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HIDROXICLOROQUINA - SEGURANÇA EM GERAL E EFICÁCIA NA PREVENÇÃO DE EVENTOS TROMBOEMBÓLICOS.

TESE DE DOUTORADO

Salvador – Bahia

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Tese apresentada ao Programa de Pós-graduação *Strictu Sensu* em Medicina e Saúde Humana da Escola Bahiana de Medicina e Saúde Pública como requisito parcial para obtenção do título de Doutor em Medicina e Saúde Humana.

Orientador: Prof. Dr. Mittermayer Barreto Santiago
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Salvador, 11 de fevereiro de 2022.

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Esta tese é dedicada à minha família, cujo apoio incondicional e irrestrito se mostrou indispensável na sua conclusão.

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“It is not the strongest of the species that survives, not the most intelligent that survives. It is the one that is the most adaptable to change.”

— Charles Darwin

RESUMO

Introdução: A cloroquina (CLQ) e a hidroxicloroquina (HCLQ) foram desenvolvidas para o tratamento da malária, mas pesquisas demonstraram que ambas seriam eficazes também para outras doenças como artrite reumatoide (AR) e lúpus eritematoso. Durante a década de 1970, foi postulado que a HCLQ poderia ter um efeito antitrombótico e alguns estudos - observacionais e experimentais - avaliaram essa teoria, sendo que a maioria encontrou um efeito positivo. Recentemente, o uso generalizado da HCLQ como um potencial tratamento para COVID-19 e os relatos crescentes de arritmias cardíacas associadas ao seu uso, levantaram suspeitas sobre sua segurança. **Objetivos:** Os objetivos deste estudo foram avaliar a segurança da CLQ e da HCLQ e a potencial eficácia da HCLQ na prevenção de eventos tromboembólicos. **Métodos:** Realizamos duas revisões sistemáticas (RS) concebidas de acordo com os Itens de Relatório Preferenciais para Revisão Sistemática e Meta-análise (PRISMA) e seus protocolos foram registrados no banco de dados PROSPERO (CRD4202019793 e CRD42021247902). Dois autores na primeira RS e quatro na segunda, examinaram independentemente todos os registros obtidos por meio de nossa estratégia de busca e, posteriormente, revisaram a elegibilidade dos textos completos dos artigos selecionados, de acordo com nossos critérios de inclusão. Na primeira RS, incluímos apenas ensaios clínicos randomizados (ECRs) relatando eventos adversos (EA) em usuários de CLQ ou HCLQ durante o tratamento para lúpus, AR, malária e COVID-19. Na segunda RS, incluímos apenas ECRs relatando eventos tromboembólicos em usuários de HCLQ em comparação com não usuários. A qualidade dos estudos incluídos, em ambas as RS, foi avaliada através da ferramenta de risco de viés Cochrane e os dados relevantes extraídos por meio de formulários de coleta de dados personalizados, de forma independente, por pelo menos dois autores. A medida de efeito foi a razão da taxa de incidência (IRR) de EA, na primeira RS, e o risco relativo (RR) de eventos tromboembólicos, na segunda RS. Ambos as medidas de efeito foram estimadas usando um modelo de efeitos aleatórios agrupando os dados extraídos dos estudos individuais. Avaliamos a heterogeneidade em ambas as RS utilizando T^2 e I^2, realizamos análise de subgrupos estratificando os ECRs de acordo com critérios pré-estabelecidos, e o viés de publicação foi avaliado por inspeção do gráfico em funil em ambos os estudos. **Resultados:** Na primeira RS, incluímos 46 ECRs em nossa análise (23.132 pacientes). Nenhuma morte foi atribuída ao uso de CLQ ou HCLQ nos ECRs incluídos. A IRR dos EA gerais durante o uso de antimaláricos foi de 1,15 [IC 95% 1,01-1,31]. Os pacientes com COVID-19 tratados com qualquer um dos antimaláricos apresentaram risco 83% e 165% maior de desenvolver EA gerais e gastrointestinais, respectivamente, em comparação com os controles. O uso de antimaláricos aumentou o risco de desenvolver EA dermatológicos em 92% em estudos de malária e reduziu em 65% em estudos de lúpus. Não encontramos um risco significativamente maior de EA cardiovasculares ou oftalmológicos em usuários de antimaláricos. Na segunda RS, incluímos 13 ECRs em nossa análise (2.663 pacientes). A HCLQ reduziu o risco de eventos tromboembólicos em 49% (RR 0,51 [IC95% 0,31-0,84]) com uma heterogeneidade média (I^2 = 67% e T^2 = 0,4948). **Conclusões:** Nossos dados reforçam a ideia de que a CLQ e a HCLQ têm um bom perfil de segurança e que a HCLQ pode reduzir significativamente o risco de eventos tromboembólicos.

Palavras chaves: Hidroxicloroquina; Cloroquina; Trombose; Anticoagulantes; Revisão sistemática; Meta-análise; Efeitos adversos relacionados a medicamentos; Toxicidade por medicamentos.

ABSTRACT

Introduction: Chloroquine and hydroxychloroquine were both developed for the treatment of malaria, but research proved that both drugs could be efficacious for other diseases as rheumatoid arthritis and lupus erythematosus. During the 1970's, it was postulated that hydroxychloroquine could have an antithrombotic effect, some observational and experimental studies evaluated that theory, and the majority suggested a positive effect. Recently, the widespread use of hydroxychloroquine as a potential treatment for COVID-19 and the growing reports of cardiac arrythmias associated with its use, raised suspicions about its safety. **Objectives:** The primary objectives of this study were to evaluate chloroquine and hydroxychloroquine safety and the potential efficacy of hydroxychloroquine for the prevention of thromboembolic events. **Methods:** We conducted two systematic reviews (SR) conceived according to the Preferred Reporting Items for Systematic Review and Meta-analysis (PRISMA) and its protocols were registered in the PROSPERO database (CRD4202019793 and CRD42021247902). Two authors in the first SR and four in second SR independently screened all the records obtained through our search strategy and later revised the selected full-text articles for eligibility, according to our inclusion criteria. In the first SR, we included only randomized controlled trials (RCTs) reporting adverse events (AE) in chloroquine or hydroxychloroquine users during treatment for lupus, rheumatoid arthritis, malaria, and COVID-19. In the second SR we included only RCTs reporting thromboembolic events in hydroxychloroquine users compared to non-users. The quality of the included studies, in both SRs, was assessed using the Cochrane risk-of-Bias tool and relevant data were extracted though customized data collection forms, independently, by at least two authors. The summary effect was the incidence rate ratio (IRR) of AE, in the first SR, and the relative risk (RR) of thromboembolic events, in the second SR. Both summary effects we estimated using a random effects model pooling the data extracted from the individual studies. We evaluated heterogeneity in both SRs using T^2 and I^2, performed subgroup analysis stratifying the RCTs according to prespecified criteria, and assessed publication bias was by funnel-plotting in both studies. **Results:** In the first SR we found forty-six RCTs which met our eligibility criteria and included them in our analysis (23132 patients). Not a single death was attributed to chloroquine or hydroxychloroquine use in the included RCTs. The IRR of general AE during antimalarial use was 1.15 [CI 95% 1.01-1.31]. COVID-19 patients treated with either antimalarial presented an 83% and 165% higher risk of developing general and gastrointestinal AE, respectively, in comparison with controls. The use of antimalarial increased the risk of developing dermatological AE by 92% in malarial studies and reduced by 65% in lupus studies. We did not find a significatively higher risk of cardiovascular nor ophthalmological AE in antimalarial users. In the second SR, we selected thirteen RCTs which met our eligibility criteria and included them in our analysis (2663 patients). We found that hydroxychloroquine reduced the risk of thromboembolic events by 49% (RR 0.51 [IC95% 0.31-0.84]) with a medium heterogeneity (I^2=67% and T^2=0.4948). **Conclusions:** Our data reinforces that chloroquine and hydroxychloroquine have a good safety profile and that hydroxychloroquine may reduce the risk of thromboembolic events.

Keywords: Hydroxychloroquine; Chloroquine; Thrombosis anticoagulants; Systematic review; Meta-analysis; Drug-related side effects and adverse reactions; Drug toxicity.

LISTA DE ILUSTRAÇÕES

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LISTA DE ABREVIATURAS E SIGLAS

AR: Artrite reumatoide

ANVISA: Agência Nacional de Vigilância Sanitária

COVID-19: *Coronavirus disease*

CLQ: Cloroquina

EA: Eventos adversos

ECRs/RCTs: Ensaios clínicos randomizados / *Randomized Controlled Trials*

EMBASE: *Excerpta Medica database*

ENL: Eritema nodoso leprosum

EULAR: Aliança Europeia de associações para reumatologia

FDA: *Food and Drug Administration*

HCLQ: Hidroxicloroquina

IRR: Taxa de razão de incidência / *Incidence rate ratio*

LES: Lúpus Eritematoso Sistêmico

MEDLINE: Base de dados da biblioteca nacional de medicina (USA)

PRISMA: *Preferred Reporting Items for Systematic Reviews and Meta-Analyses*

RR: Risco Relativo

SAAF: Síndrome do anticorpo antifosfolípide

SARS-CoV-2: *severe acute respiratory syndrome coronavirus 2*

TVP: Trombose Venosa Profunda

SUMÁRIO

1 INTRODUÇÃO

O processo de encontrar novas indicações clínicas para drogas já existentes é conhecido como redirecionamento, reaproveitamento, reperfilamento ou reposicionamento. Essa estratégia tem ganhado holofotes nos últimos anos impulsionada por um desequilíbrio entre os investimentos bilionários da indústria farmacêutica em pesquisa e desenvolvimento de novas tecnologias - como a modelagem molecular, a química combinatória, a triagem de alto desempenho e a genômica – e a relativa escassez de lançamentos de novos produtos (1).

Além dos elevados custos com pesquisas para desenvolvimento de novas drogas, a indústria farmacêutica é desafiada pela pressão global por redução de preços, pela concorrência dos medicamentos genéricos e pelos obstáculos cada vez maiores impostos pelas agências regulatórias. Este cenário tem motivado a criatividade de pesquisadores em encontrar novas indicações ou criar versões melhores de drogas já existentes (1).

Dentre as vantagens no reposicionamento de uma droga estão o menor risco de fracasso e menores custos e tempo de desenvolvimento, já que estas drogas já foram submetidas a estudos pré-clínicos e ensaios clínicos iniciais (fase I e II). Como exemplos bem sucedidos de drogas reposicionadas, podemos citar o citrato de sildenafila que originalmente foi desenvolvido como anti-hipertensivo pela Pfizer e posteriormente reposicionado para tratamento de disfunção erétil, e a talidomida que originalmente era um sedativo e foi reposicionada com sucesso para tratamento do eritema nodoso leprosum (ENL) e do mieloma múltiplo (2).

A cloroquina (CLQ) e a hidroxicloroquina (HCQA) também são consideradas drogas reposicionadas. Ambas foram desenvolvidas originalmente para o tratamento da malária, e posteriormente reposicionadas com sucesso no tratamento da artrite reumatoide (AR) e do lúpus eritematoso – ambas indicações aceitas por agências reguladoras como o *Food and Drug Administration* (FDA) nos Estados Unidos e a Agência de Vigilância Sanitária (ANVISA) brasileira (3).

Outras indicações, embora não aceitas pelas agências reguladoras, têm sido testadas em ensaios clínicos e estudos retrospectivos na tentativa de expandir as indicações de uso clínico da CLQ e HCLQ. Dentre as indicações já testadas, podemos citar a prevenção de trombose (4–9); o tratamento de osteoartrite de mãos (10,11),

alguns tipos de câncer (12–14), a síndrome do anticorpo antifosfolípide (SAAF) (15–17), dislipidemias (18), síndrome de Sjögren (19–21), e mais recentemente o tratamento da síndrome respiratória aguda grave causada pelo coronavírus 2 (Sars-CoV-2)(22–31).

A tentativa de uso da HCLQ como droga anticoagulante teve início na década de 70, com dois estudos de Carter *et al.* demonstrando uma menor incidência de trombose venosa profunda (TVP) e tromboembolismo pulmonar em pacientes tratados com HCLQ durante o período pós-operatório (6,7). Com a falta de reprodutibilidade destes resultados por outros estudos e o surgimento da heparina de baixo peso molecular e dos anticoagulantes orais, as pesquisas sobre este tema diminuíram, porém a ideia de uma possível ação anticoagulante da CLQ e HCLQ persistiram (5,32).

A Aliança Europeia de associações para reumatologia (EULAR), em uma publicação oficial, recomenda o uso da HCLQ em pacientes com a forma obstétrica da SAAF – uma trombofilia de natureza autoimune – desde que a paciente seja refratária ao tratamento combinado de heparina em dose profilática e aspirina em baixas doses. O documento, todavia, chama atenção de que esta recomendação é baseada em apenas dois estudos observacionais pequenos e de limitada representatividade (33).

Na aprovação da CLQ pelo conselho para coordenação dos estudos sobre a malária em 1946, foi concluído que a CLQ, nas doses utilizadas para o tratamento da malária, era uma droga segura, e os efeitos adversos relacionados ao seu uso - cefaleia, distúrbios visuais, prurido e queixas gastrointestinais – eram leves e reversíveis (34). Com a indicação do uso da CLQ para lúpus e AR, com doses diárias e mais elevadas, novos estudos começaram a sugerir uma maior incidência de eventos adversos, em especial a retinopatia, e já indicavam a monitorização periódica do fundo de olho em usuários crônicos da droga (35,36). Percival & Behrman demonstraram que a retinopatia associada ao uso da CLQ seria reversível, desde que fosse reconhecida precocemente e a droga interrompida de forma imediata e definitiva (37). Durante muitos anos, a CLQ e a HCLQ foram consideradas drogas seguras e o efeito colateral mais temido, embora não o mais frequente, era a retinopatia associada ao seu uso (38).

Semelhante a quinina (e o seu estereoisômero, a quinidina), a CLQ e a HCLQ, possuem propriedades inotrópicas e cronotrópicas negativas, o que justifica a ocorrência, embora rara e normalmente associada a “overdose” (em tentativas de suicídio), de cardiotoxicidade (39). Em 2018, Chatre *et al.* revisaram sistematicamente - pela primeira vez - as complicações cardíacas associadas ao uso de CLQ e HCLQ e concluíram que em geral ambas são drogas seguras, todavia raramente podem levar a manifestações cardíacas graves e potencialmente fatais (como bloqueios cardíacos e hipertrofia miocárdica)(40). Com a pandemia do Sars-CoV-2 e o uso em larga escala da HCLQ, reposicionada como potencial tratamento para doença, um novo debate emergiu acerca da sua segurança, principalmente cardiovascular (41).

Considerando a grande quantidade de estudos realizados ao longo dos quase 80 anos de uso da CLQ e HCLQ (sendo alguns inclusive com resultados conflitantes), a ausência de evidências científicas robustas sobre o efeito anticoagulante e da dúvida acerca da segurança do uso da CLQ e HCLQ, nós decidimos estudar ambos os temas utilizando para tanto a metodologia da revisão sistemática e submetendo os dados numéricos obtidos a um tratamento meta-analítico.

2 OBJETIVOS

2.1 Objetivos do artigo 1

2.1.1 Primário

Estimar a incidência total de efeitos adversos relacionados ao uso de CLQ e HCLQ em pacientes portadores de lúpus eritematoso, malária, AR e COVID-19.

2.1.2 Secundários

Estimar a incidência dos efeitos adversos categorizados em cardiovasculares, gastrointestinais, dermatológicos, neurológicos e oftalmológicos.

Avaliar se variáveis como doença de base, antimalárico utilizado (CLQ ou HCLQ), dose utilizada, uso do antimalárico em monoterapia ou associado a outras drogas, tempo de seguimento, tratamento ambulatorial ou hospitalar, influenciariam significativamente a incidência de eventos adversos.

Avaliar se a qualidade dos estudos (aferida através da ferramenta de análise de risco de viés da Cochrane) influenciaria significativamente a incidência de eventos adversos.

2.2 Objetivos do artigo 2

2.2.1 Primário

Estimar o risco relativo (RR) de eventos tromboembólicos em usuários de CLQ e HCLQ em relação aos controles.

2.2.2 Secundários

Avaliar se variáveis como doença de base (SAAF, pós-operatório ou COVID-19) influenciariam significativamente o RR de eventos tromboembólicos em usuários de CLQ e HCLQ em comparação aos controles.

Avaliar se a qualidade dos estudos (aferida através da ferramenta de análise de risco de viés da Cochrane) influenciaria significativamente a incidência de eventos adversos.

3 REVISÃO DA LITERATURA

3.1 Indicações novas para drogas antigas – Reposicionamento

Apesar de todos os investimentos bilionários da indústria farmacêutica em pesquisa básica, desenvolvimento de novas tecnologias e experimentações com novas estruturas organizacionais e de gerência, o desenvolvimento de um novo medicamento continua longo (10 a 15 anos) e extremamente caro (algo entre 500 milhões a 2 bilhões de dólares) (42). Estudos apontam que estes custos, que já eram bastante elevados, vem subindo significativamente mais rápido que a inflação global e já mais que dobraram quando comparamos a década de 1990 com a década de 1980, enquanto isso o número de novas drogas aprovadas (pelo FDA) segue o caminho inverso – vem diminuindo (43).

Se somarmos ao problema de custo e tempo, questões como as incertezas sobre o desenvolvimento de uma nova droga, as exigências – cada vez maiores - das agências regulatórias, a concorrência com indústrias dos medicamentos genéricos e as pressões globais para redução e controle de preços de medicamentos, podemos ter uma pequena noção do tamanho deste problema (43). Uma das soluções que a indústria farmacêutica tem empregado para minimizar essas dificuldades é o reposicionamento (44,45).

Reposicionamento, redirecionamento, reaproveitamento ou reperfilamento são sinônimos do processo de encontrar novos usos - fora do escopo da indicação clínica original - para uma droga já existente. Essa estratégia permite diminuir o tempo de desenvolvimento do medicamento, por conseguinte os custos, sem aumentar riscos (principalmente sobre a segurança), já que estudos iniciais (pré-clínicos, fases 1 e 2) já terão sido finalizados e ao menos a certeza sobre a segurança e perfil farmacológico da droga já estará assegurada. Além disso, os processos de manufatura muito provavelmente já terão sido estruturados – ou estarão em fases finais de estruturação - quando possíveis novas indicações para uma droga já em uso sejam propostas. Colocando em números, para se ter uma melhor perspectiva da economia deste processo, o reposicionamento reduz o processo de descoberta e desenvolvimento de uma nova droga que normalmente levaria em média 10 a 17 anos para algo em torno de 3 a 12 anos (1).

A droga duloxetina, um inibidor da recaptação da serotonina e noradrenalina, foi descoberta no início da década de 1980 como um potencial sucessor da fluoxetina,

um antidepressivo de extremo sucesso ao redor do mundo. Durante o desenvolvimento da droga, foi postulado que essa classe de medicações aumentaria o tônus do esfíncter uretral e diminuiria a atividade do músculo detrusor, levando a um aumento da resistência uretral e protegendo contra incontinência urinária. Estudos pré-clínicos demonstraram que a duloxetina potencializava os efeitos excitatórios da serotonina e noradrenalina nos neurônios motores do esfíncter urinário, e posteriormente um ensaio clínico randomizado (ECR) confirmou que a duloxetina era efetiva no tratamento da incontinência urinária de estresse (46,47).

A talidomida foi comercializada na Inglaterra e Alemanha entre 1957 e 1961 como um sedativo direcionado especificamente para o tratamento do enjoo matutino das grávidas. Na época, não era necessária a aprovação por agências regulatórias e a droga foi vendida como “totalmente segura”, o que levou a mais de 15.000 crianças, filhas de mães que utilizaram a talidomida no primeiro trimestre da gestação, a nascerem com malformações esqueléticas. Em 1964, o médico Jacob Sheskin, desesperado em encontrar um tratamento para um paciente que não dormia há semanas por conta das dores causadas pelo ENL, utilizou a única droga disponível na enfermaria do hospital que achou que poderia ter algum efeito, a talidomida (48). A droga não só curou as lesões do paciente como eliminou suas dores, o que motivou Sheskin a conduzir um ECR na Venezuela com 173 pacientes, dos quais 92% apresentaram melhora dos sintomas do ENL (49).

A sildenafila, um medicamento inibidor da fosfodiesterase-5, foi originalmente desenvolvido com o objetivo de relaxar as artérias coronárias, aumentando seu fluxo sanguíneo, o que o indicaria como tratamento para insuficiência coronariana (angina). Durante testes iniciais em voluntários sadios estes resultados não foram observados, porém foram relatas pelos participantes a ocorrência de ereções estranhamente fortes e duradouras (1). Rapidamente um estudo piloto foi realizado em pacientes com disfunção erétil sem causa orgânica definida, demonstrando a eficácia da sildenafila no seu tratamento (50). ECRs seguiram este estudo e ratificaram os resultados de eficácia e segurança da medicação (51,52). No ano de 2001, quando o Viagra (sildenafila) era o único medicamento aprovado pelo FDA para o tratamento oral da disfunção erétil nos Estados Unidos, a Pfizer chegou a faturar 1,5 bilhões de dólares (53).

A CLQ e a HCLQ, ambas drogas desenvolvidas originalmente para o tratamento da malária, vêm sendo reposicionadas ao longo dos anos como potenciais tratamentos para outras condições clínicas. Algumas indicações, como no caso do lúpus eritematoso e da AR, tem aprovação inclusive de agências regulatórias como o FDA nos Estados Unidos e a ANVISA no Brasil.

3.2 Origens da CLQ e HCLQ

O médico italiano Sebastiano Bado, em sua obra “Anastasis corticis Peruviae seu china china defensio”, conta a história da esposa do vice-rei do Peru, a condessa de Chinchón, que fora acometida no ano de 1631 por uma febre terçã (malária). Na época, a doença não tinha tratamento, e o vice-rei aguardava pelo final trágico de sua esposa. De acordo com a história, um padre jesuíta lhe trouxera então, o pó de uma árvore com propriedades medicinais conhecida pelos indígenas locais como “árvore da febre”, que crescia na região da Loja (atualmente, Equador) e cuja casca tinha cor de canela. A condessa de Chinchón, supostamente tratada com o pó desta planta, fora milagrosamente curada da malária e estendera o tratamento para outros enfermos da região, para a Espanha e o restante da Europa. Inspirado nesta história, considerada apócrifa por alguns, o botânico sueco Carl Lineu batizou, em 1742, esse grupo de plantas como *Chinchonaceas* (54,55).

Até a metade do século 17, os europeus chamavam erroneamente essa planta milagrosa de “quinquina”, uma confusão dos colonizadores espanhóis e outros europeus com outra planta chamada pelos indígenas peruanos de “quina-quina”. Essa confusão persiste até hoje, e em muitas línguas – inclusive na língua portuguesa - o nome da “árvore que cura malária” continua sendo “quina” (56).

As propriedades medicinais (antimaláricas) da quina foram reconhecidas pelos jesuítas que distribuíram o pó de sua casca por toda Europa, chegando inclusive a publicar anúncios em jornais da época. Antes dela, a malária era tratada com remédios não convencionais como olhos de caranguejo e até sangue de gato (57). Todavia, diferentes espécies da árvore produziam diferentes resultados terapêuticos (58). A solução para essa inconsistência veio de dois professores da Escola de Farmácia em Paris, – um de toxicologia (Joseph Bienaimé Caventou) e outro de química (Pierre Joseph Pelletier) – que isolaram da casca da quina uma substância alcaloide com propriedades antimaláricas que foi batizada de quinina (derivada do nome quina) (56).

Essa descoberta pavimentou o caminho para a posterior definição de uma dose adequada para o tratamento da malária (57).

A produção mundial da casca da quina até o século 19, era proveniente exclusivamente da América do Sul, notadamente do Peru e Bolívia. Essa exclusividade no cultivo da árvore gerava imprevisibilidade na oferta do produto diante de uma demanda mundial extremamente elevada. Por conta deste cenário, os ingleses tentaram o seu cultivo na região da Índia oriental enquanto os holandeses na região da ilha de Java (Indonésia). Ao final da década de 1930, 90% da demanda mundial da casca da quina já era suprida por Java (59).

O sucesso do tratamento da malária com a quinina, motivou pesquisadores a avaliarem sua eficácia ante outras condições clínicas. Em 1894, Payne descreveu com êxito o tratamento de lesões cutâneas em pacientes portadores de lúpus eritematoso com uso de quinina. Na época, ele acreditava que o lúpus era uma condição vascular, e diante da palidez observada em pacientes tratados com altas doses da quinina, ele postulou que a substância poderia ser eficaz no tratamento da enfermidade (60). Estes resultados foram posteriormente confirmados em 1938 por Davidson (61).

Embora Payne (60) tenha sido pioneiro no tratamento de pacientes com doenças reumáticas com antimaláricos, foi o trabalho de Page (62) que ganhou maior notoriedade – por ter sido o primeiro escrito na língua inglesa – ao descrever uma série de 18 pacientes com lúpus discoide, sendo dois portadores de AR e um de lúpus eritematoso sistêmico, que apresentaram melhora das lesões de pele e da inflamação articular, com uso de quinacrina. Iniciava-se então, um dos dogmas no tratamento do lúpus eritematoso, que perdura até os dias atuais.

Em 1918, Frey notou que pacientes portadores de fibrilação atrial e em uso de quinina para tratamento de malária, evoluíam com normalização do seu ritmo cardíaco. A quinina foi então aprovada pela agência regulatória americana, FDA, para tratamento de arritmias cardíacas e comercializada pela empresa farmacêutica Lilly (58).

A quinina influenciou profundamente não somente a medicina, mas toda a história mundial. Ela viabilizou que missionários, exploradores, colonizadores e militares viajassem e vivessem em áreas onde a malária era endêmica (58). Sua importância para o campo da medicina pode ser exemplificada por sua eleição por

médicos no ano de 1910 como a sétima droga mais importante da época, sendo que em 1945 foi alçada a terceira posição neste ranking, perdendo apenas para penicilina e sangue e seus derivados (63).

Apesar da sua reconhecida eficácia no tratamento da malária, os efeitos adversos e a curta duração de ação, faziam da quinina um agente profilático medíocre. Por conta disso, pesquisas na tentativa de se encontrar um medicamento alternativo à quinina, começavam a surgir. Em 1891, Paul Ehrlich – cientista alemão pioneiro nas pesquisas de antimaláricos sintéticos – observou que o corante azul de metileno era capaz de não só corar, mas de matar o plasmódio. Dois pacientes chegaram a ser curados da malária com este corante, porém sua alta toxicidade não permitiu ser utilizado em larga escala como alternativa a quinina (58).

O período das guerras motivou avanços importantes nas pesquisas em busca de um sucessor para quinina, já que a malária era um inimigo comum a todas as tropas – aliadas e inimigas – e o uso de uma medicação profilática contra a doença era uma arma indispensável. Durante a primeira guerra mundial, como os países produtores de quinina faziam parte da aliança antinazista, era previsto o aumento do custo e desabastecimento da droga na Alemanha. Este cenário impulsionou empresas alemãs no desenvolvimento de um agente antimalárico sintético, sendo formado um conglomerado de empresas químico-farmacêuticas - chamado “IG Farbenindustrie”.

A Bayer – empresa alemã produtora de corantes e participante do “IG Farbenindustrie” – se transformou em uma empresa farmacêutica pioneira em pesquisas de agentes antimaláricos, utilizando o azul de metileno como protótipo (58). Como frutos deste empenho, surgem os primeiros antimaláricos sintéticos: plasmoquina (1925), atabrina (1932), resoquina (1934) e a sontoquina (1936) (64). Na época, os testes com estas medicações eram realizados em pacientes paralíticos, internados em hospitais psiquiátricos e inoculados com o *plasmodium vivax*. Estes testes demonstraram a eficácia e a menor toxicidade da atabrina em relação às demais drogas (65). A atabrina, posteriormente sintetizada também nos Estados Unidos, foi usada compulsoriamente pelos soldados americanos na segunda guerra mundial e considerado como um importante fator na vitória dos aliados (58).

