearly all, participants randomized?	***Low risk of bias***	Outcome data for all outcomes from almost all participants, exact same numbers in the different groups.
Bias in measurement of the outcome	Was the method of measuring the outcome inappropriate? Could measurement or ascertainment of the outcome have differed between intervention groups? Were outcome assessors aware of the intervention received by study participants?	***Low risk of bias***	Appropriate and well-described analysis of the outcomes. Outcome assessors were blinded to the intervention assignment of the participants.
Bias in selection of the reported result	Were the data that produced this result analyzed in accordance with a pre- specified analysis plan that was finalized before unblinded outcome data were available for analysis? Is the numerical results being assessed likely to have been selected, on the basis of the results, from… .. multiple eligible outcome measurements (e.g. scales, definitions, time points) within the outcome domain? ... multiple eligible analyses of the data?	***Some concerns***	No information about whether the analysis was performed according to protocol.

Study ID	Owusu-Agyei (2009)	**Review ID**	(17)	**Assessor**	SMD
Experimental	RTS,S/AS01E	**Comparator**	Rabies vaccine	**Outcome**	# of SAEs, GMTs

Risk of Bias assessment: low risk of bias

Bias domain	Questions to consider	Assessor´s judgement	Support for judgement
Bias arising from the randomization process	Was the allocation sequence random? Was the allocation sequence concealed until participants were enrolled and assigned to interventions? Did baseline differences between intervention groups suggest a problem with the randomization process?	***Low risk of bias***	"Treatment numbers were assigned to vaccines with a randomization list generated using a standard SAS (Statistical Analysis System) programme" "Each [study] group was balanced for gender and age, overall and by study center"
Bias due to deviations from the intended interventions	Were participants aware of their assigned intervention during the trial? Were carers and people delivering the interventions aware of participants' assigned intervention during the trial? Was an appropriate analysis used to estimate the effect of assignment to intervention?	***Low risk of bias***	"Investigators involved in endpoint evaluation and parents/guardians were blinded to the vaccine administered, but not to the schedule". The vaccination team was not involved in any other part of the study.
Bias due to missing outcome data	Were data for this outcome available for all, or nearly all, participants randomized?	***Low risk of bias***	Outcome data for all outcomes from almost all participants, similar numbers in the different groups.
Bias in measurement of the outcome	Was the method of measuring the outcome inappropriate? Could measurement or ascertainment of the outcome have differed between intervention groups? Were outcome assessors aware of the intervention received by study participants?	***Low risk of bias***	Appropriate and well-described analysis of the outcomes. Outcome assessors were blinded to the intervention assignment of the participants, even if they knew the schedule.
Bias in selection of the reported result	Were the data that produced this result analyzed in accordance with a pre- specified analysis plan that was finalized before unblinded outcome data were available for analysis? Is the numerical results being assessed likely to have been selected, on the basis of the results, from… .. multiple eligible outcome measurements (e.g. scales, definitions, time points) within the outcome domain? ... multiple eligible analyses of the data?	***Low risk of bias***	"Analysis was carried out according to a DSMB approved report and analysis plan established before unblinding of trial data"

Study ID	RTS,S Clinical Trial Partnership (2011)	**Review ID**	(42)	**Assessor**	SMD
Experimental	RTS,S/AS01	**Comparator**	Meningococcal conjugate (6-12 weeks) or rabies vaccine	**Outcome**	# of SAEs, GMTs

Risk of Bias assessment: low risk of bias

Bias domain	**Questions to consider**	**Assessor´s judgement**	**Support for judgement**
Bias arising from the randomization process	Was the allocation sequence random? Was the allocation sequence concealed until participants were enrolled and assigned to interventions? Did baseline differences between intervention groups suggest a problem with the randomization process?	***Low risk of bias***	The information about allocation and randomization was described in a published article prior to the study. “Baseline demographic characteristics were similar in the two study groups”
Bias due to deviations from the intended interventions	Were participants aware of their assigned intervention during the trial? Were carers and people delivering the interventions aware of participants' assigned intervention during the trial? Was an appropriate analysis used to estimate the effect of assignment to intervention?	***Low risk of bias***	Study description was described and published in a previously published article. The analyses used to estimate the effect of assignment to intervention were appropriate.
Bias due to missing outcome data	Were data for this outcome available for all, or nearly all, participants randomized?	***Low risk of bias***	Outcome data were available for most participants, with good descriptions of the populations used to assess outcome, and data for why some were excluded.
Bias in measurement of the outcome	Was the method of measuring the outcome inappropriate? Could measurement or ascertainment of the outcome have differed between intervention groups? Were outcome assessors aware of the intervention received by study participants?	***Low risk of bias***	Appropriate methods of measuring the outcome that were the same in both groups. Outcome assessors were blinded to the participants` assigned intervention.
Bias in selection of the reported result	Were the data that produced this result analyzed in accordance with a pre- specified analysis plan that was finalized before unblinded outcome data were available for analysis? .. multiple eligible outcome measurements (e.g. scales, definitions, time points) within the outcome domain? ... multiple eligible analyses of the data?	***Low risk of bias***	The analysis plan was described in a published article prior to the study.