Durante a segunda guerra mundial - no ano de 1942 - a ilha de Java (atualmente Indonésia), maior produtor global do substrato da quinina, foi invadida pelo Japão e os países aliados privados de sua produção. Por conta destes eventos,

os Estados Unidos - através de uma aliança entre governo, universidades e indústria – operacionalizaram um esquema de cooperação na promoção de pesquisas para o desenvolvimento de novos agentes antimaláricos. Através desta iniciativa, três novas drogas foram desenvolvidas: CLQ, amodiaquina e, eventualmente, a primaquina (64).

Curiosamente, a CLQ era quimicamente igual a resoquina, desenvolvida anos atrás pela Bayer e patenteada nos Estados Unidos em 1941 pela empresa Winthrop Chemical Company – que operava juridicamente como uma empresa norte-americana, mas fabricava os produtos da Bayer – todavia, as pesquisas com estes medicamentos não avançaram em virtude da toxicidade observada nos estudos iniciais conduzidos na Alemanha. Porém, durante a segunda guerra mundial, alemães e franceses, realizaram novos testes ao norte da África sobre o potencial antimalárico da resoquina e da sontoquina. Somente em 1943, quando a Tunísia foi capturada por forças aliadas, um carregamento de sontoquina (uma versão metilada da resoquina) – que em pesquisas locais demonstrou excelente ação antimalárica – foi entregue às forças americanas e o medicamento finalmente foi reconhecido pelos coordenadores da comissão americana para desenvolvimento de novos antimaláricos. Surpreendentemente, anos depois, os americanos desenvolveram um composto sintético SN-7618, mais eficaz e seguro que a sontoquina, que foi batizado de CLQ. A nova droga foi amplamente distribuída ao redor do mundo e sua produção retomada na Alemanha - pela Bayer - sob o nome comercial de resoquina com o princípio ativo CLQ (66).

No início da década de 50, surgiram os primeiros estudos controlados evidenciando a eficácia do uso da CLQ em pacientes portadores de AR (67–69). Nesta mesma década, Alexander R. Surrey e Henry F. Hammer publicaram a descoberta de um novo composto antimalárico, na época chamado de 7-cloro-4-(4-(N-etil-N-β-hidroxi-etileno-amino)-1-metilbutilamina) quinolina difosfato, e mais tarde batizada de HCLQ (70). Loughlin *et al.* foram os primeiros a demonstrar a eficácia deste novo antimalárico no Haiti, ao tratar 75 pacientes acometidos pelo *plasmodium falciparum* em dose única com apenas um paciente queixando de vertigem leve após seu uso (71). Nos anos seguintes estudos controlados reafirmaram a eficácia da HCLQ no tratamento da malária, assim como da AR e do lúpus eritematoso (38).

3.3 Efeitos hematológicos da CLQ e HCLQ

Knisely *et al.* foram os primeiros a descrever um fenômeno inespecífico intravascular em capilares de cinco macacos *rhesus* infectados com *plasmodium knowlesi*. Os pesquisadores observaram, através de transiluminação com haste de quartzo, que os eritrócitos infectados pelo protozoário se tornavam cobertos por uma substância semelhante a fibrina, que os tornava aderentes uns aos outros – mas não à parede do endotélio – levando a formação de aglomerados eritrocitários que eram instantaneamente fagocitados pelos macrófagos hepáticos. Com o passar do tempo a viscosidade plasmática aumentava e a fagocitose dos aglomerados era reduzida ou não mais ocorria. Estes achados alteravam o fluxo laminar do sangue, aumentando o trabalho do coração e reduzindo – ou até mesmo interrompendo - o fluxo sanguíneo para todos os tecidos (72). Posteriormente esses achados foram observados também em humanos com diferentes condições clínicas como trauma, infecções, doenças metabólicas, reações de hipersensibilidade, doenças do colágeno, neoplasias e fenômenos idiopáticos (73).

Esse fenômeno ficou conhecido como "*sludging*" (formação de lama). Após observações seriadas em macacos infectados com o plasmódio e posteriormente tratados com antimaláricos, Knisely & Bloch, demonstraram a redução importante e até mesmo o desaparecimento do "*sludging*" que se correlacionava com melhora clínica significativa dos animais (73).

Com esses resultados, Maddow *et al.* postularam que os efeitos circulatórios dos antimaláricos se estenderiam a outras condições clínicas, além da malária. Eles avaliaram 44 pacientes com variadas doenças vasculares – doença arterial coronariana, doença cerebrovascular, tromboflebite, claudicação intermitente e anemia falciforme – e observaram que a HCLQ promoveu alterações circulatórias visíveis à conjuntiva bulbar, como aumento da velocidade do fluxo sanguíneo, redução do número de vasos obstruídos por aglomerados eritrocitários e a redução do tamanho desses aglomerados (74). No ano seguinte, Cecchi & Ferraris demonstraram a existência do fenômeno de "*sludging*" nas veias retinianas de 22 pacientes portadores de AR, e o completo desaparecimento deste em 20 pacientes após 2-8 semanas de tratamento com HCLQ (75).

Ernst *et al.* conduziram um estudo controlado e documentaram uma redução na viscosidade sanguínea em um grupo de 20 pacientes tratados com HCLQ 48 horas antes da realização de um procedimento cirúrgico (76).

Os efeitos hematológicos dos antimaláricos não parecem se limitar somente às alterações da velocidade de fluxo ou da viscosidade sanguínea. Jančinová *et al.*, em um modelo experimental com ratos, demonstraram que a CLQ era capaz de inibir a agregação plaquetária, sendo capaz, inclusive, de induzir uma “desagregação” de plaquetas estimuladas com ADP (adenosina difosfato) (77). Prowse *et al.* observaram em humanos que a exposição a antimaláricos era capaz de inibir a liberação de beta-tromboglobulina e fator 4 pelas plaquetas, evitando sua ativação e formação de trombos (78).

Bertolaccini *et al.* observaram, em um modelo experimental de SAAF obstétrica em ratos, que a HCLQ era capaz de inibir a ativação do complemento, evitando a insuficiência placentária e o desenvolvimento anormal do córtex cerebral do feto (79). Nuri *et al.* conduziram um estudo de coorte retrospectivo e demonstraram uma redução significativa nos títulos de anticorpos anticardiolipina IgG/IgM e anti-beta-2 glicoproteína I no grupo de pacientes expostos a HCLQ. Os autores também encontraram uma redução significativa na recorrência de eventos trombóticos arteriais nos pacientes com SAAF primária tratados com HCLQ (80).

3.4 HCLQ na prevenção de eventos tromboembólicos

Em 1971, Carter *et al.* decidiram testar se a capacidade de impedir a aderência de eritrócitos demonstrada pela HCLQ, poderia ser estendida às plaquetas, evitando assim a formação de trombos, sem aumentar o risco de sangramentos. Os pesquisadores randomizaram um total de 565 pacientes no período pós-operatório de procedimentos cirúrgicos variados, para tratamento com HCLQ ou controle, e observaram uma incidência significativamente menor de eventos tromboembólicos nos pacientes tratados com HCLQ (6). Esses achados foram repetidos posteriormente pelos mesmos autores (7). Chrisman *et al.* obtiveram resultados semelhantes ao analisar pacientes tratados com HCLQ no pós-operatório de cirurgias ortopédicas ou após fraturas. Os autores chegaram a sugerir que a HCLQ deveria ser utilizada em pacientes com história de sangramento digestivo ou alergia a aspirina para prevenção de trombose venosa profunda e tromboembolismo pulmonar durante o pós-operatório de cirurgias ortopédicas (4). Embora alguns estudos não tenham conseguido

demonstrar a eficácia da HCLQ em monoterapia ou em associação com aspirina na redução de eventos trombóticos (5,32), a droga chegou a ser padronizada como terapia de escolha para prevenção de trombose após cirurgias de quadril em um grande centro de referência britânico para realização de cirurgias ortopédicas (81).

Em 1987, Wallace, analisou uma coorte de 92 pacientes ambulatoriais portadores de lúpus eritematoso sistêmico (LES) e observou uma relação inversa entre a ocorrência de eventos tromboembólicos e o uso de HCLQ (82). Em uma revisão sistemática publicada em 2010 com dados de estudos observacionais, Ruiz-Irastorza *et al.* concluíram que existiam evidências científicas moderadas de que a HCLQ teria um efeito protetor contra trombose nos pacientes portadores do LES (83). Petri *et al.* em um estudo prospectivo publicado em 2021, observaram que concentrações séricas mais elevadas de HCLQ estavam associadas a um menor risco de eventos tromboembólicos em pacientes lúpicos (84).

Em 1997, Edwards *et al.* desenvolveram um modelo experimental murino da SAAF e demonstraram que nestes animais, o tratamento com HCLQ reduzia significativamente o tamanho dos trombos e o tempo de persistência destes (85). Cinco anos mais tarde, um estudo de corte transversal em humanos sugeriu uma associação entre o uso de HCLQ e diminuição de eventos trombóticos em pacientes com a SAAF (86). Resultados semelhantes foram encontrados em um estudo piloto aberto e randomizado conduzido por Kravvariti *et al.* em pacientes portadores de SAAF primária. Os autores encontraram uma redução de 85% na taxa de incidência de trombose em pacientes que fizeram uso da HCLQ (16).

3.5 Segurança do uso da CLQ e HCLQ

Uma das razões que privilegiaram a HCLQ e a CLQ para o tratamento da malária e as demais doenças, além da sua efetividade foi o seu perfil de segurança. Inicialmente, com doses semanais de 500 mg de CLQ, os efeitos adversos relatados com maior frequência eram cefaleia leve e transitória, distúrbios visuais, prurido e queixas gastrointestinais (34).

Vários estudos demonstraram a efetividade da CLQ e HCLQ no tratamento de doenças autoimunes como lúpus e AR, porém ao invés de doses semanais, nestes cenários eram utilizadas doses diárias (67–69,87–90). Scherbel & Schuchter compararam dois grupos de pacientes com AR em atividade, um tratado com sulfato de HCLQ e o outro com fosfato de CLQ. Os autores concluíram que ambas as drogas

demonstravam efetividade moderada na supressão da atividade inflamatória e um bom perfil de segurança, porém a HCLQ causava menos efeitos adversos gastrointestinais. Dentre os eventos gastrointestinais relatados pelos pacientes podemos citar: náuseas, vômitos, epigastralgia, anorexia, cólicas e diarreia (91).

A primeira descrição de retinopatia associada ao uso de CLQ data de 1957 em um paciente lúpico (92), a partir daí diversos relatos dessa complicação foram publicados na literatura o que motivou a realização de rotina de avaliações oftalmológicas de triagem em usuários crônicos da droga (35,36). A despeito de todo essa preocupação acerca da retinopatia, esta apresenta uma baixa taxa de incidência nos estudos mais recentes (1,6%) (93).

Os efeitos benéficos dos antimaláricos para pele de pacientes lúpicos é bem conhecido, assim como o seu potencial em causar reações cutâneas (83,87). A incidência de dermatites em usuários de CLQ pode chegar a até 40% dos usuários (94). Os antimaláricos estão associados a exacerbação de lesões cutâneas em pacientes portadores de psoríase (95), embora esse dado seja contestado por outros autores (96). Alguns pesquisadores relataram também o aparecimento de lesões pigmentares em região pré-tibial, palato, face e região subungueal de pacientes em uso destas medicações (97).

Efeitos adversos classificados como neurológicos – tontura, cefaleia, visão embaçada – também são relatados em até 38% dos pacientes usuários de CLQ e HCLQ (94). Existem relatos também de quadros de neuromiopatias, com sintomatologia de fraqueza progressiva da musculatura proximal, principalmente de membros inferiores, e achados eletroneuromiográficos compatíveis com comprometimento muscular e neuronal (98).

Eventos adversos cardíacos relacionados ao uso de antimaláricos, em sua maioria eram atribuídos a superdosagem, em especial em tentativas de suicídio (99). Uma revisão sistemática publicada em 2018 aponta, porém para um cenário um pouco diferente, indicando que podem sim ocorrer em indivíduos em uso crônico de doses usuais das medicações. Os autores enfatizaram que as manifestações podem ser graves e até fatais e na sua maioria se manifestam por distúrbios da condução, podendo ser divididas em dois grupos: 1. Toxicidade aguda: normalmente associadas a dosagens acima das usuais que levariam ao bloqueio dos canais de sódio, cálcio e potássio ocasionando estabilização das membranas celulares, efeito inotrópico

negativo, alargamento do intervalo QT, alargamento do intervalo QRS e vasodilatação periférica ou 2. Toxicidade cumulativa: associada ao uso crônico dos antimaláricos que ocasionaria aumento do pH lisossômico, com impedimento da degradação de proteínas lisossômicas, acúmulo de auto-fagossomos ineficazes, fosfolípides e glicogênio que ocasionariam vacuolização dos miócitos (40).

Diante da pandemia do novo coronavírus - que se iniciou no final de 2019 - em um cenário onde não existiam vacinas ou terapias específicas contra o vírus, surgiram evidências iniciais de que a HCLQ teria atividade antiviral *in vitro* em células de cultura infectadas pelo SARS-CoV-2 (100). Esses achados impulsionaram a realização de ensaios clínicos e o uso em larga escala da medicação em pacientes infectados pelo vírus, ao mesmo tempo que levantaram questionamentos acerca de sua eficácia e da segurança, em especial a segurança cardiovascular (22,24,25,29–31).

Enquanto uma revisão sistemática apontava para uma elevação no risco de prolongamento do intervalo QT induzido pela HCLQ em pacientes infectados pelo SARS-CoV-2 (101), um ensaio clínico randomizado em pacientes sadios (102) e um estudo de caso-controle aninhado (utilizando dados de uma seguradora de saúde com pacientes lúpicos) (103) não encontraram risco elevado de arritmia com uso da HCLQ.

Diante dessas dúvidas levantadas sobre a segurança do uso e o potencial efeito anticoagulante da CLQ e HCLQ, levando-se em consideração a quantidade de estudos publicados desde o início do uso dessas medicações na década de 40, o seu relativo baixo custo, e a inexistência até então de uma revisão sistemática avaliando primariamente a segurança do uso dessas medicações em diferentes cenários clínicos (lúpus, COVID-19, malária, AR) e da sua efetividade na prevenção de eventos tromboembólicos, decidimos avaliar esses dois importantes aspectos, utilizando para tanto a metodologia da revisão sistemática e submetendo os dados quantitativos a um processamento meta-analítico.

4 MÉTODOS

4.1 Desenho de estudo

Utilizamos a metodologia da revisão sistemática para elaboração dos dois artigos que compõem essa tese de doutorado. Ambos concebidos de acordo com as orientações do "*Preferred Reporting Items for Systematic Reviews and Meta-Analyses*"(PRISMA) (104) e com seus respectivos protocolos devidamente registrados no "*International prospective register of systematic reviews* – PROSPERO" sob os números de identificação abaixo:

1. Safety of chloroquine and hydroxychloroquine: a protocol for systematic review and meta-analysis (CRD42020197938)
2. Efficacy of hydroxychloroquine and chloroquine in preventing thromboembolic events: a protocol for a systematic review and meta-analysis (CRD42021247902)

4.2 Critérios de inclusão

Em ambos os estudos incluímos apenas ECRs. Os critérios de inclusão específicos de cada estudo encontram-se disponíveis nos Apêndice A (artigo 1) e Apêndice B (artigo 2).

4.3 Fontes de informação e estratégia de busca

Realizamos as buscas por estudos elegíveis, de acordo com nossos critérios de inclusão, nas bases de dados MEDLINE e EMBASE utilizando descritores (MESH) e combinações de palavras chaves. Checamos também as referências bibliográficas dos artigos incluídos e o site de registros de ensaios clínicos, https://clinicaltrials.gov/, em buscas de estudos ainda não publicados.

As estratégias de busca específicas de cada estudo encontram-se disponíveis nos Apêndice A (artigo 1) e Apêndice B (artigo 2).

4.4 Ferramentas para armazenamento e seleção dos estudos

Os registros obtidos através das nossas estratégias de buscas foram enviados para as ferramentas online Distiller SR (Artigo 1) e Covidence (Artigo 2), e para o gerenciador de referências bibliográficas ZOTERO. Dois revisores realizaram de forma independente a triagem dos títulos e resumos de acordo com os critérios de elegibilidade estabelecidos em cada estudo e excluíram os estudos considerados irrelevantes. Obtivemos os textos completos dos estudos selecionados, e estes foram então reavaliados pelos mesmos revisores que julgaram se cada um dos artigos

obtidos preenchia os critérios de inclusão do nosso estudo, em caso negativo, o artigo era excluído e a razão da exclusão registrada. Todas as discordâncias nestas fases de seleção foram resolvidas através de discussões entre os autores.

4.5 Coleta dos dados

Dois autores no artigo 1 e quatro no artigo 2 coletaram os dados de cada um dos estudos selecionados de forma independente através de um formulário online customizado. Todas as discrepâncias entre os dados coletados foram resolvidas através de discussões entre os autores, até que um consenso fosse atingido. Os dados coletados em cada um dos artigos, encontram-se disponíveis nos Apêndices A (artigo 1) e B (artigo 2).

4.6 Avaliação da qualidade dos estudos individualmente

Dois autores avaliaram de forma independente cada um dos estudos incluídos em nossas revisões sistemáticas utilizando a ferramenta da Cochrane para avaliação de risco de viés em ECR (RoB 2,0) (105). Esta ferramenta nos possibilita avaliar o risco de viés em cinco domínios diferentes:

1) Viés no processo de randomização;
2) Viés devido a desvios das intervenções pretendidas;
3) Viés devido à falta de dados de resultados;
4) Viés na aferição do resultado;
5) Viés na seleção do resultado relatado.

Semelhante às etapas anteriores, todas as discordâncias entre os autores foram resolvidas por meio de discussão e os resultados desta análise foram disponibilizados como uma tabela suplementar em cada um dos artigos.

4.7 Avaliação do risco de viés de publicação

Avaliamos a presença do viés de publicação através da inspeção visual do gráfico em funil (*funnel plot*) - plotando a medida de desfecho primário no eixo das abcissas e seu erro padrão no eixo das ordenadas. Nesta análise, é esperado que estudos com amostras maiores tenham menor dispersão dos seus resultados, que geralmente se agrupam na parte superior do gráfico, enquanto estudos menores apresentam maior dispersão e ficam na base do gráfico, na ausência de viés de publicação, esperamos um formato de funil invertido neste gráfico. Submetemos os resultados também a uma análise por regressão linear através do teste de Egger, para confirmar os resultados obtidos através da inspeção do gráfico em funil (106).

4.8 Síntese dos dados

Em ambos os estudos previmos que encontraríamos elevada heterogeneidade em nossos dados, já que analisamos drogas diferentes (CLQ e HCLQ), regimes terapêuticos diferentes, empregados em doenças diferentes e em populações heterogêneas. Por conta disso, optamos em usar um modelo de efeitos randômicos para agrupar os dados quantitativos.

Para avaliarmos a segurança do uso da CLQ e HCLQ, calculamos a razão da taxa de incidência (*Incidence Rate Ratio* - IRR) e seu erro padrão individualmente em usuários e não-usuários da HCLQ e CLQ de cada ECR e agrupamos os resultados utilizando o método de Mantel-Haenszel (107).

Para avaliarmos a eficácia da HCLQ na prevenção de eventos tromboembólicos, calculamos o RR de desenvolvimento de eventos tromboembólicos em usuários e não-usuários de HCLQ de cada ECR e agrupamos os resultados utilizando o método de Mantel-Haenszel (107).

Avaliamos a heterogeneidade em cada análise, utilizando como o T^2, I^2, o Q de Cochrane e o seu P-valor. Assumimos que os valores de I^2 de 25, 50 e 75% seriam representativos de baixa, média e elevada heterogeneidade (108).

Realizamos também análises de subgrupo estratificando os RCTs de acordo com a droga utilizada, dose utilizada, ambiente onde o estudo foi realizado (hospitalar ou ambulatorial), qualidade do estudo etc. Realizamos também análises de sensibilidade excluindo RCTs considerados “*outliers*” e repetindo as análises.

5 ARTIGOS CIENTÍFICOS

Artigo 1

Edington FLB, Gadelha SR, Santiago MB. Safety of treatment with chloroquine and hydroxychloroquine: A ten-year systematic review and meta-analysis. European Journal of Internal Medicine [Internet]. 2021 Jun; 88:63–72. Available from: https://linkinghub.elsevier.com/retrieve/pii/S0953620521000923

Artigo 2

Barros Edington FL, de Rezende DF, dos Santos LFS, Garcia RV, Gadelha SR, Santiago MB. Efficacy of hydroxychloroquine in the prevention of thromboembolic events: A systematic review and meta-analysis. Lupus [Internet]. 2022 Feb; 31(2):238–45. Available from: http://journals.sagepub.com/doi/10.1177/09612033221074192

5.1 Artigo 1 – Safety of treatment with chloroquine and hydroxychloroquine: a ten-year systematic review and meta-analysis

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Safety of treatment with chloroquine and hydroxychloroquine: A ten-year systematic review and meta-analysis

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ABSTRACT

Objective: To estimate the incidence rate ratio (IRR) of adverse events (AE) in chloroquine or hydroxychloroquine users.

Methods: We systematically reviewed randomized controlled trials (RCTs), using MEDLINE (2010-2020) and EMBASE (2010-2020) databases, reporting AE in chloroquine or hydroxychloroquine users during treatment for lupus, rheumatoid arthritis, malaria and COVID-19. The protocol for this systematic review is registered at the PROSPERO database (CRD42020197938). The quality of the included studies was assessed using the Cochrane risk-of-Bias tool and relevant data were extracted though a customized data collection form, independently, by two authors. The IRR of AE was estimated using a random-effect model meta-analysis and heterogeneity was evaluated by T^2 and I^2. Subgroup analysis was performed, and publication bias was assessed by funnel-plot.

Results: Forty-six RCTs met our eligibility criteria and were included in our analysis (23132 patients). There was not a single death attributed to chloroquine or hydroxychloroquine use in the included RCTs. The IRR of general AE during antimalarial use was 1.15 [CI 95% 1.01-1.31]. COVID-19 patients treated with either antimalarial presented an 83% and 165% higher risk of developing general and gastrointestinal AE, respectively, in comparison with controls. The use of antimalarial increased the risk of developing dermatological AE by 92% in malarial studies and reduced by 65% in lupus studies. We did not find a significatively higher risk of cardiovascular nor ophthalmological AE in antimalarial users.

Conclusions: Our data reinforces that chloroquine and hydroxychloroquine have a good safety profile though caution is advised when using higher than usual doses in hospitalized COVID-19 patients.

1. Introduction

Previously known as SN7618, chloroquine has been used since the early 1940s for the treatment of malaria [1]. In 1946 Loeb F. et al., in a statement approved by the board for coordination of malarial studies, highlighted the efficacy and safety profile of the drug – "with minor side effects as mild and transient headache, visual disturbances, pruritus, and gastrointestinal complaints". The authors also noted that none of the adverse symptoms was serious and all of them were readily reversible [2]. In 1953, Hoenkenga M.T. et al. reported encouraging results for the treatment of malaria with 7-chloro-4-[4-(N-ethyl-N-β-hydroxyethylamino)-1-methylbutylamino], later known as hydroxychloroquine. Besides its efficacy, the authors did not find any side effects during its first clinical study [3].

Since its discovery, other diseases besides malaria – like lupus [4], rheumatoid arthritis (RA) [5], hand osteoarthritis [6], and antiphospholipid syndrome [7], – have been successfully treated with chloroquine and hydroxychloroquine. In general, both drugs have been considered safe for nearly 60 years, even for long term use. Gastrointestinal complaints are the most frequent side effects, although retinopathy has been the most feared one. Dermatological, neurological, musculoskeletal, and cardiovascular side effects are also reported, though less frequently [8].

In 2018, Chatre C. et al. systematically reviewed the cardiac toxicity attributed to chloroquine and hydroxychloroquine and concluded that both drugs were safe and well-tolerated, but cardiotoxicity could be a rare, severe, and overlooked complication of such therapy [9].

In 2020, the world spread of the severe acute respiratory syndrome

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coronavirus 2 (SARS-Cov-2) – the strain of coronavirus that causes coronavirus disease 2019 (COVID-19), has demanded innovative treatment options to halt the disease progression and the already high, death toll. Wang M. et al. suggested, in February of that same year, that chloroquine was highly effective in the control of SARS-Cov-2 infection in vitro [10]. With the potential use of chloroquine and hydroxychloroquine for the treatment of COVID-19, the debate on its safety have reemerged, and studies with conflicting results have been published [11–13].

Considering that both drugs have been in the market for a long time, the extensive body of evidence supporting them, and the recent doubts on its safety, we decided to conduct a systematic review to assess the safety of hydroxychloroquine and chloroquine for the treatment of lupus, RA, malaria, and COVID-19.

2. Objectives

We systematically reviewed randomized controlled trials (RCTs) reporting adverse events (AE) in users of chloroquine or hydroxychloroquine - for the treatment of lupus, RA, malaria and COVID-19 - in comparison to non-users, to estimate the incidence rate ratio (IRR) of developing AE, in general and categorized by system.

3. Methods

This systematic review with meta-analysis was conceived according to the Preferred Reporting Items for Systematic Review and Meta-analysis (PRISMA) [14], its checklist is provided as a supplementary file and its protocol is available online at the PROSPERO database (identifier CRD42020197938).

3.1. Inclusion criteria

We included only RCTs reporting the incidence of AE in users and non-users of chloroquine or hydroxychloroquine - alone or in combination with any other drug(s) - for the treatment of lupus, RA, malaria, and COVID-19. We did not impose any restrictions to age, gender, language, type of setting, or comparator but restricted our analysis to studies published in the last 10 years.

3.2. Information source and search strategy

We searched the MEDLINE and EMBASE databases from 2010 to the present using medical subject headings (MeSH) and combinations of key terms. We also checked reference lists and citations of all primary studies, review articles and systematic reviews for additional references and ClinicalTrials.gov for unpublished data. Our MEDLINE and EMBASE search strategy are provided as a supplementary file.

3.3. Studies record and selection process

The studies obtained through the literature search were uploaded to the Distiller SR website and ZOTERO reference manager. Two authors (FLBE and SG) independently screened all titles and abstracts for relevant ones, according to our eligibility criteria, and then obtained the full text of the selected studies. The same two authors screened the full-text articles for inclusion and exclusion, recording the reasons for the last. All the disagreements were resolved by consensus and a flowchart of study selection with the characteristics of the excluded studies is provided.

3.4. Data collection process

Two review authors (FLBE and SG) extracted the following information from all eligible studies using a customized data collection form: first author's name, year of publication, country, sample size, follow up time, disease of interest (lupus, RA, malaria or COVID-19), setting, percentage of females, sample mean age and its standard deviation, drugs used in experimental and control groups and their doses, total number of AE in chloroquine or hydroxychloroquine users and non-users and the number of specific AE (nausea, vomiting, headaches, etc.) as reported in individual studies.

3.5. Risk of bias in individual studies

Two review authors (FLBE and SG) assessed independently the risk of bias using the Revised Cochrane risk-of-bias tool for randomized trials (RoB 2) [15] and the results of this analysis are provided in Table S37.

3.6. Data synthesis

It was expected a high heterogeneity among the studies, as none of the RCTs was designed to report AE as the primary objective and different drugs or combination of drugs were accessed for the treatment of four different diseases. Hence a random effect model meta-analysis was chosen, using the statistical software R (version 4.04) with the META package [16].

Some AE happened more than once in a same patient and different studies had different follow-up periods. To aggregate all this information, we estimated the IRR of each study [17].

We assumed that there would not be a loss to follow-up during studies, then we calculated the incidence rate in each experimental group, dividing the number of AE– total AE or AE categorized by system (cardiovascular, gastrointestinal, dermatological, ophthalmological and neurological) - in chloroquine / hydroxychloroquine users and non-users by the person and the time of follow-up (number of days of follow-up multiplied by the sample size of each group). We divided the incidence rate of AE in chloroquine / hydroxychloroquine users by non-users in each study, to finally obtain the IRR of each RCT. We pooled the results of each study using the Mantel-Haenszel method as suggested by the Cochrane Handbook for systematic reviews of interventions [18]. We did not exclude from the meta-analysis zero total events trials for enabling the inclusion of all RCT data, thereby providing the most generalizable estimate of the overall effect [19]. But, if the authors did not clearly state the absence of a specific AE, the data were considered "not available" (NA) and the study excluded from that specific analysis. This explains why some analysis may not have the total number of RCTs included in the meta-analysis.

Heterogeneity was assessed through T^2 and I^2 as indices of its magnitude and P-values as measures of its uncertainty. We assumed values of 25, 50 and 75% for I^2 as representative of low, medium and high heterogeneity [20].

We also performed subgroup analysis to assess the incidence rate of AE according to disease, antimalarial (chloroquine versus hydroxychloroquine) used, antimalarial drug dose, setting, and whether the antimalarial was administered as monotherapy or not.

Publication bias was assessed using funnel plotting and Egger's regression [21] and a sensitivity analysis was performed to confirm that no single study has driven our results.

4. Results

4.1. Study selection

Our research generated 938 records for evaluation. After excluding duplicates, a total of 925 titles and abstracts were screened. As we did not find any information about AE in 787 studies, they were considered irrelevant and excluded from our analysis. We retrieved 138 articles for full-text appreciation. 42 studies met our eligibility criteria and were included in our systematic review and meta-analysis.

As we found only 2 RCTs evaluating the use of hydroxychloroquine in patients with lupus, we decided to halt our restriction on the date of publication, and we were able to include other 4 studies. We conducted

our analysis with 46 studies in total. A PRISMA flowchart of the study selection process is provided as supplementary material (figS1).

4.2. Study characteristics

A total of 23132 individuals from the general population were evaluated with a follow-up period ranging from 14 to 730 days. Chloroquine was used in 32 studies and hydroxychloroquine in 14 of the included studies. A total of 15712 AE were reported throughout all studies, 9011 in patients treated with chloroquine or hydroxychloroquine. The characteristics of the studies included in our analysis are summarized in Table S37.

4.3. Meta-analysis

4.3.1. General adverse events

We pooled the results of 46 RCTs reporting the number of AE in chloroquine or hydroxychloroquine users and non-users (Fig. 1). The pooled analysis showed a significant higher incidence of AE in patients treated with chloroquine or hydroxychloroquine (IRR 1.15 [CI 95% 1.01 - 1.31]) but a high heterogeneity was observed (I^2=89%, T^2=0.12, P-value < 0.01).

When we grouped the studies according to the disease of interest a significative difference was observed between studies (P-value=0.01) (fig.S2). A higher risk for the development of AE was noted in COVID-19 (IRR 1.83 [CI95% 1.22 - 2.73]) and malaria (IRR 1.17 [CI95% 1.01 - 1.36]) studies. The heterogeneity was considered medium in COVID-19 studies (T^2=0.1546 and I^2=75%) and high in malaria studies (T^2=0.1048 and I^2=90%). In lupus RCTs (IRR 0.62 [CI 95% 0.35 –

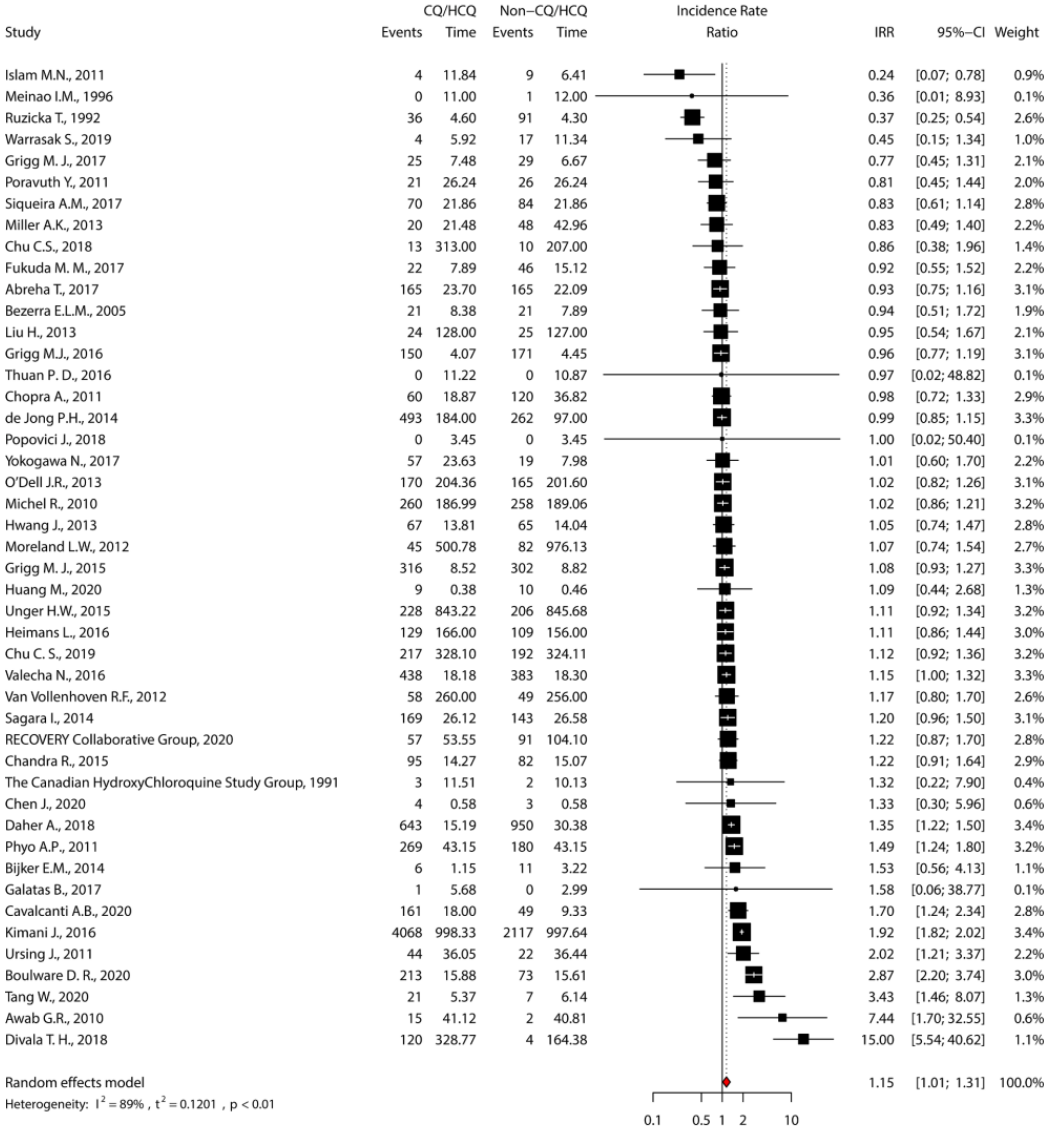

Fig. 1. Forest-plot of the pooled adverse events in chloroquine/hydroxychloroquine users and non-users.

1.10], T^2=0.2763, I^2=67%) and RA (IRR 1.03 [CI 95% 0.93 – 1.13], T^2=0, I^2=0%) we did not find a statistically significant higher IRR of AE in chloroquine or hydroxychloroquine users.

We did not find a statistically significant difference when we compared RCTs grouped by the antimalarial of choice - chloroquine versus hydroxychloroquine - (P-value=0.80) (fig.S3), the use of either antimalarial alone or in combination with other drugs (P-value =0.93) (fig.S4) or according to the setting (P-value =0.19) (fig.S5).

4.3.2. Antimalarial drug dose

In general, malaria studies analyzed doses of chloroquine of 25 mg/kg of the base drug during three consecutive days. Lupus and RA studies used fixed doses of hydroxychloroquine ranging from 200 to 400 mg/day and chloroquine ranging from 150 to 250 mg/day. Five of the six included COVID-19 studies tested higher than usual doses of chloroquine or hydroxychloroquine against conventional therapy. Three of these studies used loading doses of hydroxychloroquine ranging from 1200 to 2000 mg/day during 1 to 3 days, and maintenance doses of 400 mg to 800 mg/day during 4 to 19 days. One COVID-19 study tested 1000 mg/day of chloroquine for 10 days compared to conventional therapy.

We performed a subgroup analysis to test if higher than usual doses of chloroquine (>10 mg/kg/day) or hydroxychloroquine (>400 mg/day) would be associated with a higher IRR of adverse events. We found a statistically significant difference (p=0.02) between studies that used higher than usual doses of antimalarials – 1.86 (CI95% 1.22 – 2.85) – in comparison to studies that tested usual doses - 1.08 (CI95% 0.95 – 1.24) (fig.S6).

We repeated the same analysis above, considering only studies that tested hydroxychloroquine, as only one RCT evaluated a higher than usual dose of chloroquine. We found that the pooled IRR for the development of adverse events was also significantly higher [2.01 (CI95% 1.27 – 3.18)] in studies that used a higher than usual dose of hydroxychloroquine (Fig.S7).

4.3.3. Cardiovascular adverse events

There was a total of 161 cardiovascular AE in chloroquine or hydroxychloroquine users compared to 152 in non-users. The adverse cardiovascular events observed were bradycardia, atrial flutter/ fibrillation, and other supraventricular arrhythmias, ventricular tachycardia and fibrillation, atrioventricular blocks, myocardial infarction,

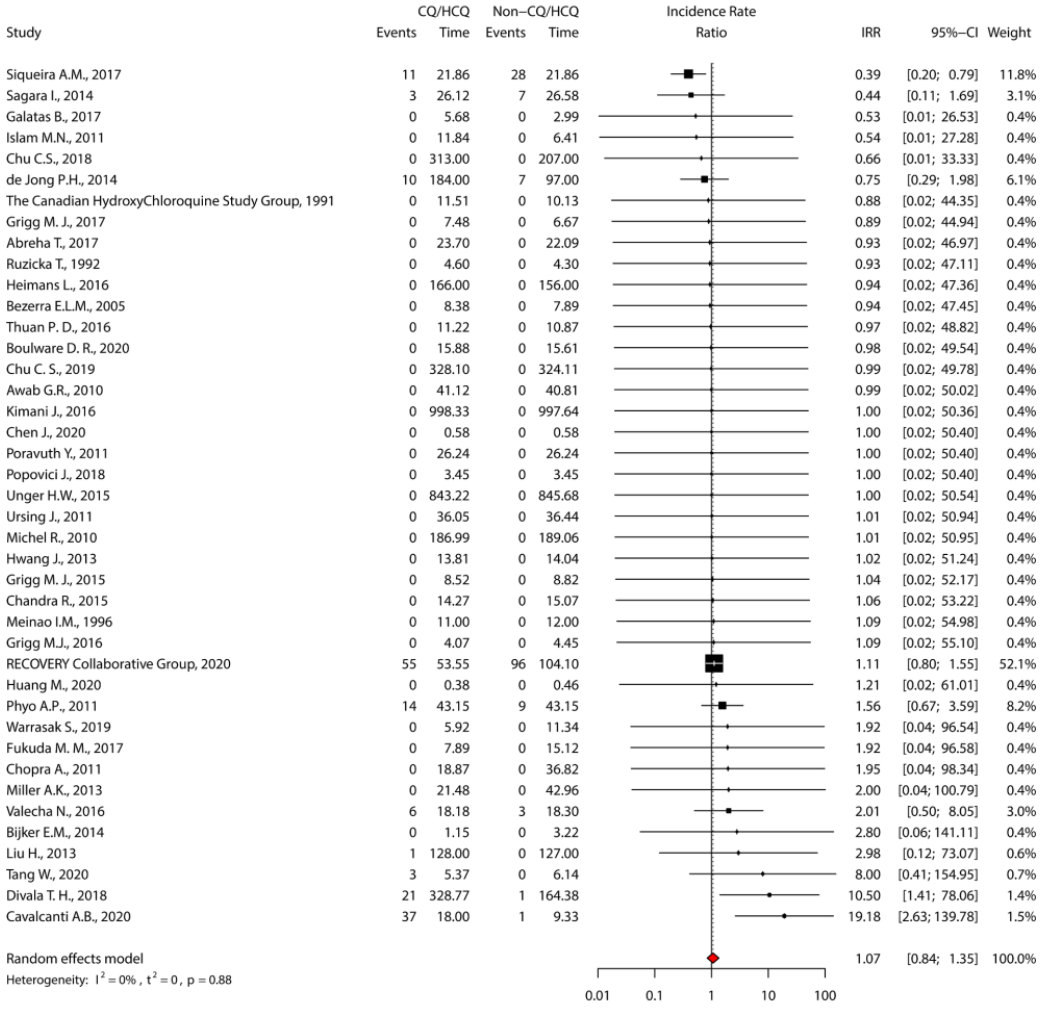

Fig. 2. Forest-plot of the pooled cardiovascular adverse events in chloroquine/hydroxychloroquine users and non-users.

prolonged QT interval, hypertension, hypotension, orthostatic hypotension, palpitations, and sinus bradycardia.

The most serious AE reported in the included studies were four episodes of ventricular tachycardia and two of ventricular fibrillation in a group of patients who received hydroxychloroquine, compared to nine episodes of ventricular tachycardia in the control group during the RECOVERY Collaborative Group study [22], although both differences were not considered statistically significant by the authors. Cavalcanti A.B. et al.[23] also reported one myocardial infarction in the group of patients treated with an association of hydroxychloroquine and azithromycin. No death was reported in the included RCTs attributed to chloroquine or hydroxychloroquine use.

Only 10 RCTs reported cardiovascular AE attributed to antimalarial use, three of them for the treatment of SARS-Cov-2. Curiously, 95 of the 161 (59%) cardiovascular AE observed in antimalarial users occurred in patients infected with the SARS-Cov-2.

The pooled IRR for cardiovascular AE was not different between chloroquine or hydroxychloroquine users and non-users (IRR 1.07 [0.84 – 1.35], T^2=0, I^2=0%) (Fig. 2).

Even when we grouped the RCTs according to the disease of interest (fig.S8), antimalarial used (fig.S9), setting (fig.S10), or if the antimalarial was administered alone or in combination (fig.S11), there were no statistically significant differences in the IRR between groups.

4.3.4. Gastrointestinal adverse events

We found a total of 4064 gastrointestinal AE, 2722 in chloroquine or hydroxychloroquine users. We pooled the results of 41 RCTs reporting gastrointestinal AE and found an IRR of 1.32 (IC 95% 0.97– 1.78) (Fig. 3) with high heterogeneity (T^2=0.61, I^2=91%).

The gastrointestinal AE reported were abdominal pain, abdominal wall hemorrhage, constipation, decreased appetite, diarrhea, dyspepsia, epigastric burning, hepatomegaly, increased alanine aminotransferase (ALT) and/or aspartate aminotransferase (AST), increased bilirubin, increased serum amylase, liver abnormality, nausea, oral ulcers, and vomiting.

The subgroup analysis of gastrointestinal AE grouped by disease revealed a statistically significant higher risk (P-value<0.01) (fig.S12) in COVID-19 patients treated with chloroquine or hydroxychloroquine (IRR 2.65 [CI 95% 1.60-4.39]. We also found a higher risk of gastrointestinal AE in hospitalized patients (IRR 1.85 [CI 95% 1.27 – 2.71]

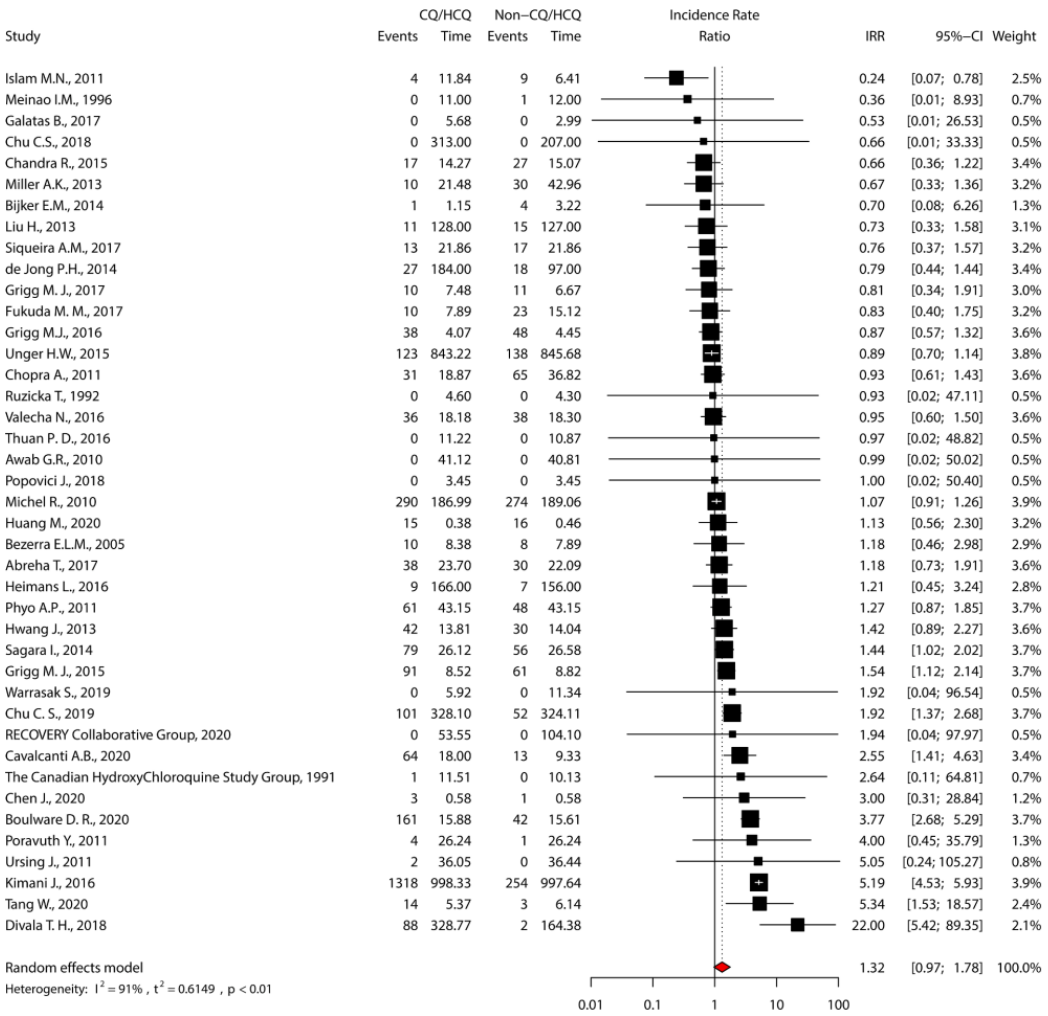

Fig. 3. Forest-plot of the pooled gastrointestinal adverse events in chloroquine/hydroxychloroquine users and non-users.

T^2=0.0586, I^2=28%) although not statistically significant different from outpatients (P-value =0.10) (fig.S13).

We found no difference in the risk of gastrointestinal AE when we grouped the RCTs according to the antimalarial used or to the use of the antimalarial alone or in combination with other drugs (fig.S14 and fig. S15)

4.3.5. Dermatological adverse events

There were 572 AE classified as dermatological, 335 in patients treated with hydroxychloroquine or chloroquine. The reported dermatological AE were alopecia, cheilitis, darkening of skin, dermatitis, eczema, facial swelling, flush, photosensitization, pretibial skin pigmentation, pruritus, rash, red-brown discoloration, scaling, and sicca complaints. The pooled IRR for dermatological AE was 1.48 (CI 95% 0.99– 2.20) (Fig. 4) with a medium heterogeneity (T^2=0.6232, I^2=61%).

We found a statistically significant difference in the IRR of dermatological AE when we grouped the RCTs according to the disease (fig S16) (P-value < 0.01). Malaria studies presented an IRR for the development of dermatological AE 92% higher [IRR 1.92(CI 95% 1.27 – 2.92)] in patients treated with hydroxychloroquine or chloroquine. On the other hand, in lupus patients, hydroxychloroquine or chloroquine seemed to prevent the development of dermatological AE [IRR 0.35(CI 95% 0.23 – 0.53)]. The use of either antimalarial in COVID-19 or RA patients did not raise the incidence rate of dermatological AE.

No statistically significant difference was found when studies were grouped according to the antimalarial of choice (fig.S17), setting (fig. S18) or the use of antimalarial in combination with other drugs or not (fig.S19).

4.3.6. Neurological adverse events

There was a total of 3116 neurological AE, 1831 in hydroxychloroquine or chloroquine users. The reported AE were agitation, anxiety, ataxia, dizziness, headache, hearing loss, insomnia or other sleep disorders, memory problems, mood disorders, psychosis, and muscle weakness.

The pooled IRR for the development of any neurological AE was 1.16 (CI 95% 0.90 - 1.50) (Fig. 5). No difference was found when RCTs were grouped according to the disease (P-value=0.50) (fig.S20), antimalarial used (P-value=0.54) (fig.S21), setting (P-value=0.26) (fig. S22) or according to the use of the antimalarial alone or in combination with other

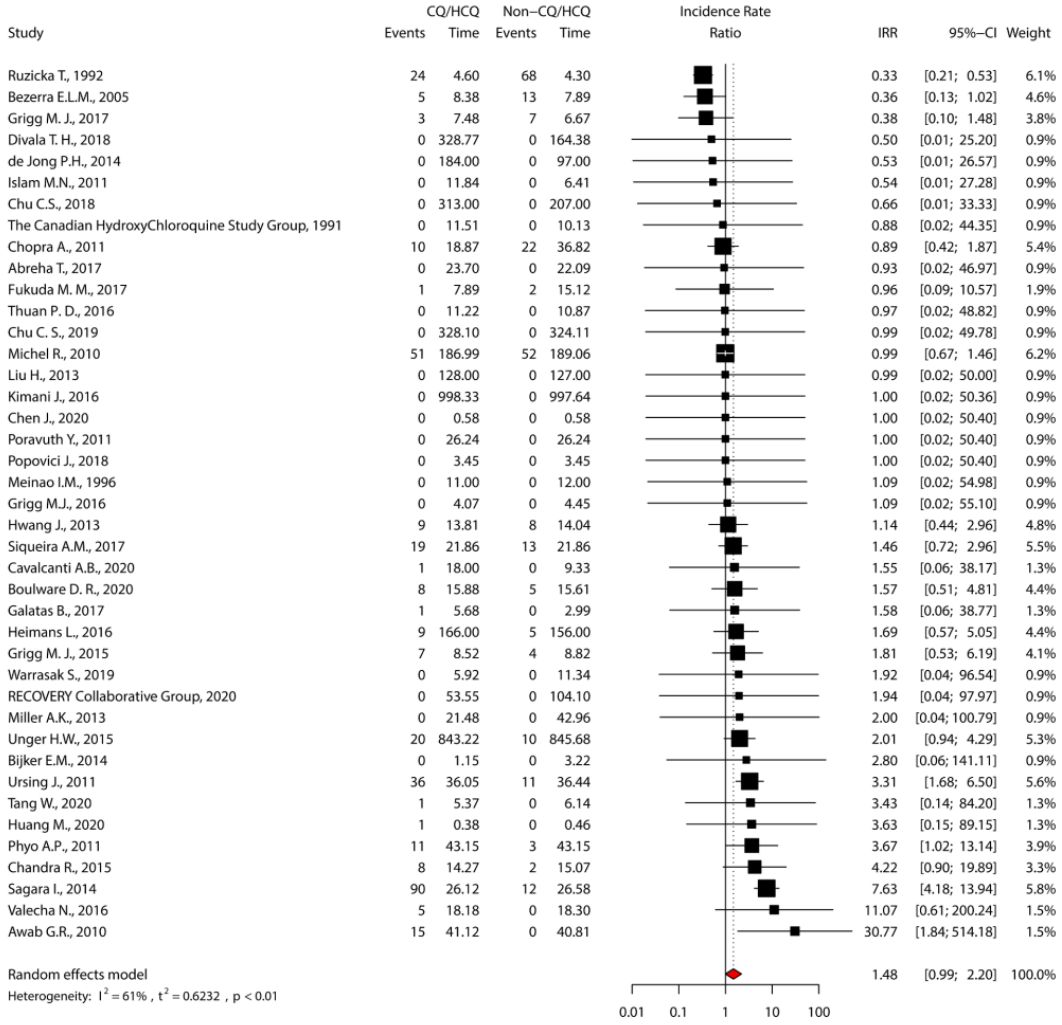

Fig. 4. Forest-plot of the pooled dermatological adverse events in chloroquine/hydroxychloroquine users and non-users.

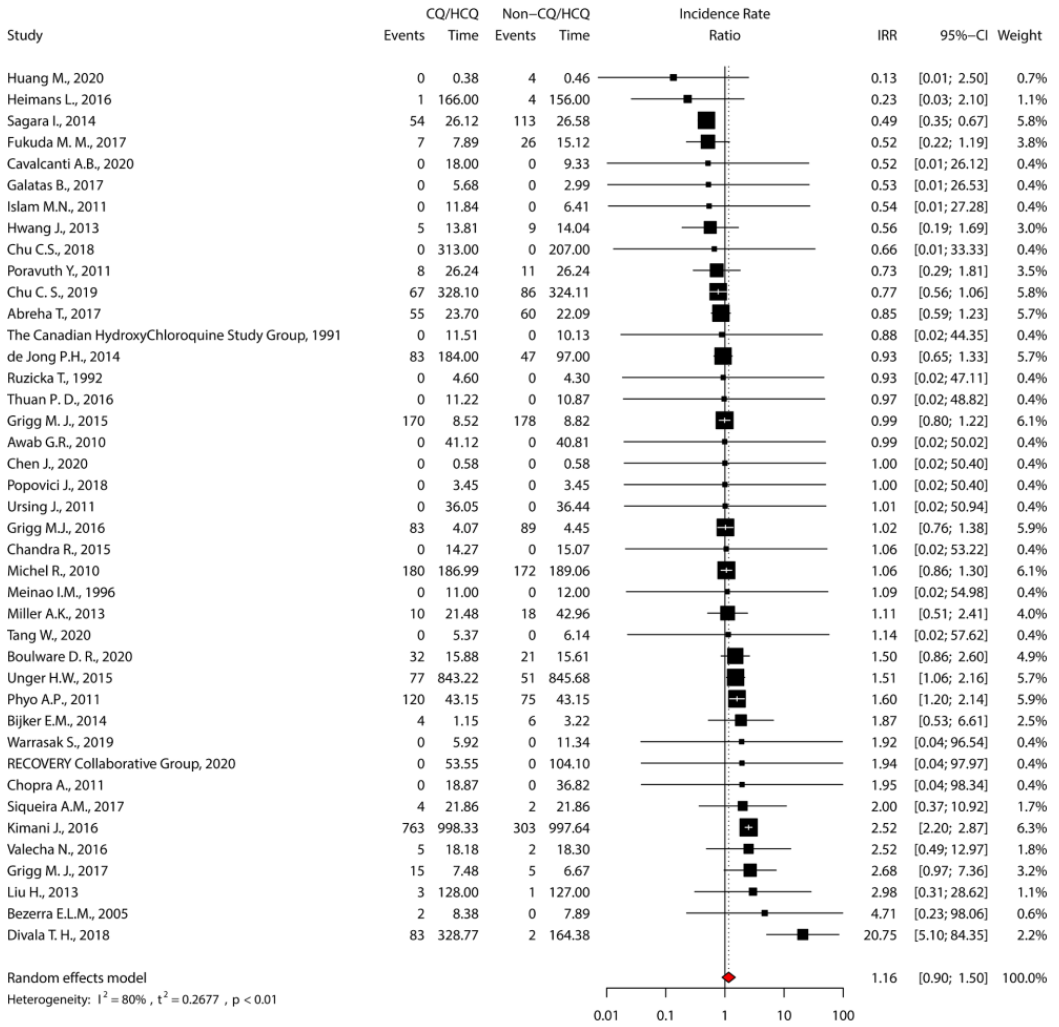

Fig. 5. Forest-plot of the pooled neurological adverse events in chloroquine/hydroxychloroquine users and non-users.

drugs (P-value=0.26)(fig.S23).