Study ID	Witte (2020)	**Review ID**	(18)	**Assessor**	SMD
Experimental	RTS,S/AS01E	**Comparator**	Hepatitis B (engerix)	**Outcome**	# of SAEs, GMTs

Risk of Bias assessment: some concerns

Bias domain	**Questions to consider**	**Assessor´s judgement**	**Support for judgement**
Bias arising from the randomization process	Was the allocation sequence random? Was the allocation sequence concealed until participants were enrolled and assigned to interventions? Did baseline differences between intervention groups suggest a problem with the randomization process?	***Some concerns***	"Treatment allocation was performed at the investigator site using a SAS programmed randomization list generated at GSK vaccines, Belgium. Randomization was not stratified, but ensured equal number distribution of subjects across treatment groups"
Bias due to deviations from the intended interventions	Were participants aware of their assigned intervention during the trial? Were carers and people delivering the interventions aware of participants' assigned intervention during the trial? Was an appropriate analysis used to estimate the effect of assignment to intervention?	***Some concerns***	"The study was a phase II, open, randomized controlled trial with 8 groups" There were no deviations from the intended intervention because of the trial context that were likely to have affected the outcome.
Bias due to missing outcome data	Were data for this outcome available for all, or nearly all, participants randomized?	***Low risk of bias***	Outcome data were available for most participants, with good descriptions of the populations used to assess outcome, and data for why some were excluded.
Bias in measurement of the outcome	Was the method of measuring the outcome inappropriate? Could measurement or ascertainment of the outcome have differed between intervention groups? Were outcome assessors aware of the intervention received by study participants?	***Low risk of bias***	The measurement methods of the outcomes were appropriate and well-described. Same methods for all study groups.
Bias in selection of the reported result	Were the data that produced this result analyzed in accordance with a pre- specified analysis plan that was finalized before unblinded outcome data were available for analysis? Is the numerical results being assessed likely to have been selected, on the basis of the results, from… .. multiple eligible outcome measurements (e.g. scales, definitions, time points) within the outcome domain? ... multiple eligible analyses of the data?	***Low risk of bias***	"All analyses were conducted according to a predefined analysis plan"

Appendix 3: Output of Egger test and Begg's test for small-study effects for RTS,S/AS01 studies

STATA output of Egger test and Begg's test for small-study effects for RTS,S/AS01 studies:

```
Regression-based Egger test for small-study effects
Random-effects model
Method: REML

H0: beta1 = 0; no small-study effects
            beta1 =      4.36
      SE of beta1 =     4.967
                z =      0.88
       Prob > |z| =    0.3806

. meta bias, begg

  Effect-size label: Mean diff.
        Effect size: _meta_es
          Std. err.: _meta_se

Begg's test for small-study effects

Kendall's score =      1.00
    SE of score =     5.323
              z =      0.00
     Prob > |z| =    1.0000
```