4.3.7. Ophthalmological adverse events

The included studies reported ophthalmological AE as conjunctivitis, keratopathy, retinopathy, or as a generic term "visual problems". There was a total of 207 ophthalmological AE in hydroxychloroquine or chloroquine users compared to 84 in non-users. In 30 of the 41 included RCTs there was not a single reported ophthalmological AE. Pooling the RCTs we found an IRR of 1.10(CI 95% 0,62 – 1.96) with a medium heterogeneity (T^2=1.2220, I^2=49%) (Fig. 6).

We performed subgroup analysis to test if the disease being treated (fig.S24), the antimalarial used (fig.S25), the use of the antimalarial alone or in combination (fig.S26), and the setting (fig.S27) would modify the IRR. None of these analyses revealed a statistically significant result. Even when we pooled the RCTs according to the follow-up period (RCTs with a follow-up of one year or more versus RCTs with a follow-up of less than one year) no significant difference was found (fig. S28).

4.4. Evaluation of quality in individual studies

We considered the risk of bias as low in 25 studies, with some concerns in 19 studies and 2 studies were considered with a high risk of bias. A table with the results of the RoB 2 analysis is provided (Table S37).

The subgroup analysis did not suggest that the quality of individual studies modified the pooled effect size (P-value=0.83) (fig.S29).

Eight studies did not explicitly state if the allocation sequence was random and sixteen did not state if the allocation sequence was concealed until participants were enrolled and assigned to interventions. In Twenty-six studies the participants were aware of the intervention being tested (open studies).

5. Publication bias

The visual inspection of the funnel plot suggested some asymmetry, later confirmed in a weighted linear regression of the treatment effect on its standard error (P-value = 0.003229) (fig.S30).

Considering the asymmetry observed in our funnel plot, we performed Duval S. and Tweedie R.'s "Trim and fill" procedure [24] to

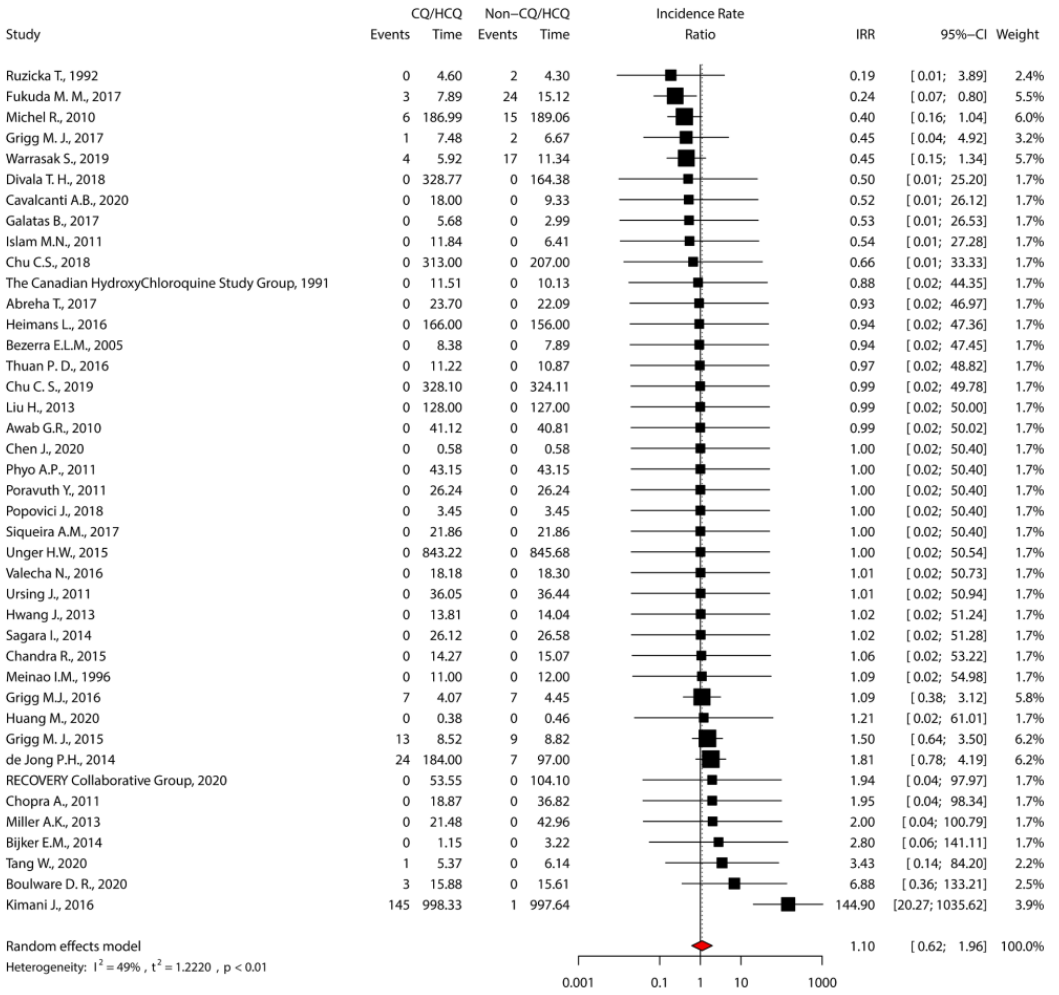

Fig. 6. Forest-plot of the pooled ophthalmological adverse events in chloroquine/hydroxychloroquine users and non-users.

estimate potentially missing studies and adjust the overall effect estimate. This analysis revealed an IRR of 1.41(CI 95% 1.25 - 1.59) suggesting that we may have underestimated the true effect size due to publication bias.

6. Sensitivity analysis

We performed a sensitivity analysis to assess the robustness of our results. Firstly, we repeated our main analysis excluding outliers as described by Viechtbauer and Cheung [25]. This procedure significantly reduced the heterogeneity of our analysis (I^2 from 89% to 35%) (fig.S31) and the pooled IRR still suggested a statistically significant higher risk of developing any AE in hydroxychloroquine and chloroquine users (IRR 1.11 [CI 95% 1.04 – 1.18]).

We conducted the same analysis for cardiovascular (fig.S32), gastrointestinal (fig.S33), neurological (fig.S34), dermatological (fig. S35), and ophthalmological (fig.S36) AE. We did find significantly different results for the risk of gastrointestinal (IRR 1.14 [CI 95% 1.01 – 1.29]) and dermatological AE (IRR 1.35[CI 95% 1.09– 1.66]).

7. Discussion

We found a higher IRR of total AE in hydroxychloroquine or chloroquine users. This risk was 83% higher in patients infected with COVID-19 in comparison with non-users.

A higher IRR of gastrointestinal AE was observed in patients treated with either antimalarials, after exclusion of outliers (IRR 1.14 [CI 95% 1.01 – 1.29]). This risk was significantly higher in COVID-19 patients (IRR 2.65[CI 95% 1.60– 4.39]). Gastrointestinal complaints are the most reported AE in hydroxychloroquine and chloroquine users [8]. Recently, Mao R. et al. systematically reviewed the prevalence and prognosis of digestive system involvement in patients with COVID-19 and estimated a 15% prevalence of gastrointestinal symptoms, with nausea or vomit, diarrhea and loss of appetite being the three most reported complaints. The combination of antimalarial use, COVID-19 and other drugs utilized for its treatment (azithromycin, lopinavir, ritonavir, etc.) may explain the higher risk of gastrointestinal AE in COVID-19 patients treated with chloroquine or hydroxychloroquine [26].

The risk for dermatological AE was significantly higher in patients with the diagnosis of malaria treated with either antimalarial (IRR 1.92 [CI 95% 1.27 – 2.92]) and significantly lower in patients with lupus (IRR

0.35 [CI 95% 0.23 – 0.53). The effect of antimalarials in the skin of lupus patients is well known for a long time [27,28] and recently its efficacy to other skin diseases has been suggested [29].

Although not a single fatal event attributed to the use of chloroquine and hydroxychloroquine was reported in the included studies nor we found a significantly higher IRR of cardiovascular AE in antimalarial users, four episodes of ventricular tachycardia, two of ventricular fibrillation and one myocardial infarction were reported. All these AE occurred in RCTs which enrolled hospitalized COVID-19 patients and used a higher than usual daily dose of hydroxychloroquine. None of the authors attributed these events to antimalarial use, but caution is advised when using antimalarials in patients with those characteristics.

Chen D. et al. found hypokalemia in 85% of severely ill patients infected with SARS-Cov-2 [30] and Shi S. et al. reported that 19.7% of patients with confirmed COVID-19 developed cardiac injury during hospitalization [31]. Both these findings suggest, in theory, that the viral infection itself could exacerbate the incidence of arrhythmias regardless the use of antimalarial drugs.

In 1958 Burrell Z.L. and Mantinez A.C. conducted a prospective study to assess the effectiveness of antimalarials in the treatment of supraventricular paroxysmal tachycardia, multifocal ventricular extrasystoles and atrial fibrillation [32]. The authors concluded that 50 of 73 arrhythmias responded favorably to either chloroquine or hydroxychloroquine with minor AE.

In 2014, Teixeira R.A. et al. conducted a cross sectional study with 317 lupus patients to evaluate the cardiac safety and the antiarrhythmic potential of chloroquine. They observed a high incidence of non-sustained and sustained supraventricular tachyarrhythmias in the whole population, but the frequency of these arrhythmias were lower the longer the patients used chloroquine [33].

Our data together with those previous studies reinforce the notion that chloroquine and hydroxychloroquine have a general good cardiovascular safety profile, but rarely may be associated with serious cardiovascular adverse events. The recent RCTs failing to prove the efficacy of chloroquine or hydroxychloroquine against COVID-19 and our findings of potential increased risk of AE when using higher than usual doses in COVID-19 hospitalized patients should discourage such strategy.

There are some pitfalls to conduct systematic reviews on AE, such as the variation in the completeness of AE detection across trials, the inconsistent definitions of AE, inconsistent adjudication methods, the variation in reporting effort for unexpected AE, and scant information on withdrawals or loss to follow-up because of AE [34]. As an example of such pitfalls in our analysis, Thuan P.D.et al.[35] reported that they did not find any serious AE in either arm of the study while Popovici J. et al. [36] reported no AE at all. Neither of the two studies had a significant impact on the pooled results of our analysis. It is also important to consider that none of the included RCTs was primarily designed to assess the safety of antimalarials and in some cases, chloroquine and hydroxychloroquine, corresponded to the control arm of the study [37–41].

Our study has some strengths that are worth mentioning. To our knowledge, this is the first study to systematically review the safety of chloroquine and hydroxychloroquine using data exclusively from RCTs in 4 different diseases, including COVID-19. We also conducted a random-effects meta-analysis with the data obtained and estimated the general risk for the development of AE while using chloroquine or hydroxychloroquine, and estimated the risk of AE categorized by system - cardiovascular, gastrointestinal, dermatological, neurological, and ophthalmological AE.

Some limitations of the present study, though, should also be addressed. The high heterogeneity found in our main analysis, although predicted and attributed to the different diseases, drugs and primary outcomes assessed by the included studies, may have some influence in our results. This heterogeneity was significantly reduced after removing outliers without modifying the direction of the overall effect.

The analysis of our funnel plot suggested publication bias, later confirmed by Egger's regression method. We adjusted the point estimate of our overall effect size according to the "Trim and fill" procedure which suggested that our estimate of the overall effect of hydroxychloroquine or chloroquine was higher than we predicted.

8. Conclusions

We found that chloroquine and hydroxychloroquine have in general a good safety profile, as most of their side effects were mild and not fatal. COVID-19 patients seem to have a higher risk of general and gastrointestinal AE during their use. These AE may be related to an interaction between virus, host and polypharmacy, not the antimalarial use itself. Although our analysis did not suggest a significantly higher IRR of cardiovascular AE in antimalarial users when compared to non-users, myocardial infarction (one patient), ventricular tachycardia (four patients) and fibrillation (two patients) were reported in hospitalized COVID-19 patients who received higher than usual antimalarials doses - a finding that should discourage its prescription in such scenario.

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Availability of data and materials

The authors declare that this systematic review is an original work and all the data will be available from the corresponding author on request.

Ethics approval and consent to participate

As all the data used in this systematic review is available for public access, there is no need of ethics committee approval.

Consent

Not applicable.

Author's contribution

FLBE conceptualized, drafted and is the guarantor of this manuscript. FLBE and SG developed the selection criteria, the risk of bias assessment strategy and data extraction criteria. MBS and SG reviewed the manuscript. All authors read, provided feedback and approved this systematic review.

Declaration of Competing Interest

The authors declare that they have no competing interest.

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5.2 Artigo 2 – Efficacy of hydroxychloroquine in the prevention of thromboembolic events: A systematic review and meta-analysis

Paper

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Efficacy of hydroxychloroquine in the prevention of thromboembolic events: A systematic review and meta-analysis

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Abstract

Objective: To estimate the risk ratio (RR) of thromboembolic events in chloroquine and hydroxychloroquine users compared to non-users.

Methods: We systematically reviewed randomized controlled trials (RCTs), using MEDLINE and EMBASE databases from inception to the present, reporting thromboembolic events in chloroquine and hydroxychloroquine users compared to non-users. Four authors independently screened all the records obtained through our search strategy and later revised the selected full-text articles for eligibility, according to our inclusion criteria. The same four authors independently extracted relevant data through a customized data collection form while two other authors assessed the quality of the included RCTs using the Cochrane risk-of-bias tool (Version 2.0). All the disagreements were resolved through discussions among the authors. We calculated the risk ratio (RR) and its respective standard error of developing thromboembolic events in hydroxychloroquine users and non-users for each individual study and pooled the results using a random effects model meta-analysis. We assessed Heterogeneity using the Tau^2 and I^2, and publication bias using funnel plotting and Egger's regression. The protocol for this systematic review is registered at the PROSPERO database (CRD42021247902).

Results: Thirteen RCTs met our eligibility criteria and were included in our analysis (2663 patients). We found that hydroxychloroquine—no study on chloroquine was found—reduced the risk of thromboembolic events by 49% (RR 0.51 [IC 95% 0.31–0.84]) with a medium heterogeneity (I^2 = 67% and T^2 = 0.4948). We did find some asymmetry in the inspection of the funnel plot, which was ruled out through an Egger's regression (p-value = 0.1025).

Conclusion: Our data reinforce the idea that hydroxychloroquine reduces the risk of thromboembolic events.

Keywords

Hydroxychloroquine, thrombosis, anticoagulants, systematic review, meta-analysis

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Introduction

Chloroquine was introduced in the early 1940s followed by hydroxychloroquine in the early 1950s for the treatment of malaria, with both drugs demonstrating efficacy and good safety profile in their respective initial studies.[1,2] In 1953, Goldman L. published a series of 21 cases of lupus erythematosus successfully treated with chloroquine, and by 1957, three other studies documented hydroxychloroquine efficacy in the same disease.[3] The interest in both drugs has increased over the years, and studies assessing their efficacy in rheumatoid arthritis,[4] hand osteoarthritis,[5] lipid disorders,[6] diabetes,[7] and antiphospholipid syndrome (APS)[8] have been published.

In 1941, Knisely, M.H. et al.[9] described the aggregation of fibrin-coated erythrocytes observed during trauma, infection, hypersensitivity reactions, metabolic diseases,

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collagen diseases, and neoplasia, a phenomenon that the authors called red cell "sludging." Almost two decades later, Madow B.P. observed blood circulation changes—increased linear velocities of blood flow, reduction in the number of vessels plugged with red cells aggregates, and reduction in the size of such aggregates—in the conjunctiva of patients diagnosed with vascular diseases and treated with hydroxychloroquine and suggested that the antimalarial drug could have a "desludging" effect.[10]

Based on these results, Carter A. E. et al.[11] postulated that this "desludging" effect could also decrease the propensity of platelets—and not only erythrocytes—to adhere and form clots, reducing the incidence of deep venous thrombosis (DVT) and pulmonary embolism (PE). The authors were the first to suggest, in a randomized controlled trial (RCT), that hydroxychloroquine could significantly reduce the incidence of postoperative DVT.[11] Since then, other observational and experimental studies have assessed the efficacy of hydroxychloroquine in the prevention of thrombosis in different clinical scenarios, and even a systematic review of observational studies (without a meta-analysis) suggested that antimalarials may have a protective effect against thrombotic events in lupus patients.[12]

Considering their low cost, general good safety profile,[13] the body of literature already published and the unavailability of a systematic review of RCTs on this theme, we decided to systematically review the efficacy of chloroquine and hydroxychloroquine in the prevention of venous and arterial thromboembolic events.

Objectives

The objective of this systematic review is to estimate the risk ratio (RR) of thromboembolic events in hydroxychloroquine and chloroquine users compared to non-users without restrictions to age, disease of interest, or clinical setting.

Methods

This systematic review and meta-analysis was conceived according to the Preferred Reporting Items for Systematic Review and Meta-analysis (PRISMA) 2015.[14] Its checklist is provided as a supplementary file and its protocol is available online at the PROSPERO database (identifier CRD42021247902).

Inclusion criteria

We included only RCTs reporting the occurrence of thromboembolic events—DVT, stroke, myocardial infarction (MI) and PE—in patients treated with hydroxychloroquine or chloroquine compared to placebo, any other drug, or no drug, without any restrictions to the disease being studied, age, gender, type of setting, comparator, or language of publication.

Information source and search strategy

We searched the MEDLINE and EMBASE databases from inception to the present using medical subject headings (MeSH) and combinations of key terms. We also checked reference lists and citations of all primary studies, review articles and systematic reviews for additional references and ClinicalTrials.gov for unpublished data. Our MEDLINE and EMBASE search strategy are provided as a Supplementary file.

Study record and selection process

The studies obtained through the literature search process were uploaded to the Covidence online tool and Zotero reference manager. FLBE, RG, DR and LFS independently screened for relevant studies, all titles and abstracts, according to our eligibility criteria, and then retrieved the full text of the selected ones. The same authors reviewed the full-text articles for inclusion and exclusion, recording the reasons for the last. All the disagreements were resolved through discussion among the review authors.

Data collection process

We designed a customized extraction form, which FLBE, RG, DR, and LFS used independently to extract relevant data from eligible studies. The extracted data were compared, and all discrepancies resolved through discussion.

Risk of bias of individual studies

Two review authors (FLBE and SG) assessed independently the risk of bias in individual studies using the revised Cochrane tool for accessing risk of bias in randomized trials (RoB tool 2).[15] This tool provides a framework for assessing risk of bias in five different domains (1. bias arising from the randomization process, 2. bias due to deviations from intended interventions, 3. bias due to missing outcome data, 4. bias in measurement of the outcome and 5. bias in selection of the reported result). All disagreements were resolved through discussion and the results of this analysis are provided as a table (Table 1—supplementary material).

Data synthesis

We expected high heterogeneity between the studies, as we decided to include RCTs studying different diseases (postoperative thrombosis, APS, etc.), using different dosing regimens of antimalarial drugs and different comparators. Hence, we used a random effects model meta-analysis using R (version 4.1.2) with the META package.

We calculated the RR—and its standard error—of developing thromboembolic events in antimalarials users compared to non-users in each individual study and pooled the results using the Mantel–Haenszel method.[16]

We assessed heterogeneity through T^2, I^2 and its p values and assumed 25, 50 and 75% for I^2 as representative of low, medium, and high heterogeneity.[17] We also assessed publication bias using funnel plotting and Egger's regression,[18] and conducted a sensitivity analysis to confirm that no single study has driven our results.

Results

Study selection

Our search strategy generated 246 records, from which 36 duplicates were removed. We screened 210 titles and abstracts, considered 160 studies as irrelevant and excluded them from our analysis. We retrieved 50 articles for full-text appreciation from which 13 met our eligibility criteria and were included in our meta-analysis. A PRISMA flowchart detailing the study selection process is provided (Figure 1).

We found a total of 12 RCTs registered at Clinicaltrials.gov evaluating the use of hydroxychloroquine for the prevention of thromboembolic events. Two of those studies had already been published and were found through our search in MEDLINE and EMBASE databases, one study was prematurely terminated and another one was withdrawn. Two RCTs had their status classified as unknown. The remaining RCTs are still recruiting patients or not yet recruiting patients. None of these studies disclosed any preliminary results and were not included in our analysis.

Study characteristics

We evaluated a total of 2663 individuals (51% males) from the general population with a follow-up period ranging from 7 to 936 days. All the studies used hydroxychloroquine—we did not find a single RCT using chloroquine—with daily doses ranging from 200 mg to 1200 mg. Eleven studies were conducted in a hospital setting while only two studies evaluated outpatients.

The effectiveness of hydroxychloroquine as an anticoagulant was assessed in different scenarios: eight RCTs evaluated its use for postoperative thrombosis prophylaxis, 2 RCTs for prophylaxis of thrombosis in APS patients, and one study evaluated the use of hydroxychloroquine for the prevention of thrombosis after major fractures. We also included two RCTs which evaluated the effectiveness of hydroxychloroquine for the treatment of COVID-19 and disclosed data on thromboembolic events.

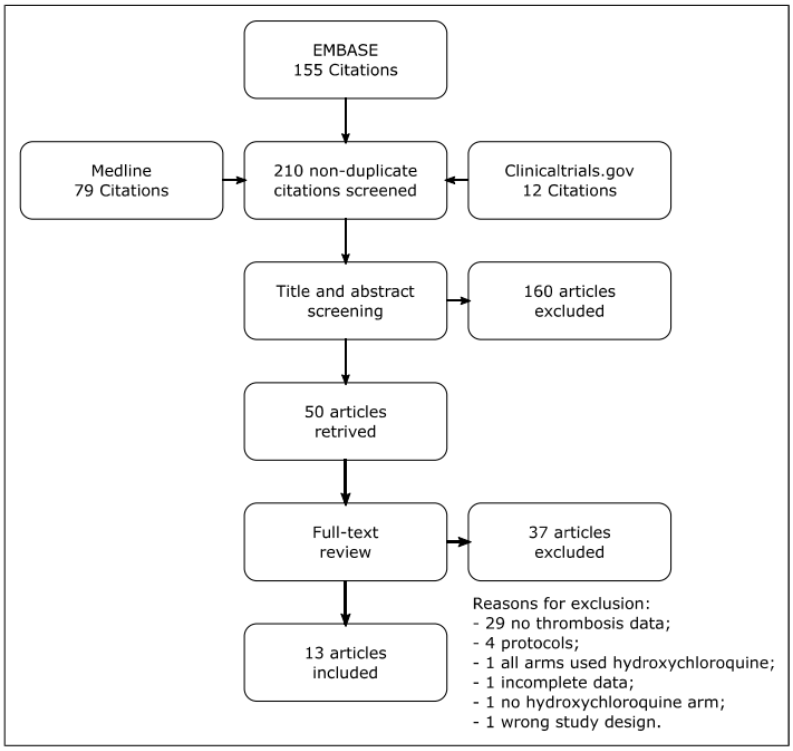

Figure 1. PRISMA flowchart.

A total of 325 thromboembolic events were reported (268 DVTs, 50 PE, 3 MI, and 4 strokes) and the main characteristics of the studies included in our analysis are summarized in Table 2 (supplementary file).

Meta-analysis

We pooled the results of 13 RCTs (as one study presented 0 events in intervention and control arms, it did not appear in the meta-analysis) and found a significant reduction in the RR of thromboembolic events in hydroxychloroquine users (RR 0.51 [CI 95% 0.31–0.84]) with a medium heterogeneity (I^2 = 67% and T^2 = 0.4948) (Figure 2).

Subgroup analysis

We grouped the RCTs according to the disease to which hydroxychloroquine effectiveness was being assessed and compared the potential anticoagulant effect of hydroxychloroquine in COVID-19 patients against the remaining diseases.

Only two RCTs studying COVID-19 fulfilled our eligibility criteria and yielded a pooled RR of 0.83 (CI 95% 0.30–2.30) against 0.44(CI 95% 0.24–0.81) for the remaining 10 RCTs. The test for subgroup difference did not yield a significant difference (Figure 3).

Evaluation of quality in individual studies

We considered the risk of bias as low in 4 studies and with some concerns in 8 studies (Table 2—supplementary file). Nine studies did not clearly state if the allocation sequence was concealed until participants were enrolled and assigned to interventions, five studies did not mask patients nor assessors and only three studies published a pre-specified analysis plan and clearly stated the use of an intention to treat analysis (ITT). These aspects increased the risk of bias in the randomization process, deviation from intended interventions and selection of the reported result domains.

We performed a subgroup analysis stratifying the RCTs according to their risk of bias, but no statistically significant difference was found (Figure 4).

Publication bias

Although the visual inspection of the funnel plot suggested some asymmetry (Figure 5), it was not confirmed through a weighted linear regression of the treatment effect on its standard error (Egger's test) (p-value = 0.1025).

Sensitivity analysis

We performed a sensitivity analysis excluding studies considered outliers—studies with a 95% confidence interval that lied outside the 95% confidence interval of the pooled effect—from the analysis. The study from Carter, A.E. et al.[11] was considered as outlier and was excluded from the analysis. Then, the pooled result was recalculated yielding a RR of 0.69 (IC 95% 0.52–0.92) with less heterogeneity (I^2 = 44%) (Figure 6).

Discussion

We found a statistically significant reduction in the RR of thromboembolic events in hydroxychloroquine users when compared to non-users. Our findings reinforce the current knowledge that the use of hydroxychloroquine may benefit patients with high risk for thromboembolic events, potentially as adjunctive therapy.

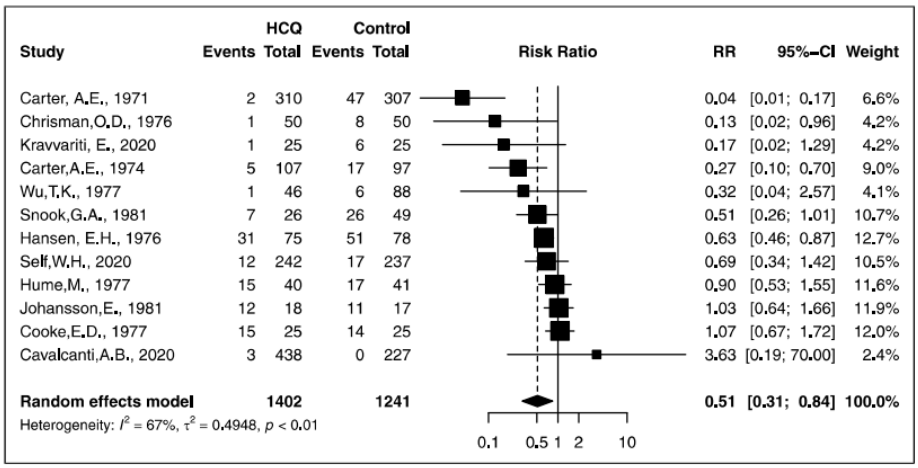

Figure 2. Forest plot of the pooled RR of thromboembolic events in hydroxychloroquine users compared to non-users.

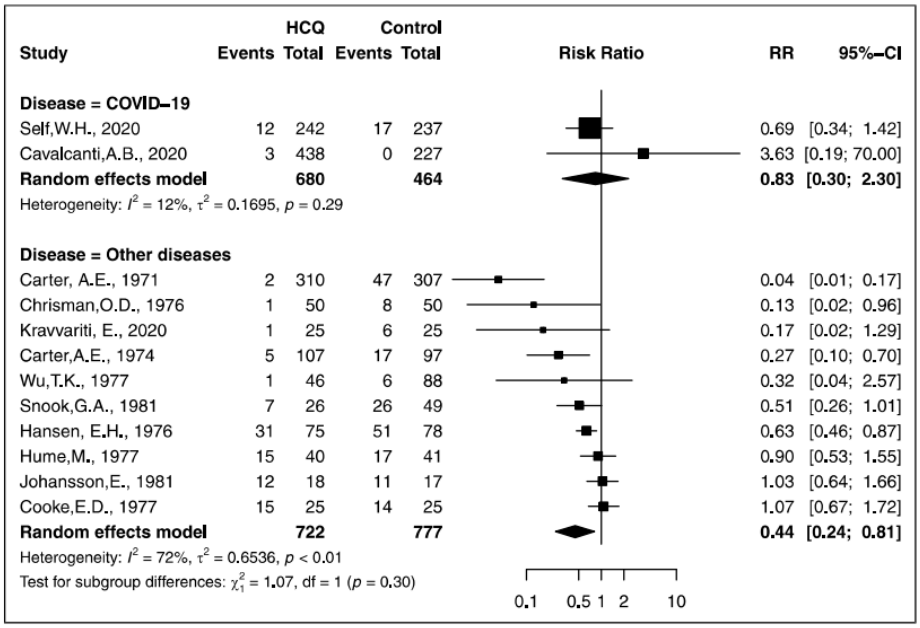

Figure 3. Forest plot of the pooled RR grouped by disease in hydroxychloroquine users and non-users (COVID-19 versus other diseases).

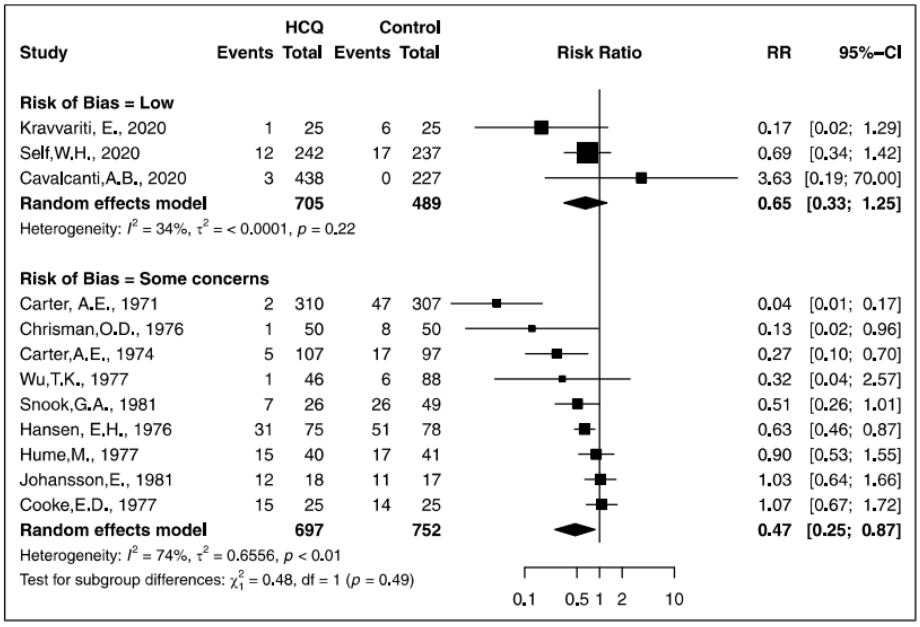

Figure 4. Forest plot of the pooled RR grouped by risk of bias.

Most of the studies evaluating the potential use of hydroxychloroquine as an anticoagulant were conducted during the 70s and 80s, after which a gap of nearly 40 years remained without any publication.

Erkan D. et al.[19] prematurely terminated in 2015 their international, multicenter, RCT that attempted to assess the efficacy of hydroxychloroquine for primary thrombosis prevention in persistently antiphospholipid (APL)

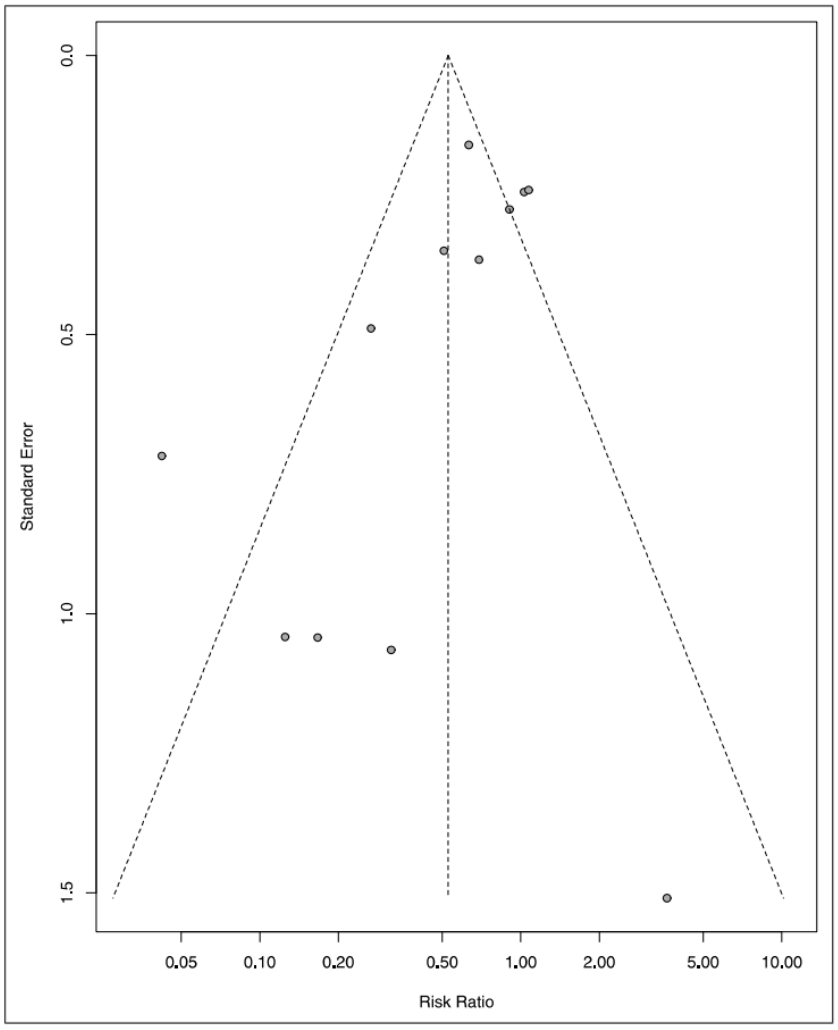

Figure 5. Funnel Plot.

antibodies positive patients with no other systemic autoimmune diseases. The reasons that led the authors to such decision were the low recruitment rate, prolonged manufacturing shortage and significant price increase of hydroxychloroquine in the United States. One hospital, though, obtained approval to continue the study as a single-center and published their results in 2020.[8] The authors found that hydroxychloroquine use was associated with an 85% reduction in the incidence rate of thrombosis, which until the date this manuscript was written, was the last RCT to primarily evaluate hydroxychloroquine use for the prevention of thromboembolic events.

The European Alliance of Associations for Rheumatology (EULAR) recommends the addition of hydroxychloroquine in women with "criteria" obstetric APS with recurrent pregnancy complications despite combination treatment with low-dose aspirin and heparin at prophylactic dosage though the authors advise that the evidence supporting such recommendation is based on two small observational studies with little representativeness.[20] The authors also propose, as a research agenda, the evaluation of the role of hydroxychloroquine as adjunctive treatment for primary thrombosis prevention in subjects with high-risk APL profile and in patients with history of first or recurrent arterial thrombosis.

Although the results of our analysis are encouraging, some limitations of our study should be addressed. Seven of the thirteen RCTs included in our analysis were conducted in the 70s and used I-125-tagged fibrinogen scanning and clinical exam for the diagnosis of DVT—an outdated diagnostic procedure—whose lack of specificity could have biased our results.

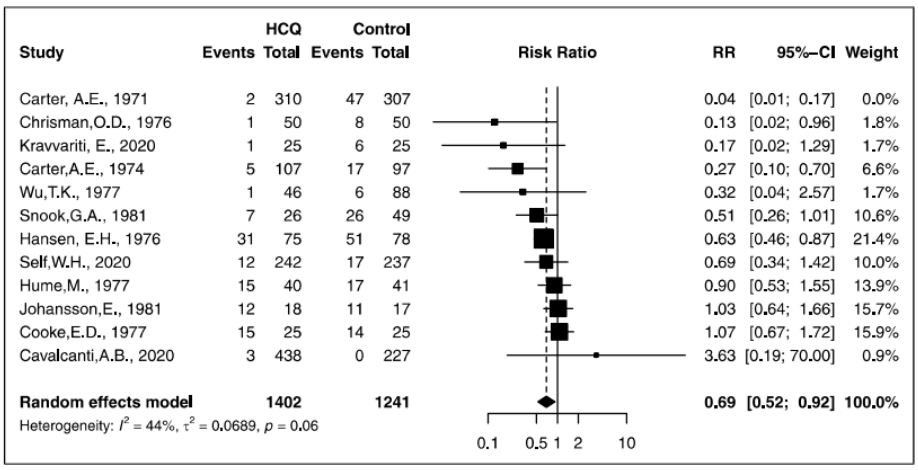

Figure 6. Sensitivity analysis plot after exclusion of outliers.

Two RCTs included in our study were not primarily designed to evaluate the risk of thromboembolic events—they assessed the efficacy of hydroxychloroquine for the treatment of COVID-19—but their exclusion from our analysis did not significantly alter the intensity nor the direction of the pooled effect.

We found considerable heterogeneity (I^2 = 67%, T^2 = 0.4948) in our primary analysis, possibly due the fact that we pooled RCTs conducted in different clinical scenarios and using different dosing regimens. We then performed a sensitivity analysis to explore such heterogeneity. The analysis deemed one of the RCTs as an outlier, and after repeating our main analysis with the exclusion of that study, the direction of the pooled effect remained the same (RR 0.69[CI 95% 0.52–0.92]), though with less heterogeneity (I^2 = 44%, T^2 = 0.0689).

Even with such limitations, our study has strengths that are worth mentioning. To our best knowledge, this is the first systematic review primarily assessing the efficacy of hydroxychloroquine for the prevention of thromboembolic events in different clinical scenarios. We included data from RCTs only and conducted a random effects meta-analysis estimating the RR of thromboembolic events in hydroxychloroquine users compared to non-users.

Hydroxychloroquine has a relatively low cost and a good safety profile. Such characteristics combined with the current trend on drug repurposing and the results our study may encourage new RCTs to primarily reassess the effectiveness of hydroxychloroquine as an anticoagulant with current diagnostic methods.

Conclusion

Our results suggest that hydroxychloroquine users have a lower risk of developing thromboembolic events compared to non-users. Considering that most of the RCTs included in our analysis used outdated methods for the diagnosis of thromboembolic events, we encourage new RCTs re-assessing our results utilizing more advanced coagulation diagnostic techniques.

Author Contributions

FLBE conceptualized, drafted, and is the guarantor of this manuscript.

FLBE, SG, RG, LFS, and DR developed and conducted the study selection strategy, the risk of bias assessment strategy and data extraction.

MBS and SG reviewed the manuscript.

All authors read, provided feedback, and approved this systematic review.

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Supplemental Material

Supplemental Material—Efficacy of hydroxychloroquine in the prevention of thromboembolic events: A systematic review and meta-analysis

Supplemental material for Efficacy of hydroxychloroquine in the prevention of thromboembolic events: A systematic review and meta-analysis by Fernando Luiz Barros Edington, Daniel Fraga de Rezende, Luis Fernando Simões dos Santos, Rayssa Valandro Garcia, Sandra Rocha Gadelha, and Mittermayer Barreto Santiago in Lupus

Availability of data and materials

The authors declare that this systematic review is an original work, and all the data will be available from the corresponding author on request.

Supplemental Material

Supplemental material for this article is available online.

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6 DISCUSSÃO

6.1 Segurança do uso da CLQ/HCLQ

Nossos dados indicam um maior risco de EA gerais em usuários de CLQ e HCLQ quando comparados aos não usuários [IRR 1.15 (IC95% 1,01 – 1,31)], e esse risco, se mostrou 83% mais elevado nos pacientes portadores da COVID-19 (IRR 1.83 [CI95% 1.22 - 2.73]). Todavia os EA observados são, em sua grande maioria, leves e autolimitados.

Encontramos um risco significativamente maior de efeitos adversos gastrointestinais em usuários de CLQ e HCLQ portadores de COVID-19 (IRR 2.65[CI 95% 1.60– 4.39]). Dentre os efeitos adversos relacionados ao uso de antimaláricos, os gastrointestinais são os mais frequentes, embora os oftalmológicos e cardiovasculares sejam os mais temidos (38). Mao *et al.* estimaram, em uma revisão sistemática, que 15% dos pacientes infectados pelo SARS-CoV-2 apresentaram efeitos adversos gastrointestinais, sendo náuseas, vômitos, diarreia e perda de apetite os mais frequentes (109). Acreditamos que a associação entre uso dos antimaláricos e outras drogas utilizadas para o tratamento da COVID-19 (como azitromicina, por exemplo) e a própria infecção pelo SARS-CoV-2 explicariam essa maior incidência de EAs gastrointestinais.

Observamos uma menor incidência de efeitos adversos dermatológicos em pacientes portadores de lúpus eritematoso, um achado que reforça o conhecimento já bem estabelecido dos efeitos benéficos destas medicações no tratamento de lesões cutâneas de pacientes portadores da doença (87,110,111).

Não foi relatada uma única morte relacionada ao uso de CLQ ou HCLQ nos ECRs que incluímos em nossa meta-análise e nem observamos um maior risco de EAs cardiovasculares em usuários de CLQ/HCLQ (IRR 1.07 [0.84 – 1.35]). Todavia, foram reportados quatro episódios de taquicardia ventricular, dois episódios de fibrilação ventricular e um infarto agudo do miocárdio em pacientes usuários de HCLQ, embora os autores não tenham relacionado esses eventos ao uso dos antimaláricos (25,31).

Nossos dados sugeriram um maior risco de EA [IRR 1.86 (CI95% 1.22 – 2.85)] em pacientes que utilizaram doses de antimaláricos mais altas que as usuais (> 10

mg/kg/dia de CLQ ou >400 mg/dia de HCLQ). Cinco dos seis ECRs que estudaram pacientes com COVID-19, utilizaram doses de antimaláricos acima das usuais.

No passado, os antimaláricos já foram utilizados com sucesso no tratamento de algumas arritmias (112), porém numa revisão sistemática recente, Tleyjeh *et al.* sugeriram que o uso de antimaláricos em pacientes portadores de COVID-19 estaria associado com um importante risco de prolongamento do intervalo QT e uma incidência relativamente aumentada de *torsades de pointes*, taquicardia ventricular ou parada cardíaca (101).

Chen *et al.* observaram hipopotassemia em 85% dos pacientes graves infectados pelo SARS-CoV-2, enquanto Shi *et al.* relataram que 19,7% dos pacientes com infecção confirmada pelo SARS-CoV-2 desenvolveram complicações cardiovasculares durante o internamento hospitalar (113,114). É possível que a toxicidade cardíaca observada por Tleyjeh *et al.* esteja relacionada ao vírus SARS-CoV-2, ao estado crítico que se encontrava o paciente ou ao uso de elevadas doses de antimaláricos, e não ao uso dos antimaláricos per si.

Esse estudo tem pontos fortes que merecem destaque. Esta é a primeira revisão sistemática a analisar dados de segurança sobre o uso da CLQ e HCLQ utilizando exclusivamente ECRs de 4 doenças diferentes realizados nos últimos 10 anos. Além disso, nós conduzimos uma meta-análise de efeitos randômicos e conseguimos estimar a IRR de EA (gerais e categorizados por sistemas) em usuários e não-usuários de CLQ e HCLQ.

Todavia algumas limitações deste estudo devem ser levadas em consideração ao analisar os nossos resultados. Embora tenha sido prevista, encontramos uma elevada heterogeneidade em nossa análise principal, que pode ser atribuída às diferentes doenças e desfechos primários dos ECRs analisados, assim como as diferentes drogas, doses, variados períodos de seguimento etc. Embora tenhamos observado a presença de viés de publicação no gráfico em funil e na regressão de Egger, o ajuste dos nossos resultados utilizando o método de "*trim and fill*" mostraram que as nossas estimativas subestimaram a real IRR de EA

6.2 Eficácia da HCLQ na prevenção de eventos tromboembólicos

Nossos dados sugerem uma redução estatisticamente significativa no RR de eventos tromboembólicos em usuários de HCLQ quando comparamos com os não usuários. Esses achados reforçam a noção atual de que a HCLQ pode beneficiar

pacientes de alto risco para eventos tromboembólicos, potencialmente como uma terapia adjuvante.

O uso da HCLQ é inclusive indicado nas recomendações do EULAR para tratamento de mulheres com a forma obstétrica da SAAF a despeito da terapia combinada de aspirina em baixas doses e heparina em doses profiláticas (33). Todavia, os autores advertem que esta recomendação é baseada em apenas dois estudos observacionais com representatividade reduzida.

Nossos resultados estão em consonância com estudos realizados na década de 70, que sugeriam um efeito protetor na HCLQ no desenvolvimento de eventos tromboembólicos pós operatórios (4,6,7,9). O efeito anticoagulante da HCLQ também foi demonstrado em pacientes portadores do lúpus eritematoso e SAAF (16,86).

Esta é a primeira revisão sistemática a analisar exclusivamente ECRs com o objetivo primário de estimar a eficácia da HCLQ na prevenção de eventos tromboembólicos em diferentes cenários clínicos.

Embora nossos resultados sejam bastante animadores, vale destacar que sete dos treze estudos incluídos na nossa meta-análise foram realizados na década de 70 e utilizaram cintilografia com fibrinogênio marcado com I-125 e exame clínico para o diagnóstico de TVP, uma técnica diagnóstica ultrapassada cuja falta de especificidade pode ter enviesado nossas análises.

Outra limitação importante que deve ser levada em consideração ao interpretar nossos resultados, é a alta heterogeneidade que observamos em nossa análise principal (I^2=75%, T^2=0.37). Exploramos essa elevada heterogeneidade através da análise de sensibilidade e encontramos um possível “*outlier*”, após sua exclusão da análise, conseguimos reduzir sensivelmente a heterogeneidade (I^2=49%, T^2=0.11).

7 CONCLUSÕES

7.1 Conclusões do artigo 1

Nossos dados reforçam o entendimento de que tanto a CLQ quanto a HCLQ possuem um bom perfil de segurança embora o uso de doses acima das usuais em pacientes hospitalizados por COVID-19 deva ser evitado.

7.2 Conclusões do artigo 2

Nossos resultados sugerem que usuários de HCLQ têm um menor risco de desenvolver eventos tromboembólicos quando comparados a não usuários. Considerando que a maioria dos estudos incluídos em nossa análise utilizou técnicas diagnósticas ultrapassadas para detectar esses eventos, esperamos que nossos resultados encorajem a realização de novos ECRs reavaliando estes resultados e utilizando técnicas diagnósticas correntes.

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APÊNDICES

Apêndice A – Protocolo artigo 1

Safety of treatment with Chloroquine and Hydroxychloroquine: A protocol for a systematic review and meta-analysis.

1 INTRODUCTION:

In December 2019 a new virus was identified following a cluster of patients with pneumonia of unknown cause who were epidemiologically linked to a seafood market in the city of Wuhan, China (1). This new highly contagious RNA virus was later named severe acute respiratory syndrome Coronavirus 2 (SARS-CoV-2) and has spread fast since its identification. According to World Health Organization (WHO), by May 2020 new coronavirus infections have surpassed 5 million cases and 357688 deaths were confirmed around the world (2).

Currently, there is no specific treatment or vaccine for this new coronavirus disease (COVID-19) though some drugs have been used by extrapolation from similar diseases, as chloroquine and hydroxychloroquine. Savarino et al. was the first to suggest in 2003 that both drugs could be of some use in the treatment of another coronavirus, responsible for the SARS-CoV, another severe respiratory disease that emerged in China in 2002 (3). Keyaerts et al. and Vincent et al. later demonstrated in vitro anti-virus effect of chloroquine phosphate against SARS-CoV infected Vero E6 cells (4,5). Wang et al. and Yao et al. have evaluated in vitro the effects of chloroquine (6,7) and hydroxychloroquine (7) against SARS-CoV-2 infected Vero E6 cells and both have confirmed the viral inhibitory effect of the drugs.

The promising in vitro effects of chloroquine and hydroxychloroquine inspired researchers to evaluate if the results could be replicated in clinical trials. A randomized controlled pilot study conducted by Chen et al. found no significative difference in negative conversion rate, median duration from hospitalization, median time for temperature normalization or adverse effects in 15 patients treated with hydroxychloroquine compared to conventional treatment (8). Huang et al. have studied chloroquine against a combination of lopinavir and ritonavir in small randomized controlled study with 22 patients. The authors have found faster negative conversion rate and quicker recovery time in the chloroquine group, without serious side effects

(9). Gautret and colleagues also found faster negative conversion rates in patients treated with hydroxychloroquine. Mehra et al. in a recent multinational registry analysis studied 96032 patients and found no benefit in the treatment of COVID-19 with chloroquine or hydroxychloroquine alone or in combination with a macrolide, instead the authors suggested an increased risk of in-hospital mortality and ventricular arrythmia when treating COVID-19 patients with either drug (10).

These preliminary results have generated debate over the world about efficacy and safety of antimalarial drugs for the treatment of COVID-19. WHO does not officially recommend use of chloroquine or hydroxychloroquine for COVID-19 and recently have paused an ongoing international clinical trial due to concerns of the safety of these drugs, in special the occurrence of cardiac arrythmia (11). Until now chloroquine or hydroxychloroquine have been considered safe for treatment of malaria and rheumatic diseases patients (3).

Considering these recent concerns on antimalarials safety in a COVID scenario, we decided to systematically review the safety of these drugs in for FDA approved diseases and patients infected with SARS-CoV-1 and 2.

2 OBJECTIVES

The objective of this systematic review is to estimate the risk of adverse events in patients using chloroquine or hydroxychloroquine in ambulatory and hospital settings for the treatment of Systemic Lupus Erythematosus, Chronic Discoid Lupus, Rheumatoid arthritis, malaria and SARS-COV-1 and SARS-COV-2.

3 DEFINITIONS

This systematic review and meta-analysis was conceived according to the Preferred reporting items for systematic review and meta-analysis (PRISMA) 2015 (12), its checklist will be provided as supplementary file and its protocol submitted to the PROSPERO database.

1) Inclusion Criteria:

a) Studies designs:

i) Randomized controlled trials (RCT);

b) b. Participants:

i) Studies evaluating humans' beings treated with chloroquine, hydroxychloroquine, or chloroquine diphosphate with no restrictions to age or gender.

c) Interventions:

i) Any study comparing the incidence of adverse effects between Chloroquine or Hydroxychloroquine or Chloroquine diphosphate users and non-users.

d) Comparator:

i) Any other drug or combination of drugs, except chloroquine or hydroxychloroquine or chloroquine diphosphate

e) Outcomes:

i) Any measure of association (relative ratio [RR], hazard ratio [HR] or odds ratio [OR] with its 95% confidence interval [CI]), or the absolute (or relative) number of patients who have developed adverse events in antimalarials users and non-users' groups. We will also group the adverse events in one of these categories:

(1) Cardiovascular: Palpitations, arrythmias, etc.;

(2) Gastrointestinal: Abdominal pain, nauseas, vomiting, elevated liver enzymes, diarrhea, etc.;

(3) Musculoskeletal: joint or muscle pain, elevated Creatine phosphokinase (CPK), etc.;

(4) Ophthalmological: blurred vision or any visual disturbances;

(5) Neurological: Headache, dizziness, convulsions, Insomnia, etc.;

(6) Dermatological: Skin rashes, itching, hair loss, etc.

f) Timing:

i) Any eligible publication from 2010 until the present day.

g) Setting / Language:

i) There will be no restriction based on language or type of setting.

4 INFORMATION SOURCE AND SEARCH STRATEGY:

We will search the MEDLINE and EMBASE databases from 2010 to the present, with no restrictions on language of publication, using medical subject headings (MeSH) and combinations of key terms. In addition to the databases previously mentioned, we will also check reference lists and citations of all primary studies, review articles or systematic review for additional references. We will also check ClinicalTrials.gov for unpublished data. Our Medline and EMBASE search strategy, respectively, are provided below:

Medline:

(((chloroquine [Title/Abstract]) OR (Chloroquine [Title/Abstract])) OR (hydroxychloroquine [Title/Abstract]) OR (antimalarials [Title/Abstract])) OR (Hydroxychloroquine [Title/Abstract]) Filters: Clinical Trial, Meta-Analysis, Randomized Controlled Trial, Systematic Reviews, in the last 10 years

Embase:

('chloroquine':ab,ti OR 'hydroxychloroquine':ab,ti OR 'antimalarial':ab,ti) AND ('lupus erythematosus':ab,ti OR 'rheumatoid arthritis':ab,ti OR 'malaria':ab,ti OR 'covid 19':ab,ti OR 'coronavirus':ab,ti OR 'sars coronavirus':ab,ti) AND [randomized controlled trial]/lim AND [humans]/lim AND [embase]/lim AND [2010-2020]/py

5 STUDY RECORD AND SELECTION PROCESS

The titles and abstracts obtained through the literature search process will be uploaded to the Distiller SR online tool and Mendeley reference manager. Two review authors will screen independently for relevant studies, all titles and abstracts according to our eligibility criteria, and then the full text of the selected studies will be obtained. The same two authors will screen the full text for identifying studies for inclusion and exclusion, recording the reasons for the last. Any disagreement will be resolved by discussion between the two review authors; if no agreement can be reached, a third author will arbitrate.

A flow-chart of study selection with the characteristics of excluded studies will be provided as recommended by PRISMA.

6 DATA COLLECTION PROCESS

Two review authors will independently collect the following data from every study using a customized data collection form:

h) First author name;
i) Year of publication;
j) Sample size;
k) Country;
l) Type of antimalarial (Chloroquine, Hydroxychloroquine or Chloroquine diphosphate), dosage and period of use;
m) Disease for which the antimalarial is used (Lupus, Rheumatoid arthritis, malaria or COVID);
n) Age;
o) Gender;
p) Follow-up duration;
q) Setting;
r) Number of adverse effects in antimalarials users and non-users' groups;
s) Number of patients who developed adverse effects in antimalarials users and non-user groups;

7 PRIMARY OUTCOME:

The primary outcome will be to estimate the relative risk of development of any adverse effect in chloroquine or Chloroquine Phosphate or Hydroxychloroquine users in comparison to non-users.

Secondary Outcomes:

We plan to compare the risk of developing adverse effects after using antimalarials for the treatment of COVID-19 versus Lupus Erythematosus (Systemic and Discoid), rheumatoid arthritis and malaria.

We also plan to analyze the risk of development of the main antimalarial adverse effects in categories (cardiovascular, gastrointestinal, musculoskeletal, ophthalmological, etc.) according to the type of antimalarial, dosage and period of use.

8 RISK OF BIAS OF INDIVIDUAL STUDIES:

Two review authors will assess independently the risk of bias in individual studies using the Cochrane tool for accessing risk of bias in randomized trials tool (RoB tool 2) (14) for randomized-control studies. Disagreements will be resolved first by discussion and then by consulting a third author for arbitration. A table with the individual results of these analysis will be provided.

9 DATA SYNTHESIS

If the selected studies are sufficiently homogeneous in design and comparator, we shall conduct a meta-analysis. We plan to calculate the Odds Ratio for the development of antimalarials side effects, with its 95% confidence interval, from each individual study using the raw data provided (15). Then, we will pool the individual results using a random-effects meta-analysis model.

In case of missing data for quality assessment or quantitative synthesis, we will attempt to contact the study authors by email at least two times in a two-week interval. If following this procedure, there is still insufficient data, the study will be excluded from the analysis.