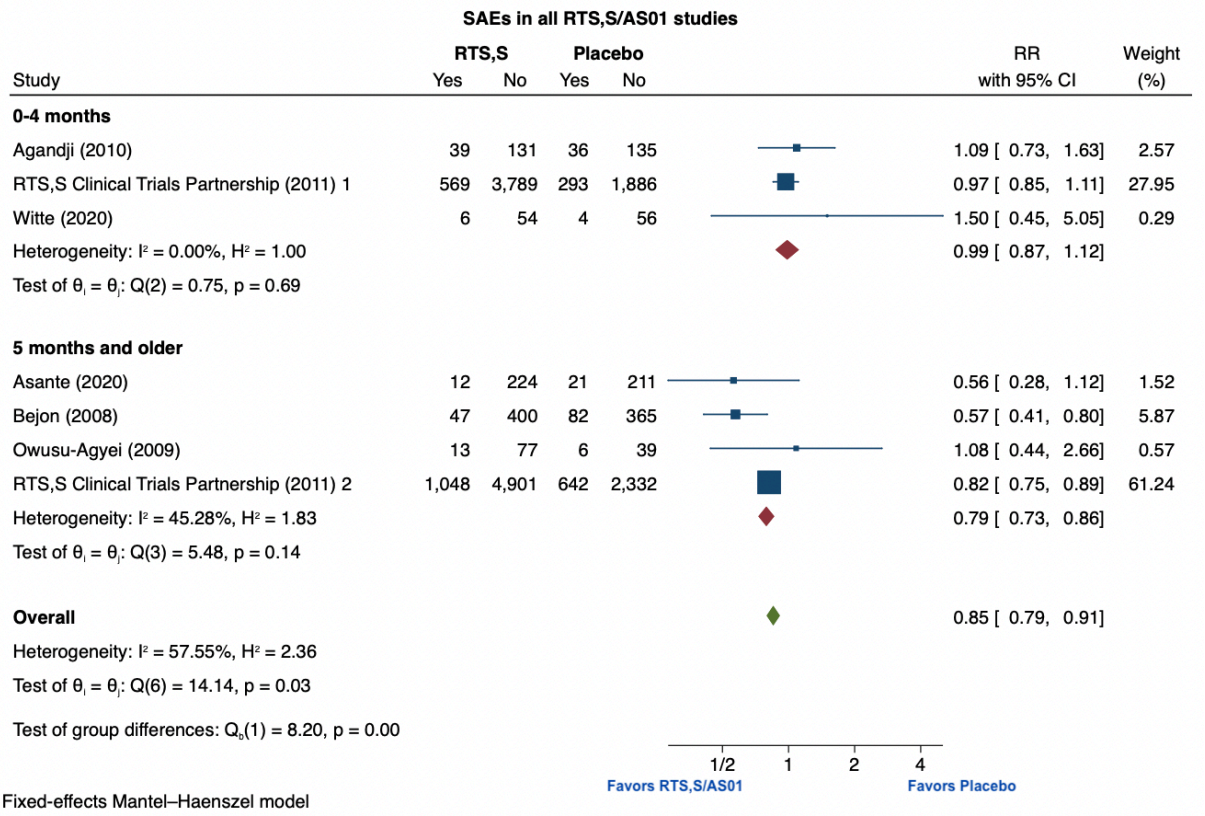
Appendix 4: Output of Egger test and Begg's test for small-study effects for RTS,S/AS02 studies

STATA output of Egger test and Begg's test for small-study effects for RTS,S/AS02 studies:

```
Regression-based Egger test for small-study effects
Random-effects model
Method: REML

H0: beta1 = 0; no small-study effects
            beta1 =      6.09
      SE of beta1 =     3.405
                z =      1.79
       Prob > |z| =    0.0736

. meta bias, begg

  Effect-size label: Mean diff.
        Effect size: _meta_es
          Std. err.: _meta_se

Begg's test for small-study effects

Kendall's score =      0.00
    SE of score =     2.944
              z =     -0.34
     Prob > |z| =    1.0000
```