Heterogeneity will be assessed through T^2 and I^2 as indices of its magnitude and Q and its p-value as measures of its uncertainty. We will assume I^2 values of 25, 50, and 75% being representative of low, medium, and high heterogeneity (16).

Subgroup analysis is predicted for comparing summary effects in studies evaluating patients with different diseases, different antimalarials, different dosages, different periods of use and different settings.

10 ETHICS AND DISSEMINATION

Ethical approval is not required for this systematic review and meta-analysis as only a secondary analysis of data already available in scientific databases will be conducted. The results of this review will be submitted for peer-reviewed publication and will be presented at relevant conferences.

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Apêndice B – Protocolo artigo 2

Efficacy of hydroxychloroquine and Chloroquine in the prevention of thrombotic events: A protocol for a systematic review and meta-analysis

1 INTRODUCTION:

Chloroquine was introduced in the early 1940's followed by hydroxychloroquine in the early 1950's for the treatment of malaria with both drugs demonstrating efficacy and good safety profiles in their respective initial studies [1,2]. In 1953, Goldman L. et al. published a series of 21 cases of lupus erythematosus successfully treated with chloroquine, and by 1957 three other studies documented hydroxychloroquine efficacy in same disease [3]. The interest in both drugs has increased over the years and studies assessing their efficacy in rheumatoid arthritis [4], hand osteoarthritis [5], lipid disorders [6], diabetes [7] and antiphospholipid syndrome [8] have been published.

In 1960, Madow B.P. observed reduction in plugging of vessels, reduction in the size of aggregates and increase in linear-flow velocities of blood in patients with coronary artery disease treated with hydroxychloroquine [9]. Based on these results, Carter A. E. et al. postulated that this "desludging" effect could decrease the propensity of platelets to adhere and form clots, reducing the incidence of deep venous thrombosis and pulmonary embolism [10].

In 1971 Carter A. E. et al. conducted the first randomized controlled trial (RCT) to test the efficacy of hydroxychloroquine in preventing postoperative deep venous thrombosis (DVT) [10]. The authors found a statistically significant reduction of postoperative DVT in patients treated with hydroxychloroquine, a finding later confirmed in a second similar trial [10]. Since then, other studies have assessed the efficacy of hydroxychloroquine in the prevention of thrombosis in different clinical scenarios but to our best knowledge none using a systematic review approach.

Considering their low cost, general good safety profile, the body of literature already published and the unavailability of a systematic review on this theme, we decided to systematically review the efficacy of chloroquine or hydroxychloroquine in the prevention of venous and arterial thrombosis.

2 OBJECTIVES

The objective of this systematic review is to evaluate the efficacy of hydroxychloroquine and chloroquine in ambulatory and hospital settings for the primary and secondary prevention of thromboembolic events in adult patients.

3 DEFINITIONS

This systematic review and meta-analysis was conceived according to the Preferred reporting items for systematic review and meta-analysis (PRISMA) 2015 [11], its checklist will be provided as supplementary file and its protocol submitted to the PROSPERO database.

4 INCLUSION CRITERIA

a) Studies designs:

i) Randomized controlled trials (RCTs).

b) Participants:

i) Studies evaluating humans with no restrictions to age or gender.

c) Interventions:

i) Studies comparing the incidence of primary or secondary thromboembolic events between chloroquine or hydroxychloroquine users and non-users.

d) Comparator:

i) Placebo, no drug or any other drug or combination of drugs, except antimalarial drugs.

e) Outcome:

i) Any measure of association (relative risk [RR], hazard ratio [HR] or odds ratio [OR] with its 95% confidence interval [CI]) or the absolute number of patients who have developed thrombotic events in antimalarials users and non-users' groups.

f) Timing, Setting and Language:

i) There will be no restriction based on timing, language, or type of setting.

5 INFORMATION SOURCE AND SEARCH STRATEGY:

We will search the MEDLINE and EMBASE databases from inception to the present, with no restrictions on language of publication, using medical subject headings (MeSH) and combinations of key terms. In addition to the databases

previously mentioned, we will also check reference lists and citations of all primary studies, review articles or systematic review for additional references. We will also check ClinicalTrials.gov for unpublished data. Our Medline and EMBASE search strategy, respectively, are provided below:

Medline:

(chloroquine OR hydroxychloroquine) AND (thrombosis OR “thrombotic events” OR “myocardial infarction” OR “myocardial ischemia” OR “cerebral infarction” OR “cerebral ischemia” OR stroke OR embolism OR “pulmonary embolism” OR “pulmonary thromboembolism” OR “sars-cov-2” OR “covid-19” OR coronavirus OR “deep venous thrombosis” OR “deep vein thrombosis”) AND (randomized controlled trial [Publication Type] OR “clinical trial” [Publication Type] OR “controlled clinical trial” [Publication Type])

Embase:

#1 `human’/exp

#2'chloroquine':ti, ab OR 'hydroxychloroquine':ti,ab

#3 'randomized controlled trial'/exp OR 'controlled clinical trial'/exp

#4 'thromboembolism'/exp OR 'thrombosis'/exp OR 'lung embolism'/exp OR 'deep vein thrombosis'/exp OR 'heart infarction'/exp OR 'cerebrovascular accident'/exp OR 'coronavirus disease 2019'/exp

#5 (#1 AND #2 AND #3 AND #4)

6 STUDY RECORD AND SELECTION PROCESS

The titles and abstracts obtained through the literature search process will be uploaded to the Distiller SR online tool and Mendeley reference manager. Two review authors will screen independently for relevant studies, all titles and abstracts according to our eligibility criteria, and then the full text of the selected studies will be obtained. The same two authors will screen the full text for identifying studies for inclusion and exclusion, recording the reasons for the last. Any disagreement will be resolved by discussion between the two review authors; if no agreement can be reached, a third author will arbitrate.

A flow-chart of study selection with the characteristics of excluded studies will be provided as recommended by PRISMA.

7 DATA COLLECTION PROCESS

Two review authors will independently collect the following data from every study using a customized data collection form:

g) First author name;

h) Year of publication;

i) Sample size;

j) Country;

k) Type of antimalarial (Chloroquine or Hydroxychloroquine), dosage and period of use;

l) Clinical scenario in which the antimalarial is used (Postoperative, Lupus, Antiphospholipid Syndrome, pregnancy, etc.);

m) Territory of the thrombotic event (Arterial or Venous);

n) Diagnosis of the thrombotic event (DVP, pulmonary thromboembolism (PTE), myocardial infarction, etc.);

o) Criteria used for the diagnosis of the thrombotic event (Clinical, ultrasound, angio-tomography, arteriography, etc.);

p) Mean age;

q) Gender;

r) Follow-up duration;

s) Setting;

t) Number of patients in antimalarials users and non-users' groups;

u) Number of thrombotic events in antimalarials users and non-users' groups;

8 PRIMARY OUTCOME

The primary outcome will be the Relative Risk (RR) for the development of any thrombotic event in antimalarial users in comparison to non-users.

9 RISK OF BIAS OF INDIVIDUAL STUDIES:

Two review authors will assess independently the risk of bias in individual studies using the Cochrane tool for accessing risk of bias in randomized trials tool (RoB tool 2) for randomized-control studies [12]. Disagreements will be resolved first by discussion and then by consulting a third author for arbitration. A table with the individual results of these analysis will be provided.

10 DATA SYNTHESIS

If the selected studies are sufficiently homogeneous in design and comparator, we shall conduct a meta-analysis. We plan to calculate the Relative Risk for the development of antimalarials side effects, with its 95% confidence interval, from each individual study using the raw data provided. Then, we will pool the individual results using a random-effects meta-analysis model.

In case of missing data for quality assessment or quantitative synthesis, we will attempt to contact the study authors by email at least two times in a two-week interval. If following this procedure, there is still insufficient data, the study will be excluded from the analysis.

Heterogeneity will be assessed through T^2 and I^2 as indices of its magnitude and Q and its p-value as measures of its uncertainty. We will assume I^2 values of 25, 50, and 75% being representative of low, medium, and high heterogeneity [13].

Subgroup analysis is predicted for comparing summary effects in studies evaluating patients with different clinical scenarios, different antimalarials, different dosages, different periods of use and different settings.

11 ETHICS AND DISSEMINATION

Ethical approval is not required for this systematic review and meta-analysis as only a secondary analysis of data already available in scientific databases will be conducted. The results of this review will be submitted for peer-reviewed publication and will be presented at relevant conferences.

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Apêndice C – Ilustrações suplementares do artigo 1

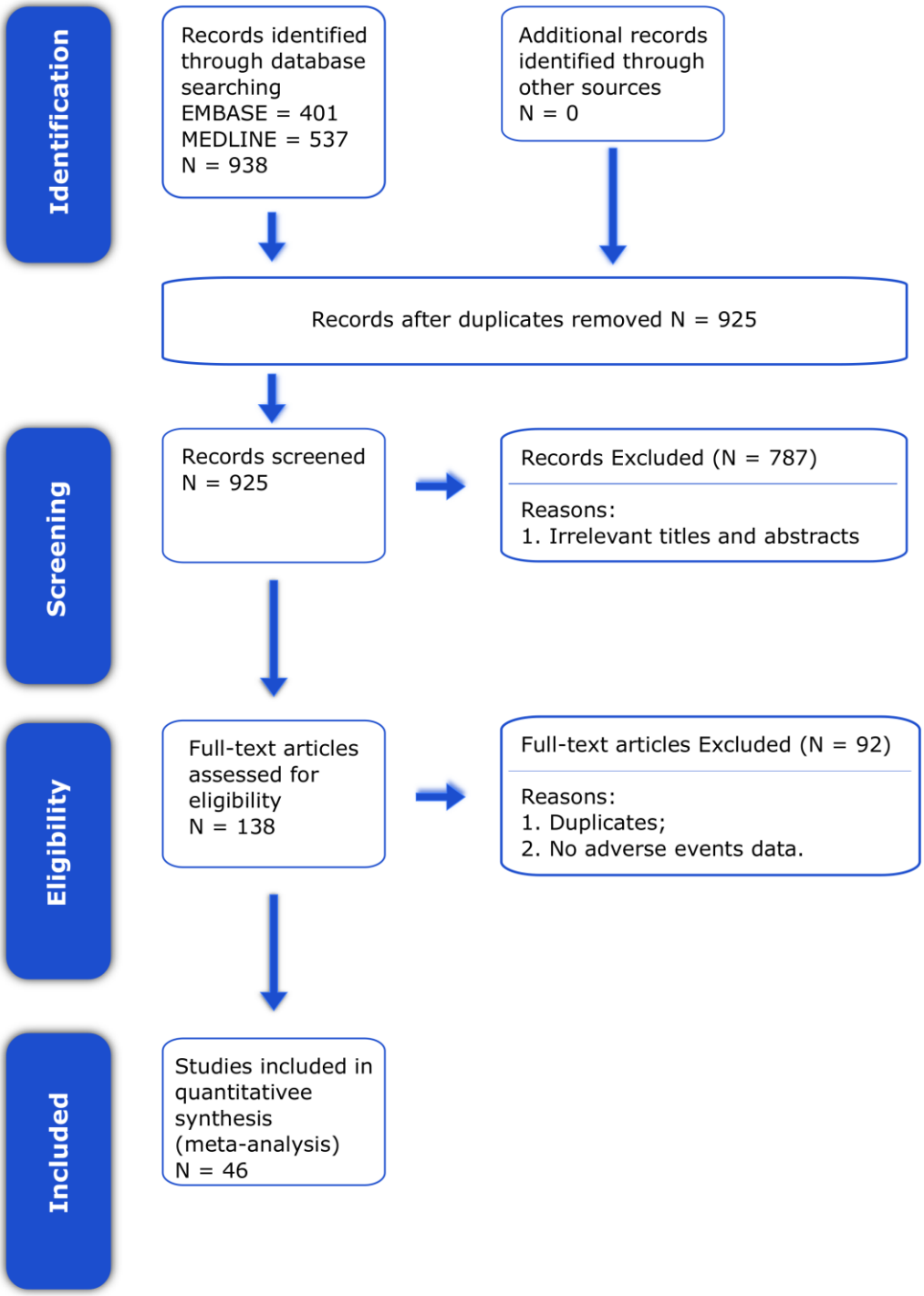

Figure S1. PRISMA flowchart.

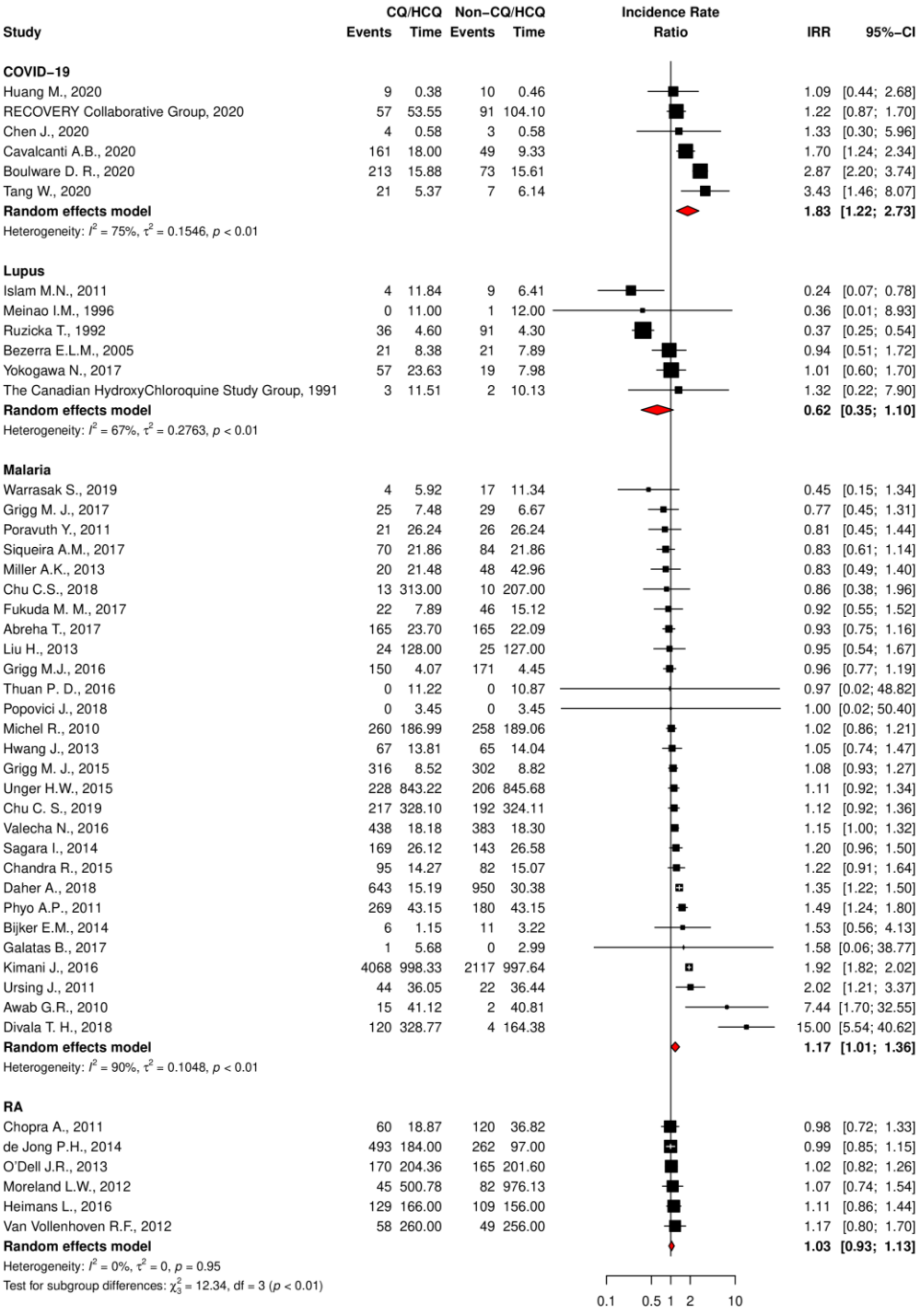

Figure S2. Forest-plot of the pooled adverse events grouped by disease in chloroquine/hydroxychloroquine users and non-users.

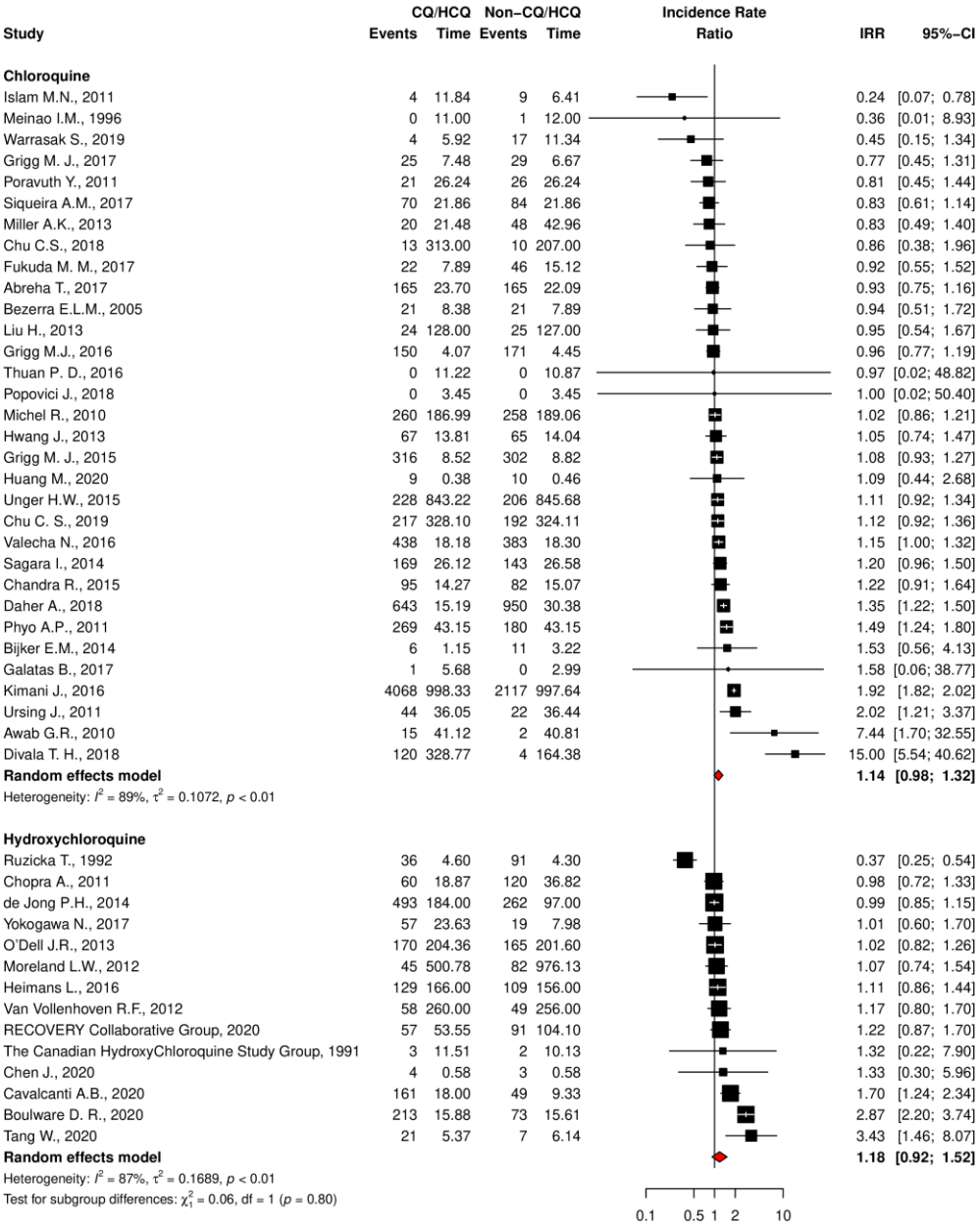

Figure S3. Forest-plot of the pooled adverse events grouped by antimalarial of choice in chloroquine/hydroxychloroquine users and non-users.

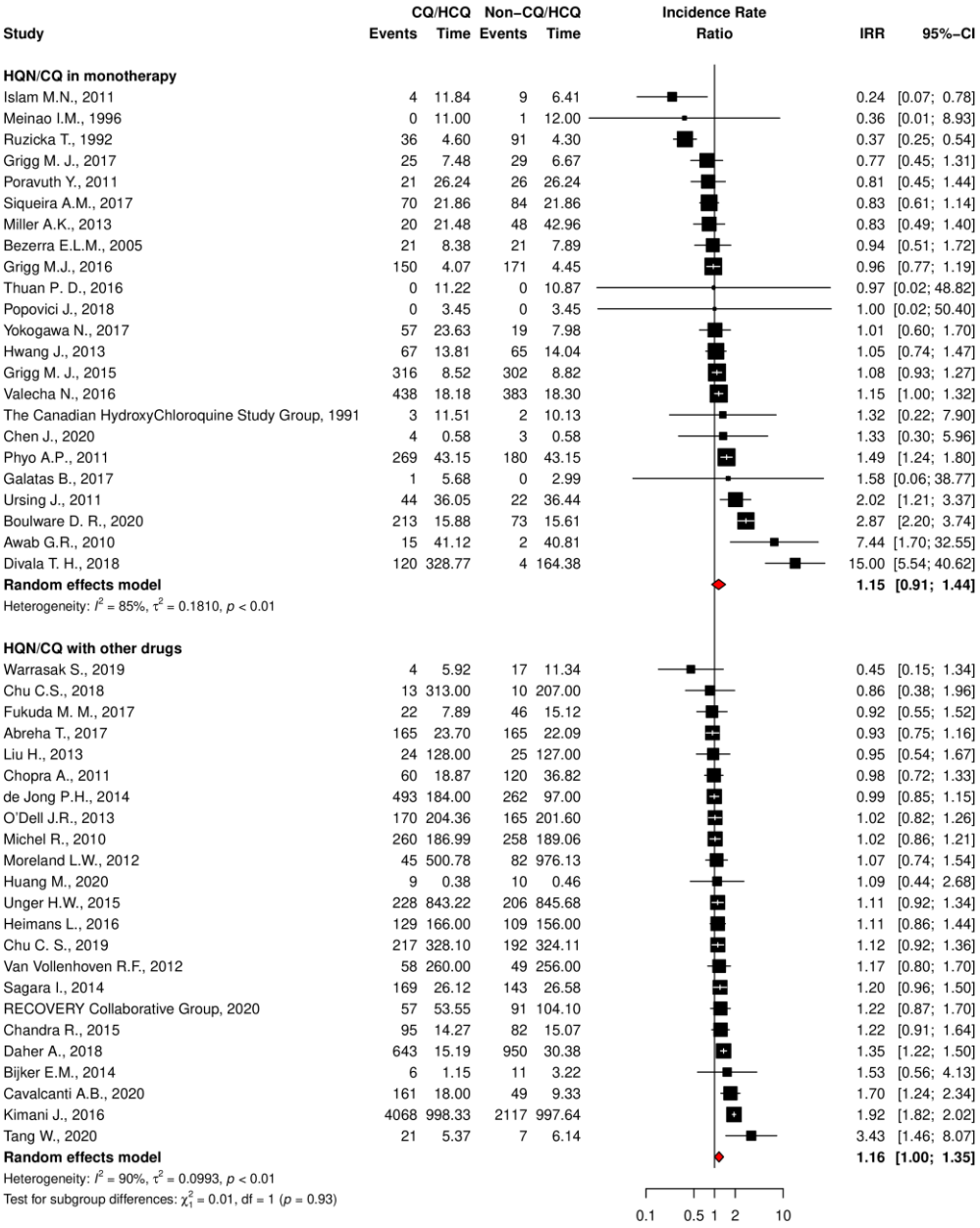

Figure S4. Forest-plot of the pooled adverse events grouped by antimalarial used alone or in combination with other drugs in chloroquine/hydroxychloroquine users and non-users.

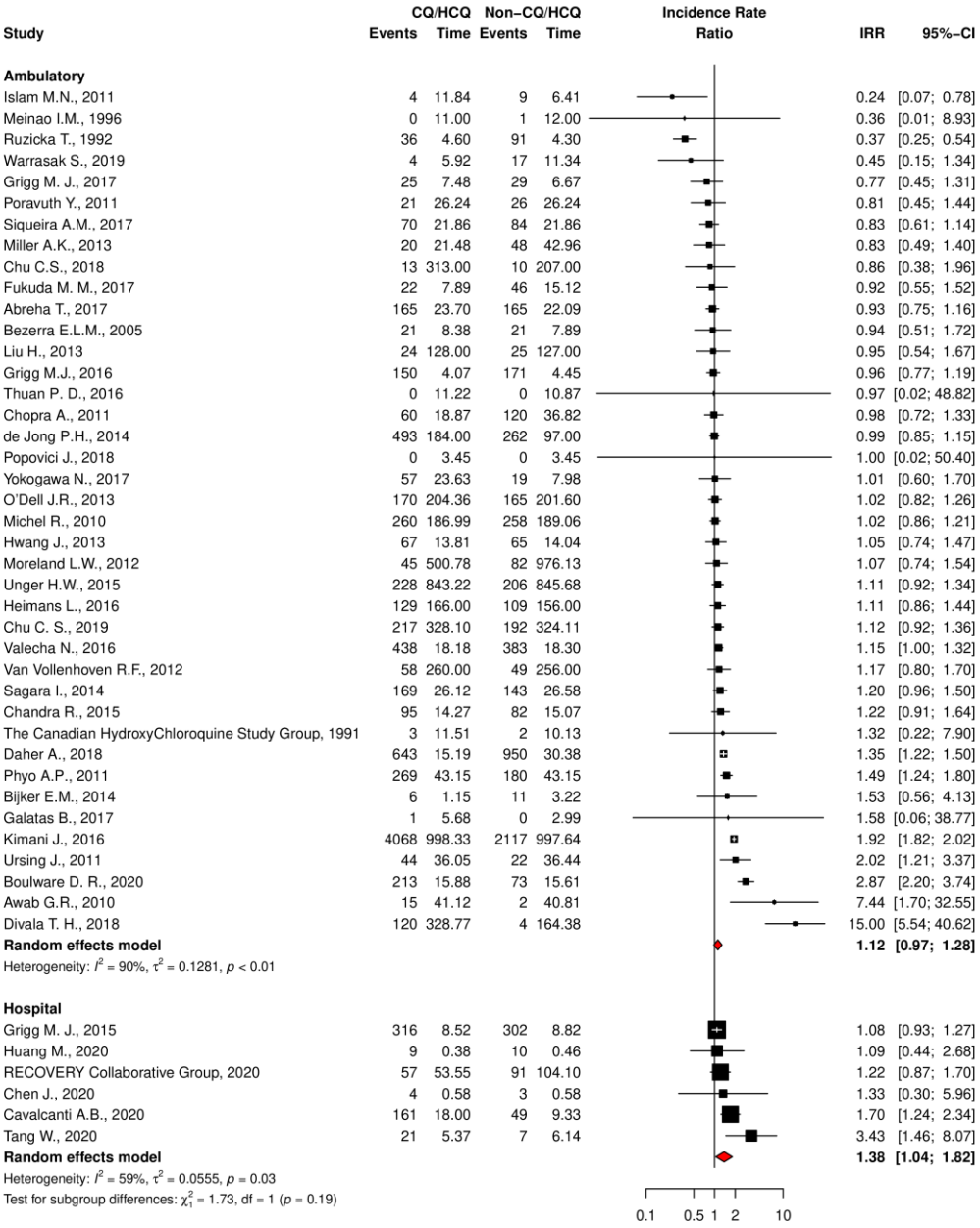

Figure S5. Forest-plot of the pooled adverse events grouped by the setting (inpatient [Hospital] versus outpatient [ambulatory]) in chloroquine/hydroxychloroquine users and non-users.