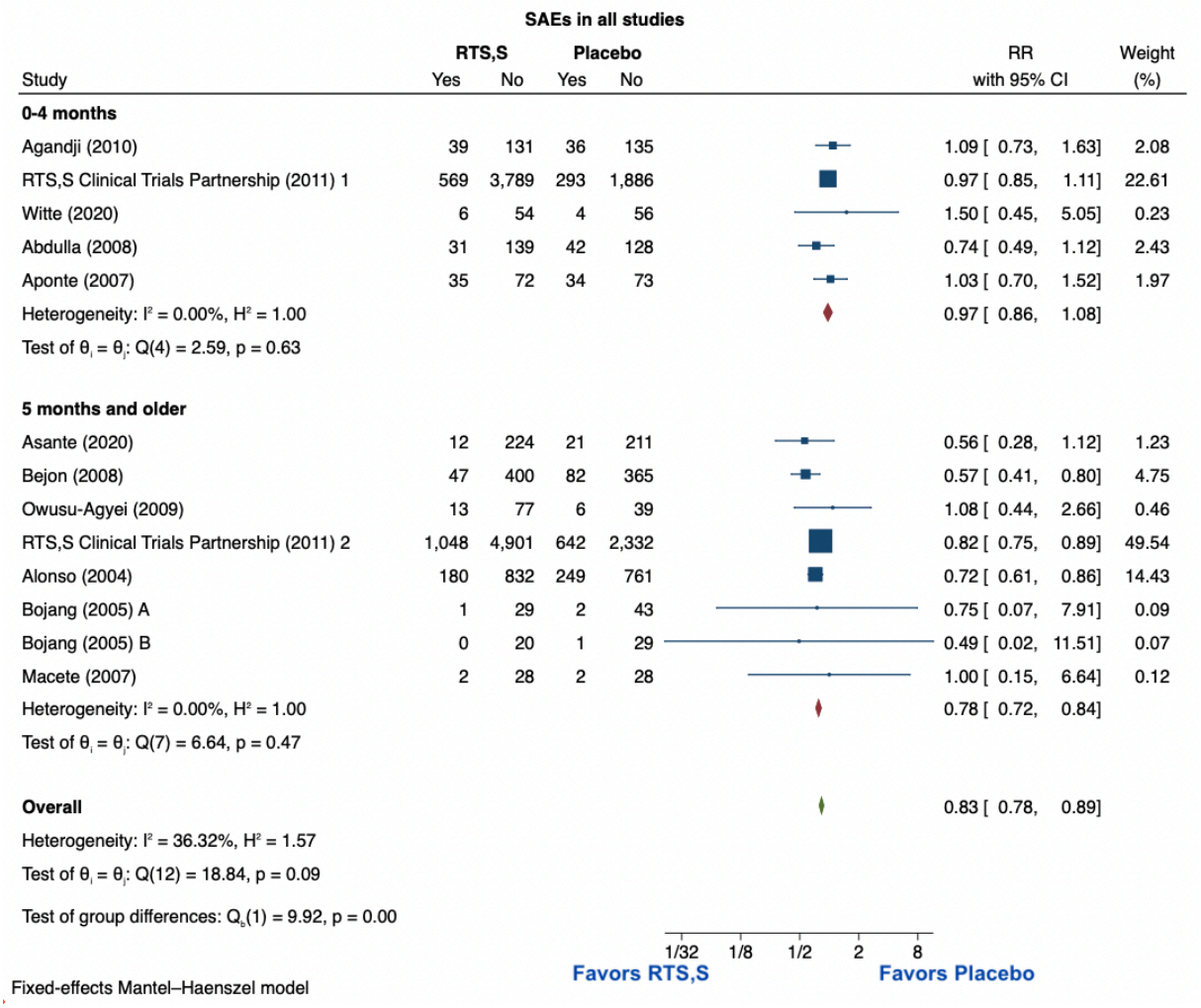
Appendix 5: Sensitivity analysis of SAEs in all RTS,S/AS01 studies

Forest plot from a meta-analysis of SAEs in all RTS,S/AS01-studies, including the study with a high risk of bias:



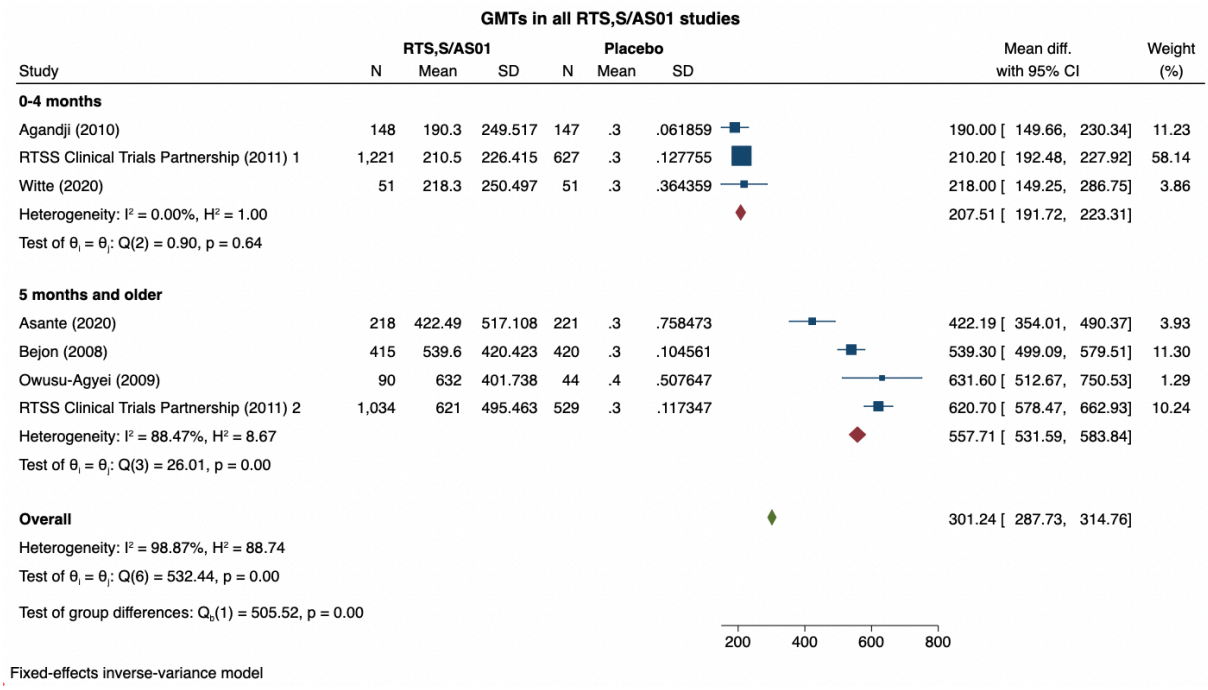
Appendix 6: Sensitivity analysis of SAEs in all studies

Forest plot from a meta-analysis of SAEs in all studies, without differing between RTS,S/AS01 and RTS,S/AS02:



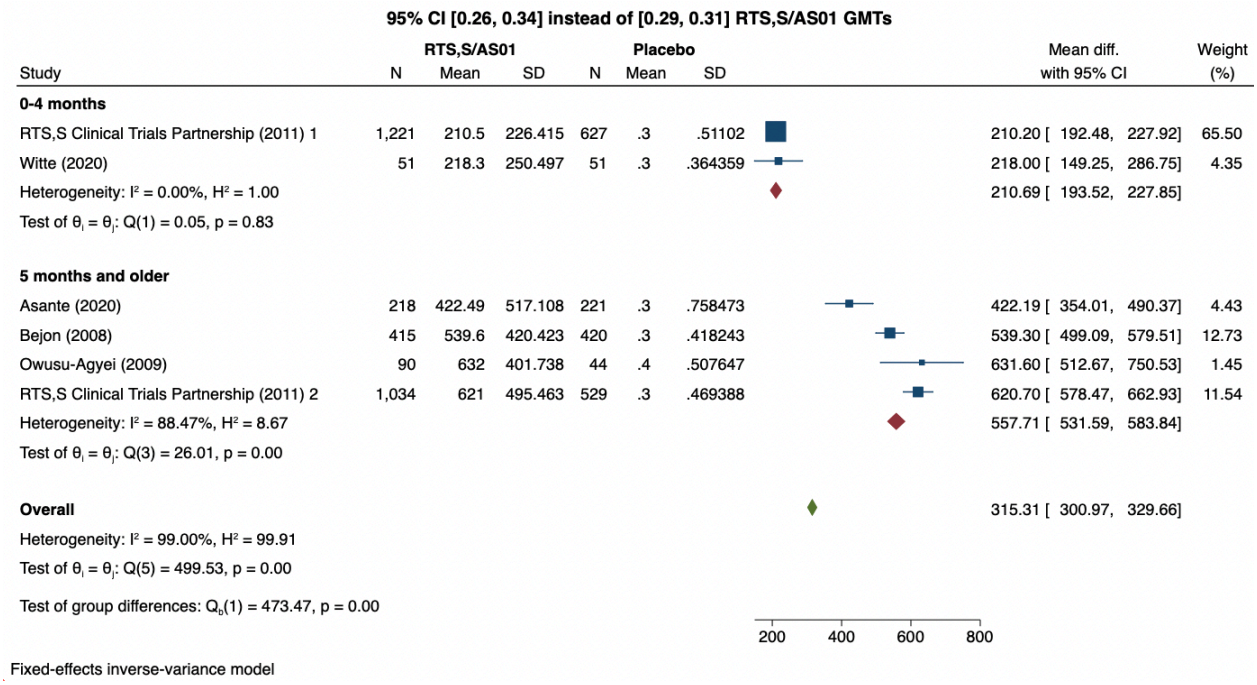
Appendix 7: Sensitivity analysis of GMTs in all RTS,S/AS01 studies