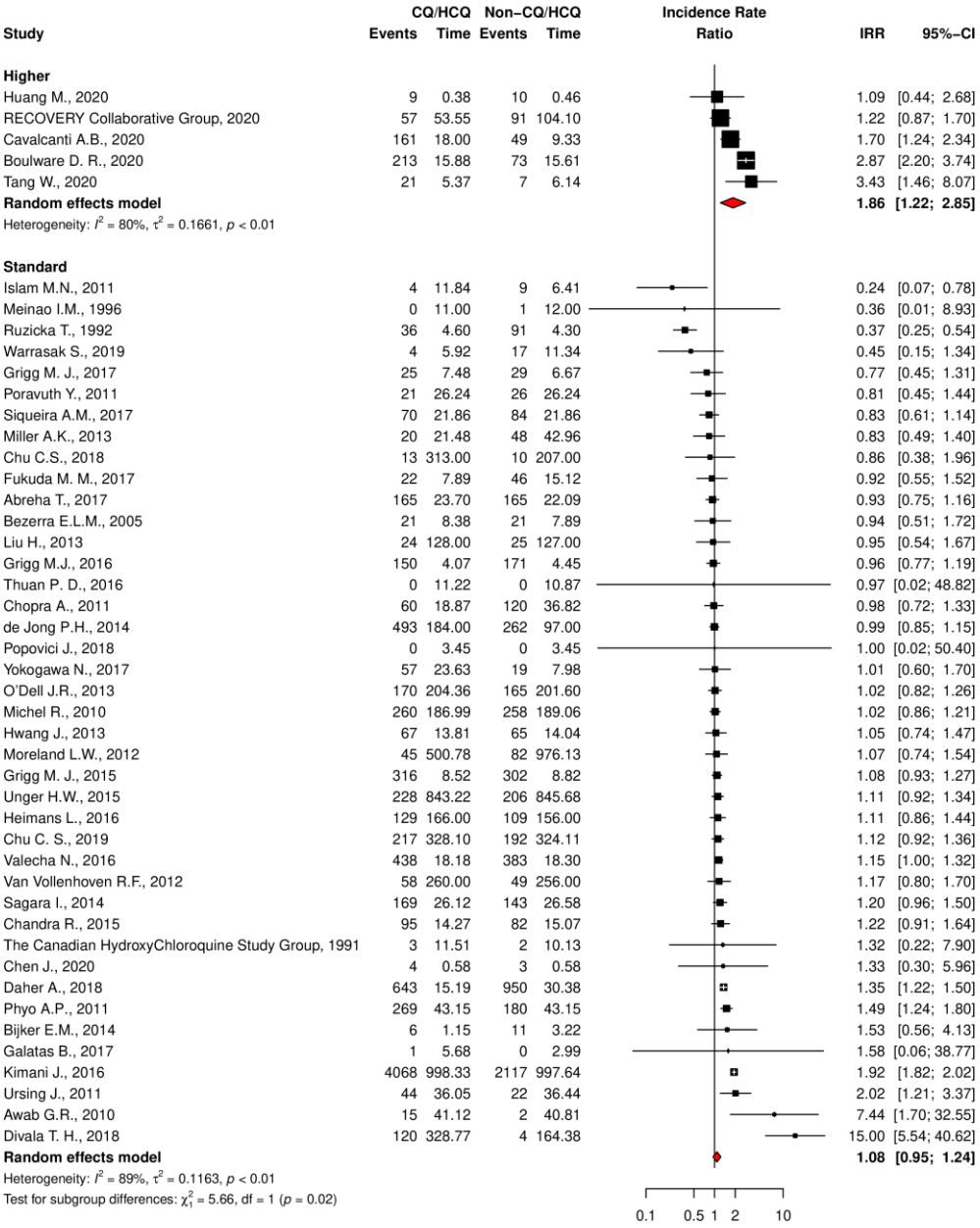

Figure S6. Forest-plot of the pooled adverse events grouped by the antimalarial dose (Usual dose versus Higher than usual) in chloroquine/hydroxychloroquine users and non-users.

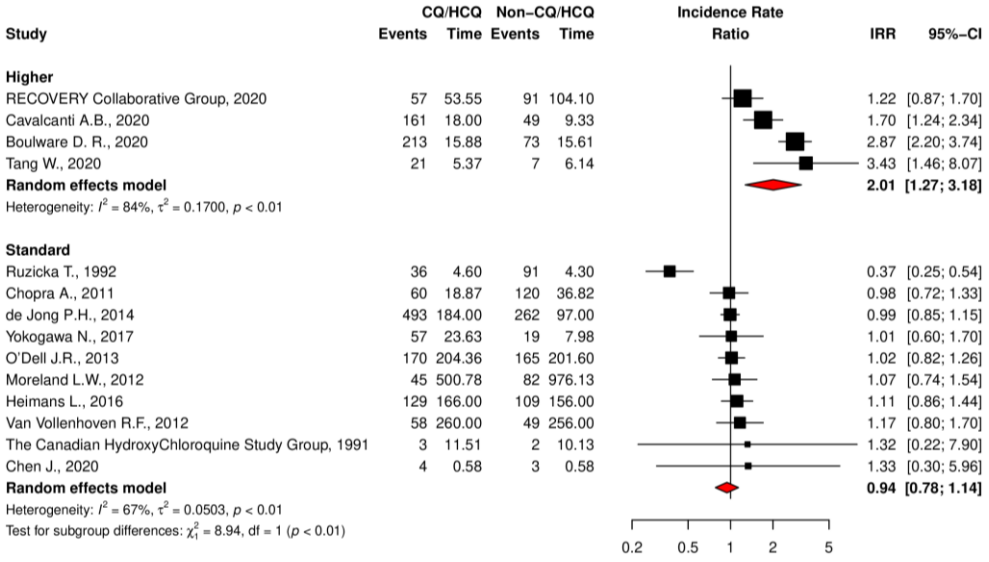

Figure S7. Forest-plot of the pooled adverse events only in hydroxychloroquine users and non-users grouped by the antimalarial dose (Usual dose versus Higher than usual).

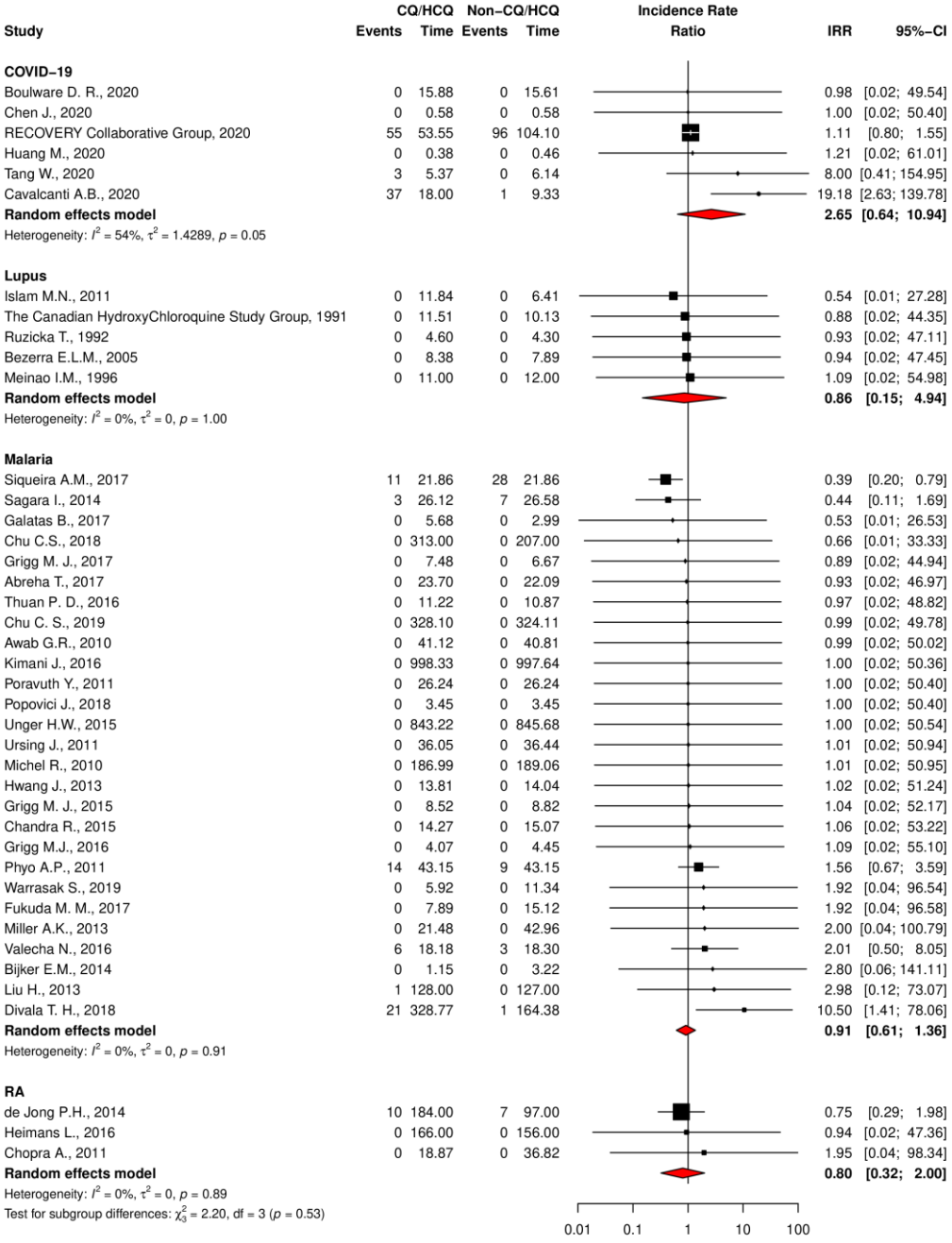

Figure S8. Forest-plot of the pooled cardiovascular adverse events grouped by disease in chloroquine/hydroxychloroquine users and non-users.

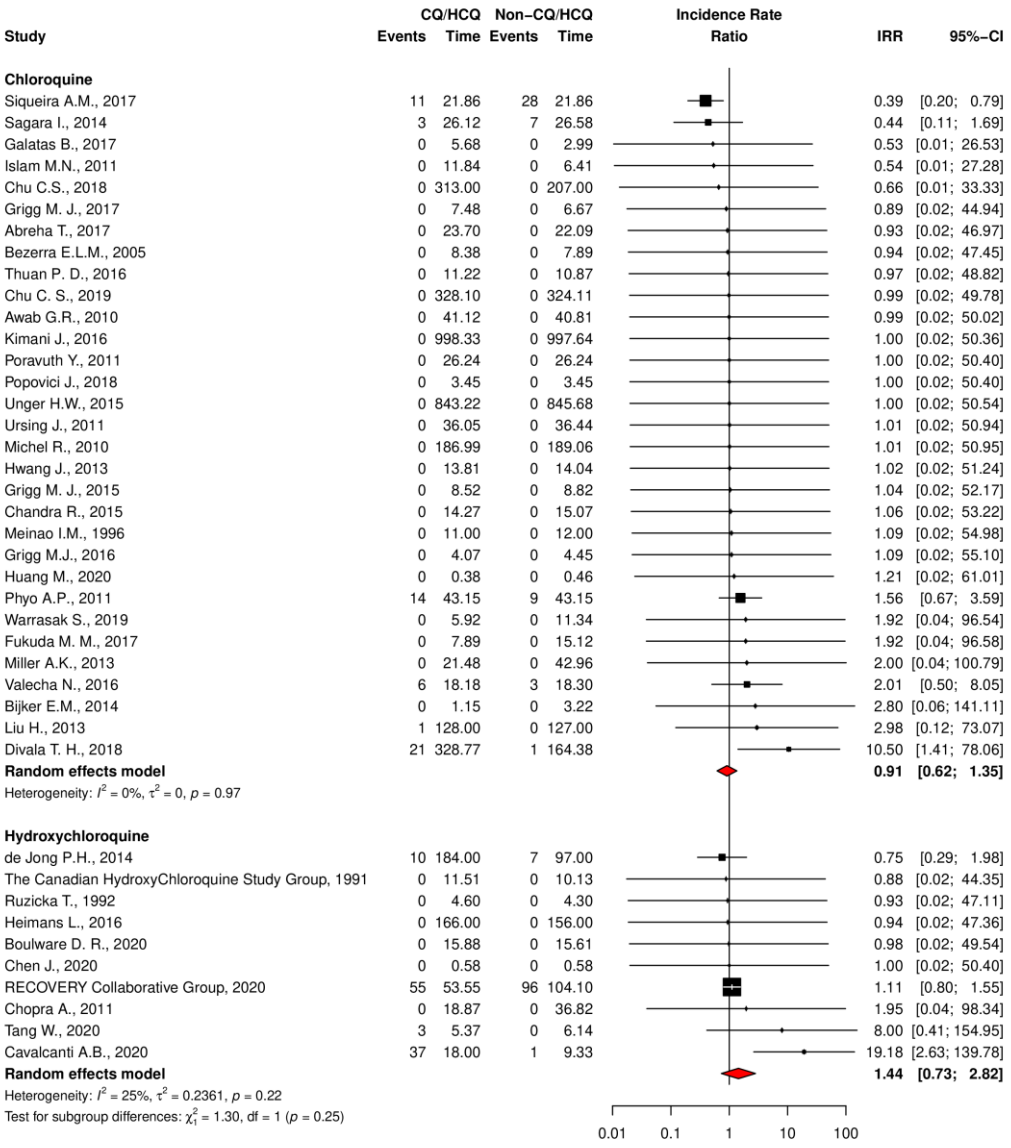

Figure S9. Forest-plot of the pooled cardiovascular adverse events grouped by antimalarial in chloroquine/hydroxychloroquine users and non-users.

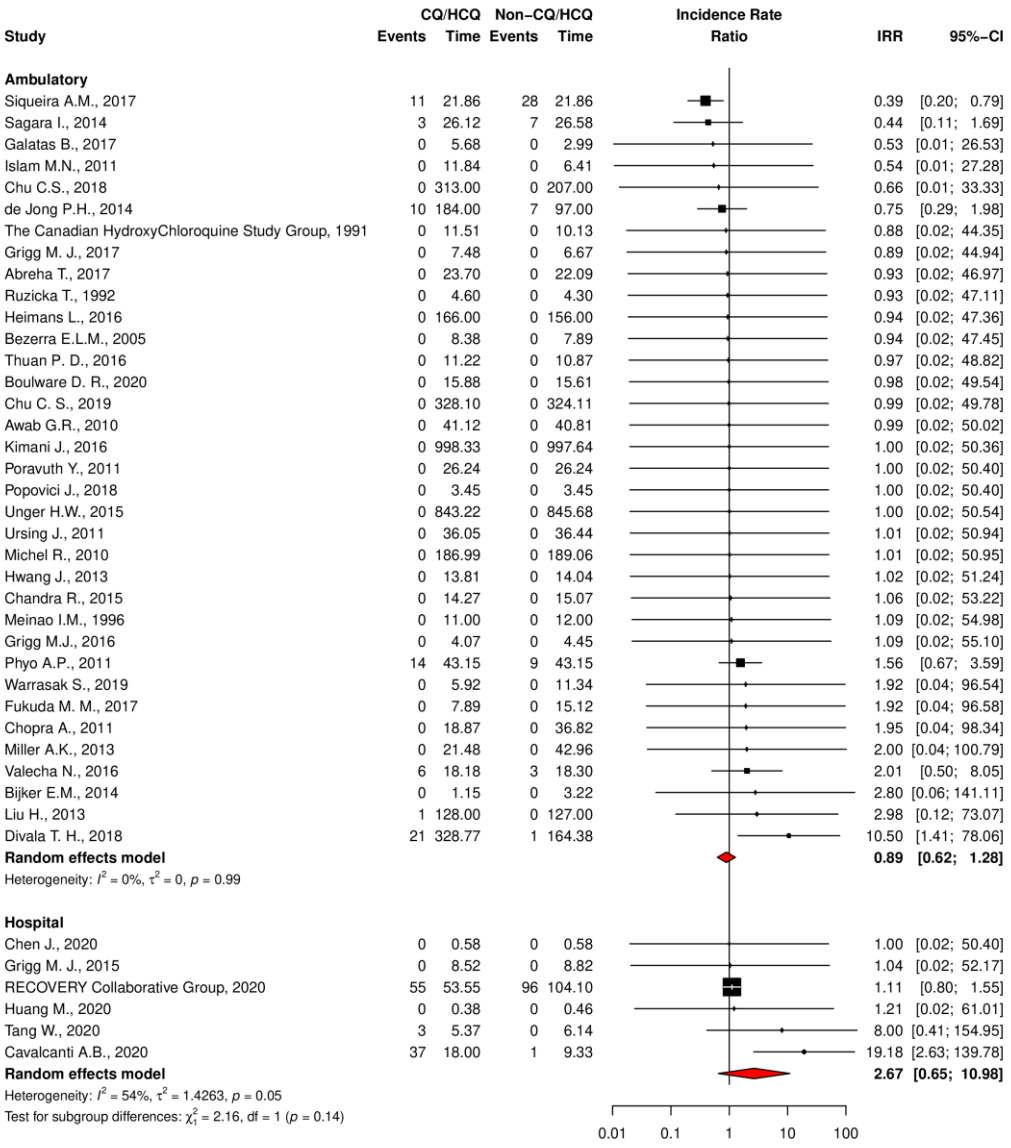

Figure S10. Forest-plot of the pooled cardiovascular adverse events grouped by setting in chloroquine/hydroxychloroquine users and non-users.

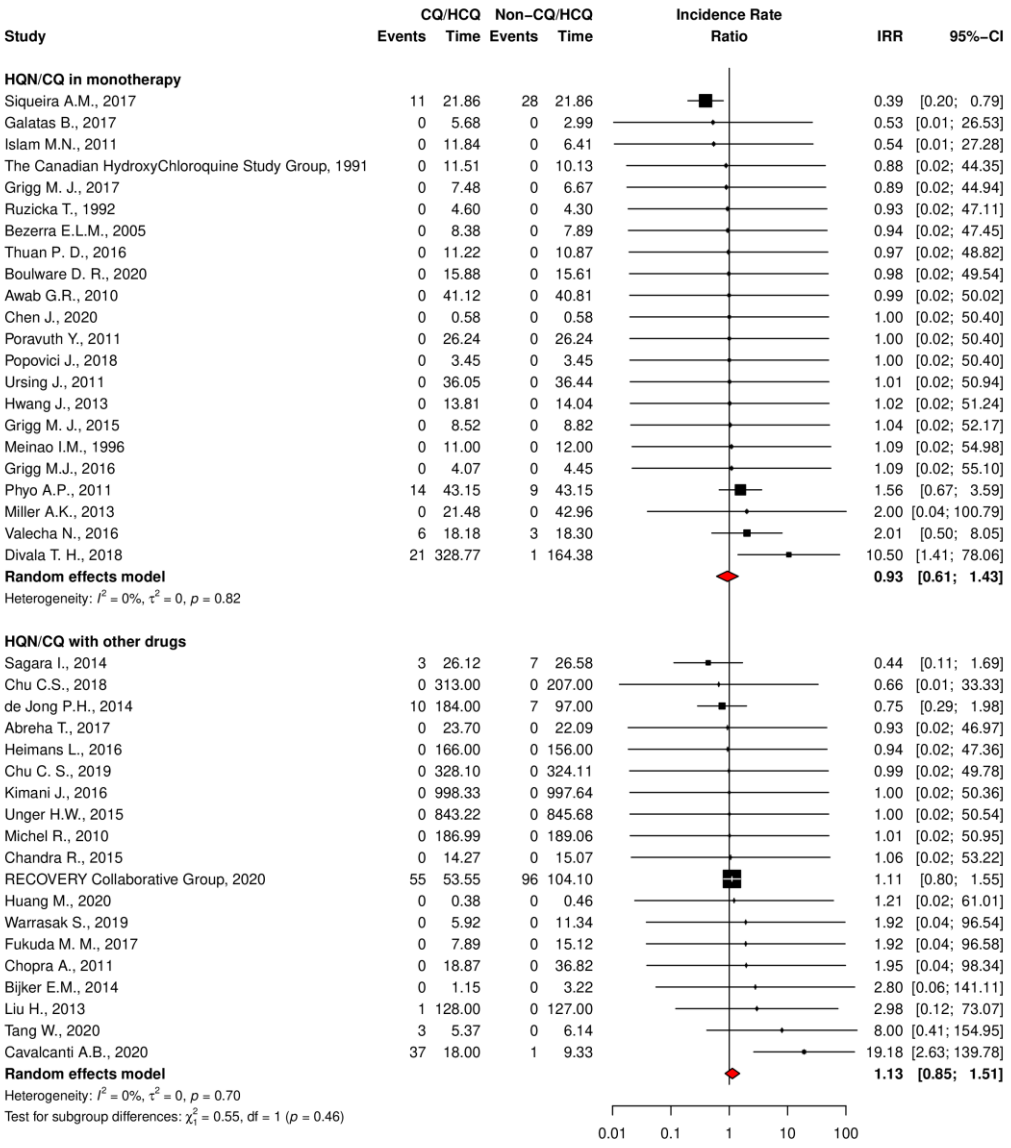

Figure S11. Forest-plot of the pooled cardiovascular adverse events grouped by antimalarial used alone or in combination with other drugs in chloroquine/hydroxychloroquine users and non-users.

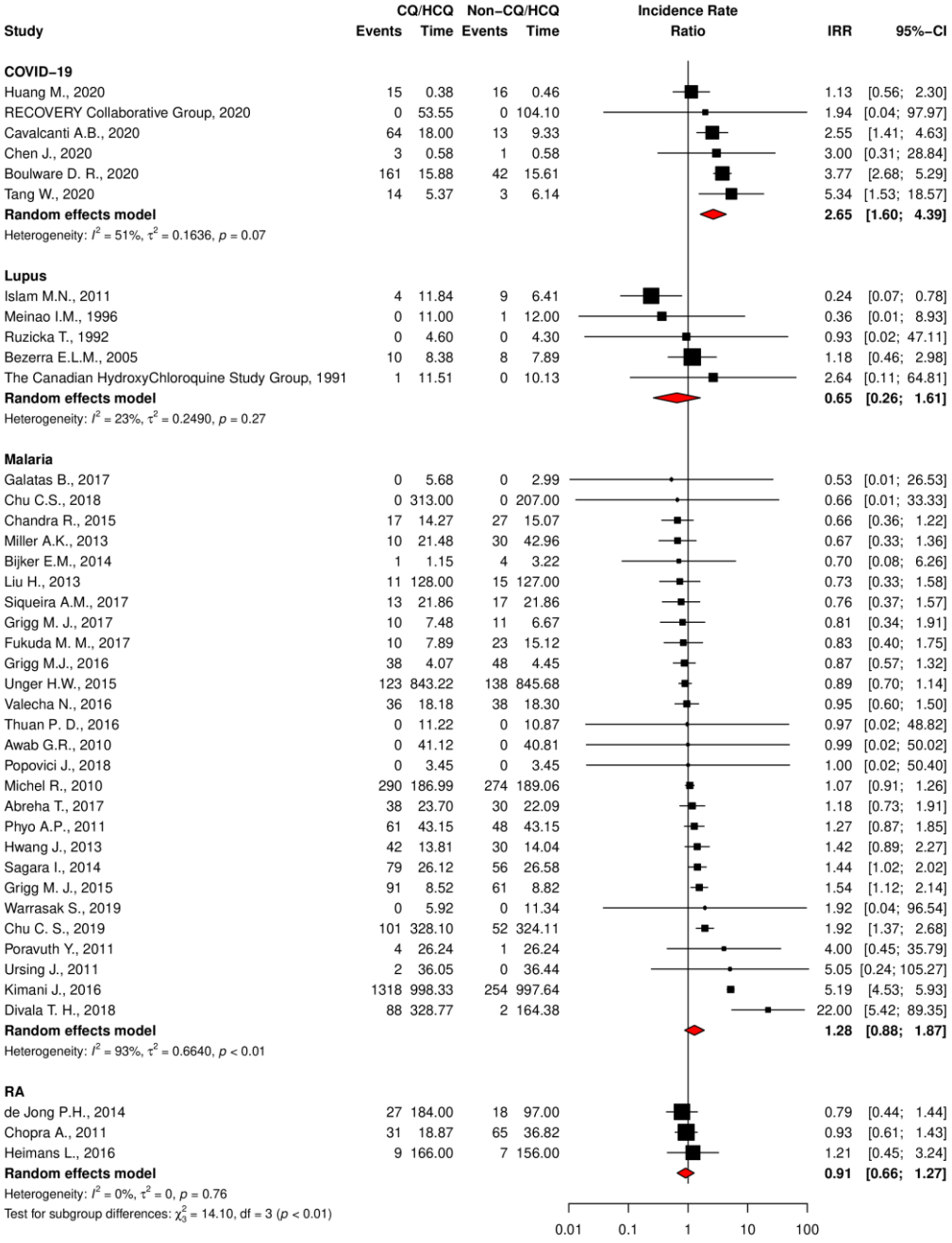

Figure S12. Forest-plot of the pooled gastrointestinal adverse events grouped by disease in chloroquine/hydroxychloroquine users and non-users.

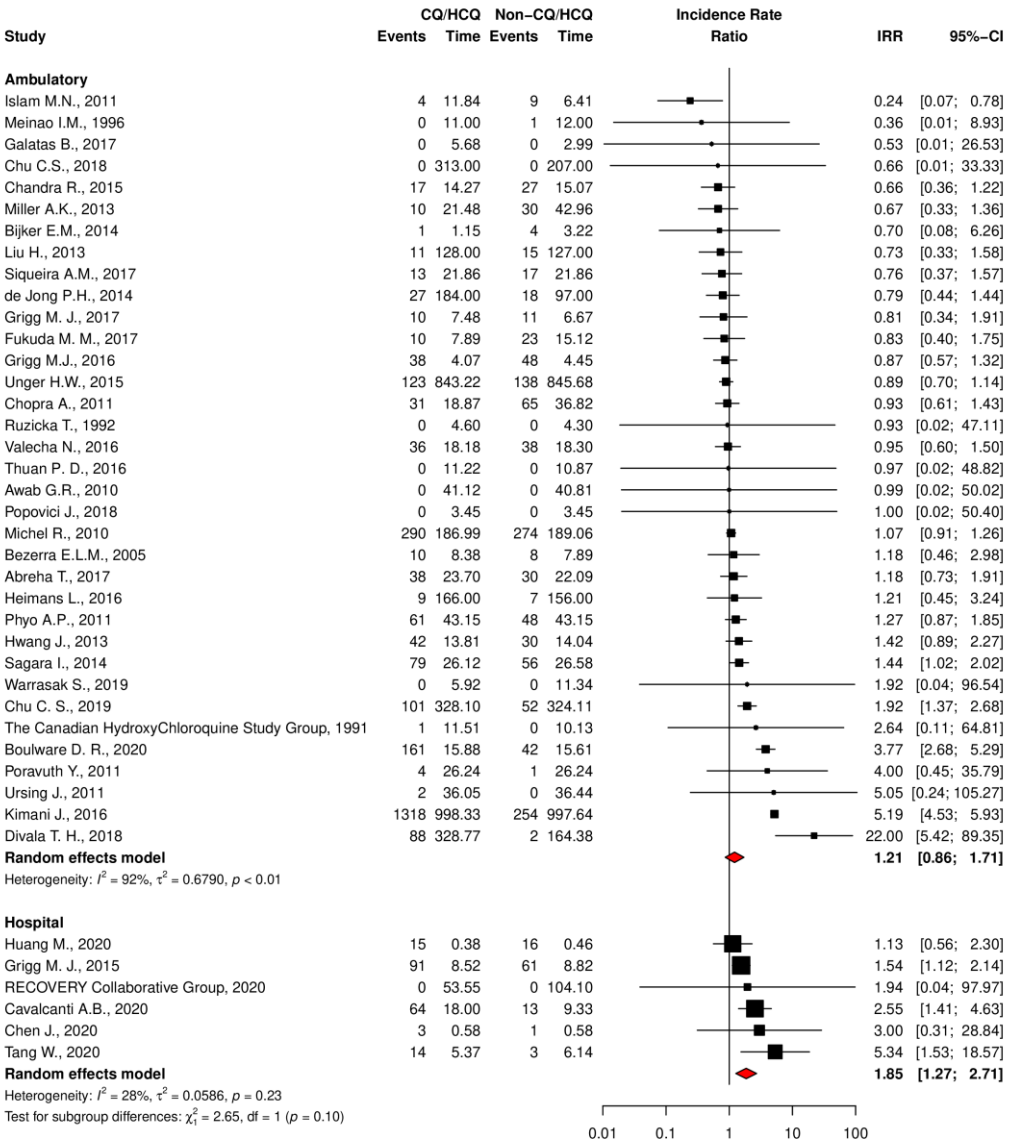

Figure S13. Forest-plot of the pooled gastrointestinal adverse events grouped by setting in chloroquine/hydroxychloroquine users and non-users.