Forest plot from a meta-analysis of GMTs in all RTS,S/AS01-studies, including the study with a high risk of bias:



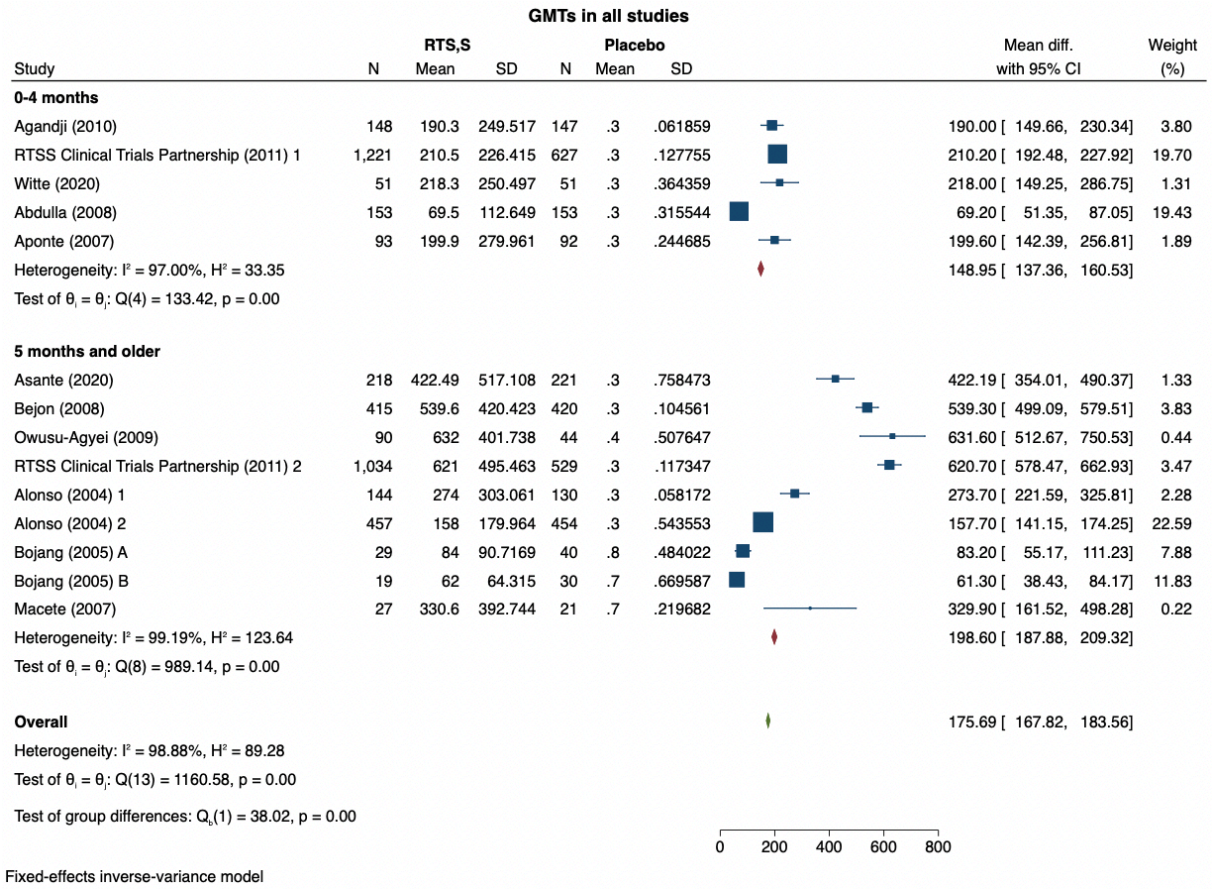
Appendix 8: Sensitivity analysis of GMTs in RTS,S/AS01 studies with a different 95% CI estimate

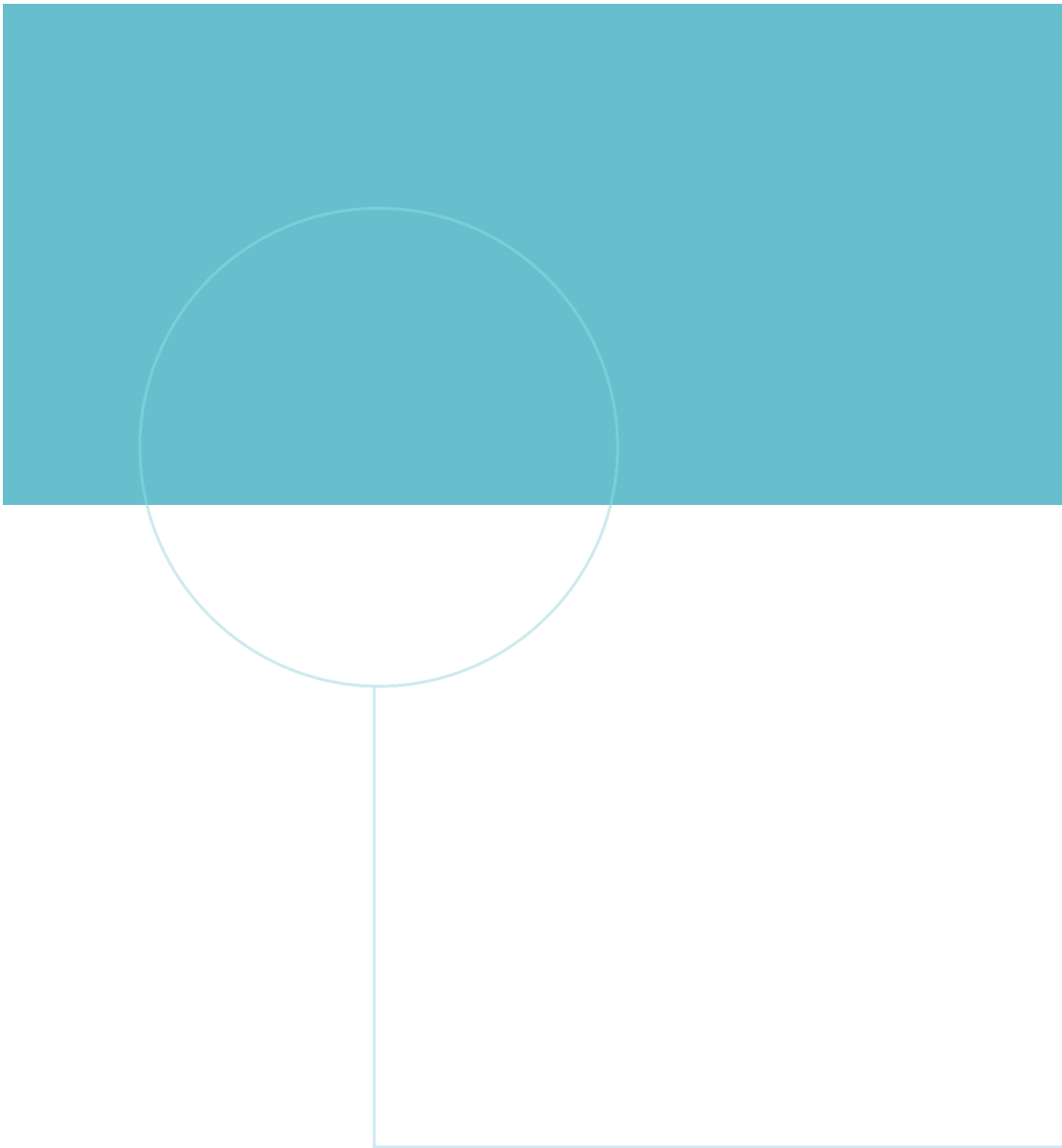
Forest plot from a meta-analysis of GMTs in the included RTS,S/AS01-studies, but with a 95% CI estimate of [0.26, 0.34] instead of [0.29, 0.31]:



Appendix 9: Sensitivity analysis of GMTs in all studies

Forest plot from a meta-analysis of SAEs in all studies, without differing between RTS,S/AS01 and RTS,S/AS02 (and including the RTS,S/AS01 study with a high risk of bias and the 3 RTS,S/AS02-studies that used another unit than the rest of the studies:





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