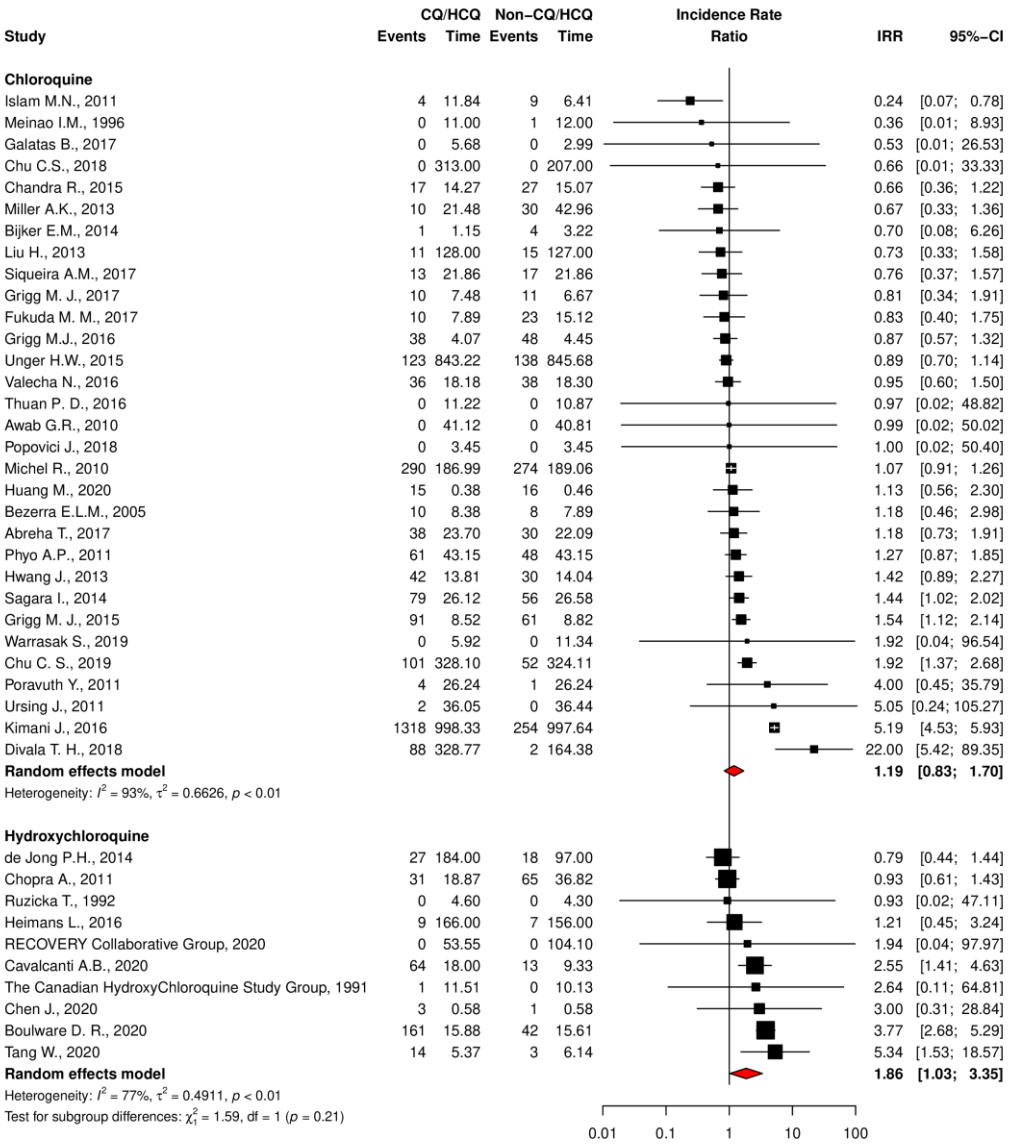

Figure S14. Forest-plot of the pooled gastrointestinal adverse events grouped by antimalarial in chloroquine/hydroxychloroquine users and non-users.

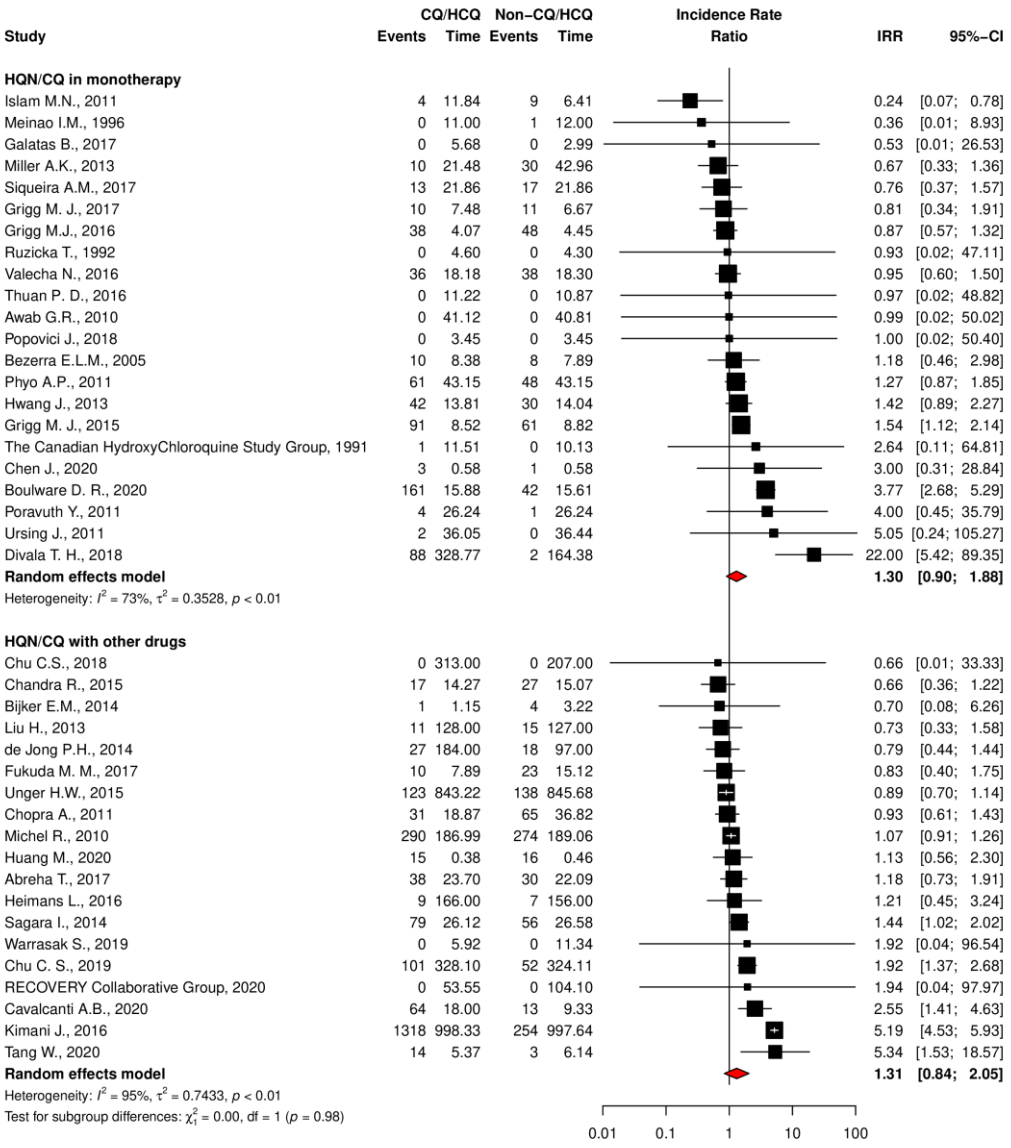

Figure S15. Forest-plot of the pooled gastrointestinal adverse events grouped by antimalarial used alone or in combination with other drugs in chloroquine/hydroxychloroquine users and non-users.

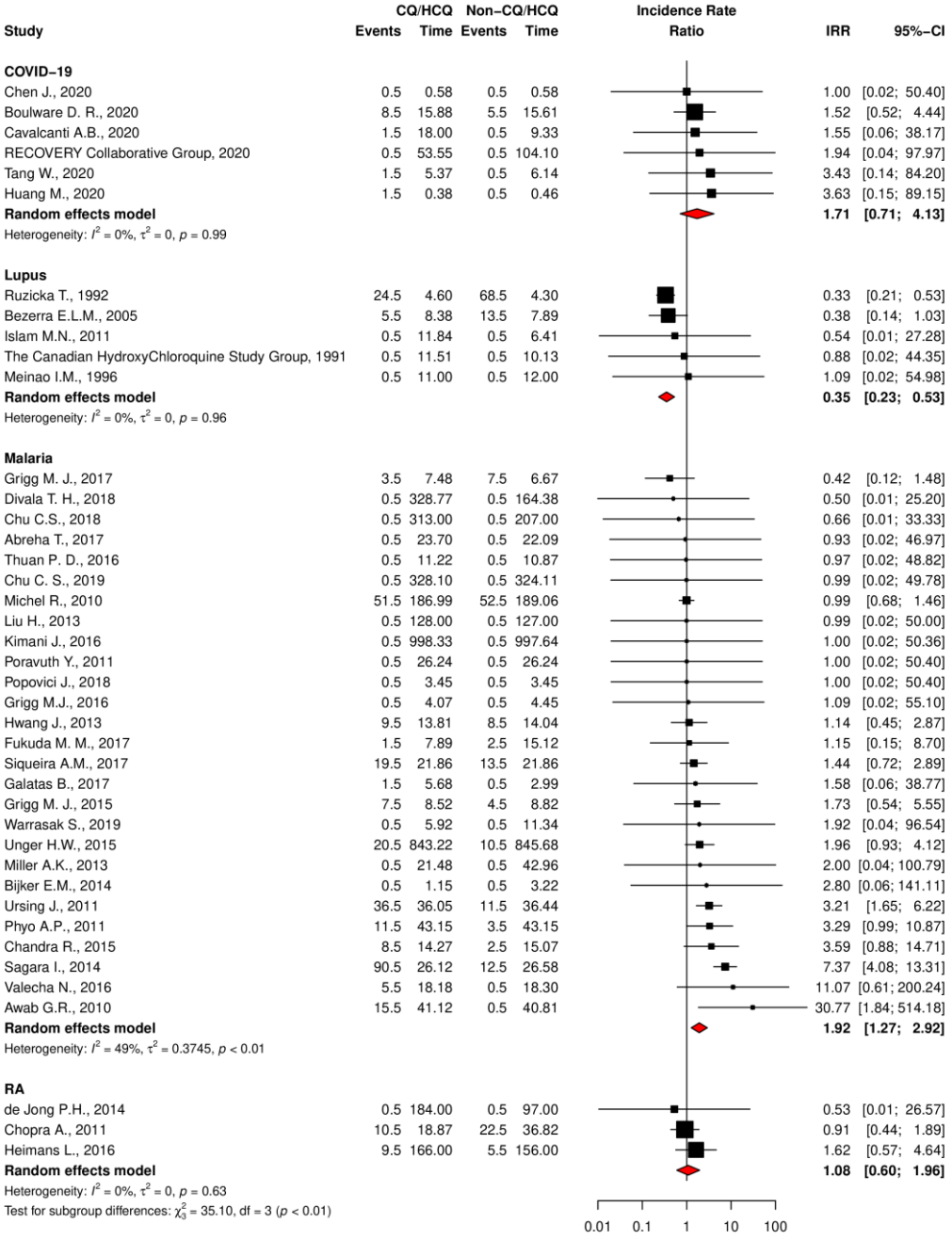

Figure S16. Forest-plot of the pooled dermatological adverse events grouped by disease in chloroquine/hydroxychloroquine users and non-users.

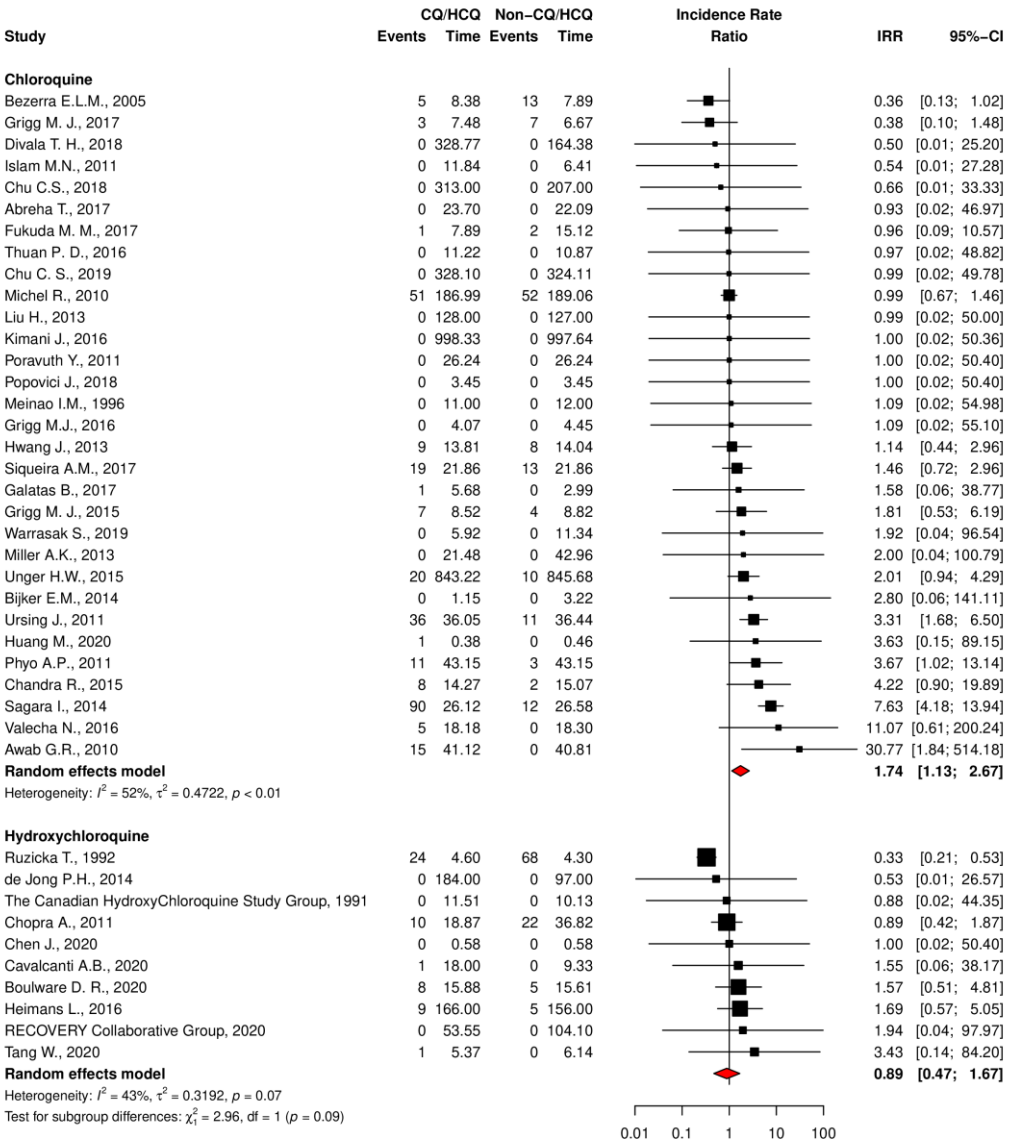

Figure S17. Forest-plot of the pooled dermatological adverse events grouped by antimalarial in chloroquine/hydroxychloroquine users and non-users.

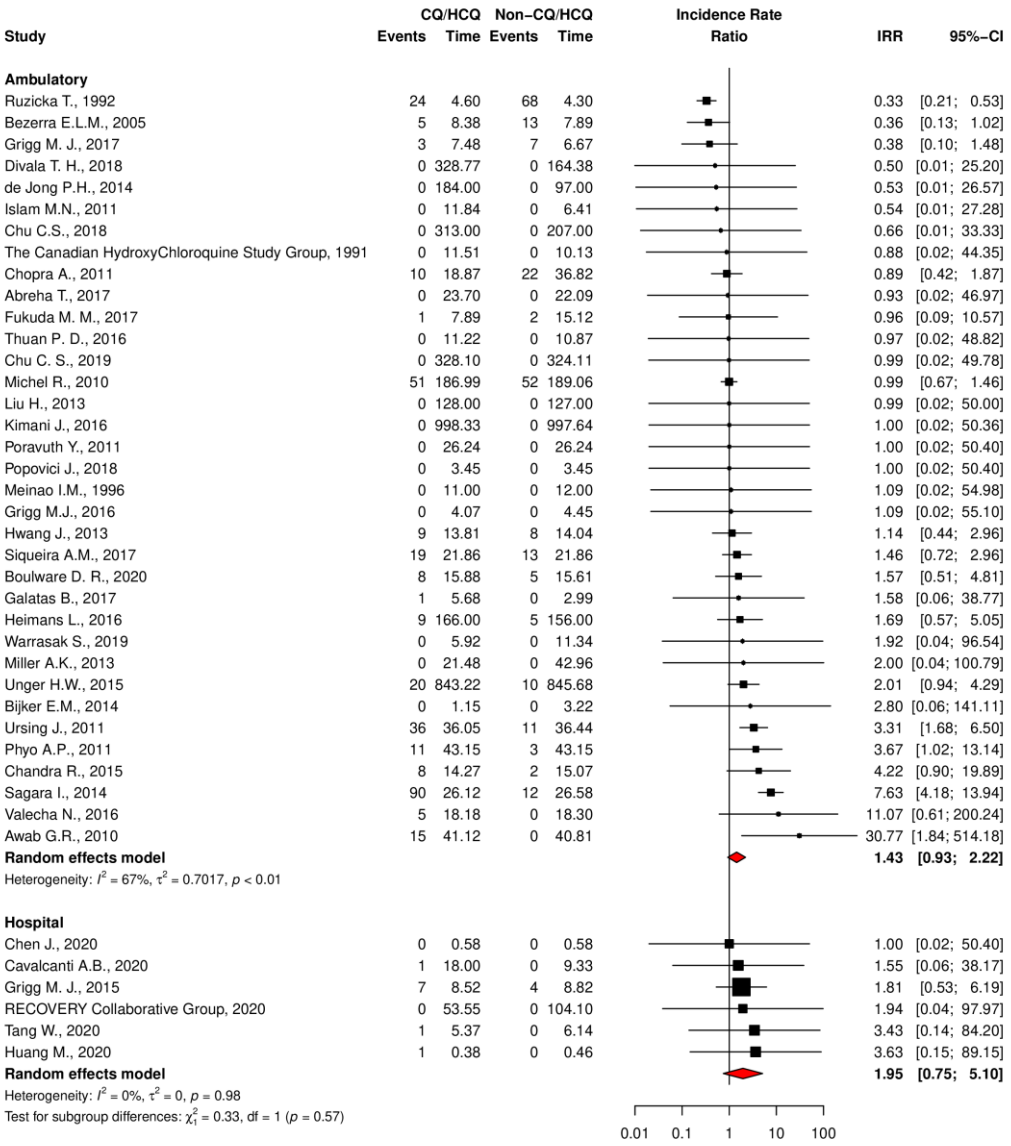

Figure S18. Forest-plot of the pooled dermatological adverse events grouped by setting in chloroquine/hydroxychloroquine users and non-users.

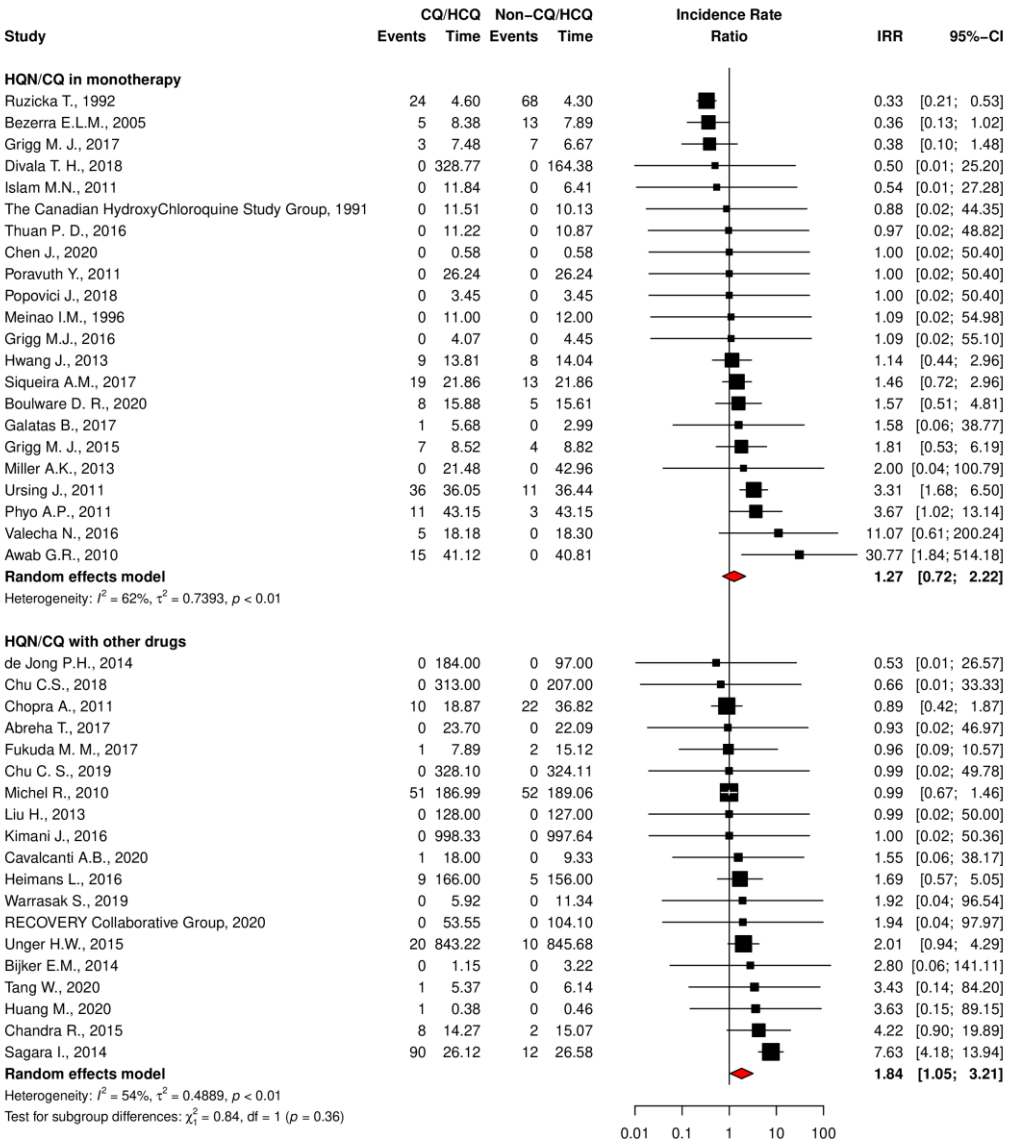

Figure S19. Forest-plot of dermatological adverse events grouped by antimalarial used alone or in combination with other drugs in chloroquine/hydroxychloroquine users and non-users.

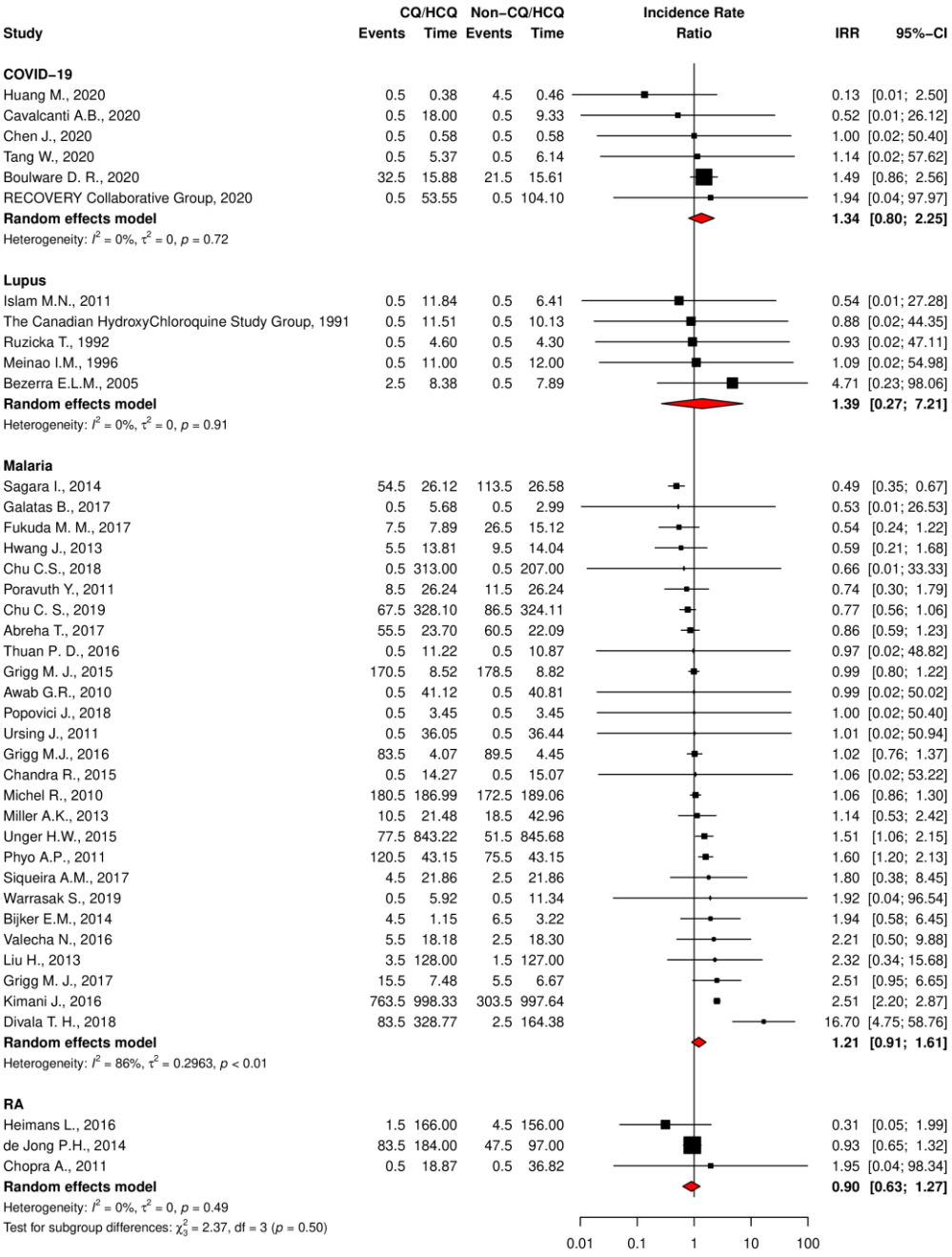

Figure S20. Forest-plot of neurological adverse events grouped by disease in chloroquine/hydroxychloroquine users and non-users.

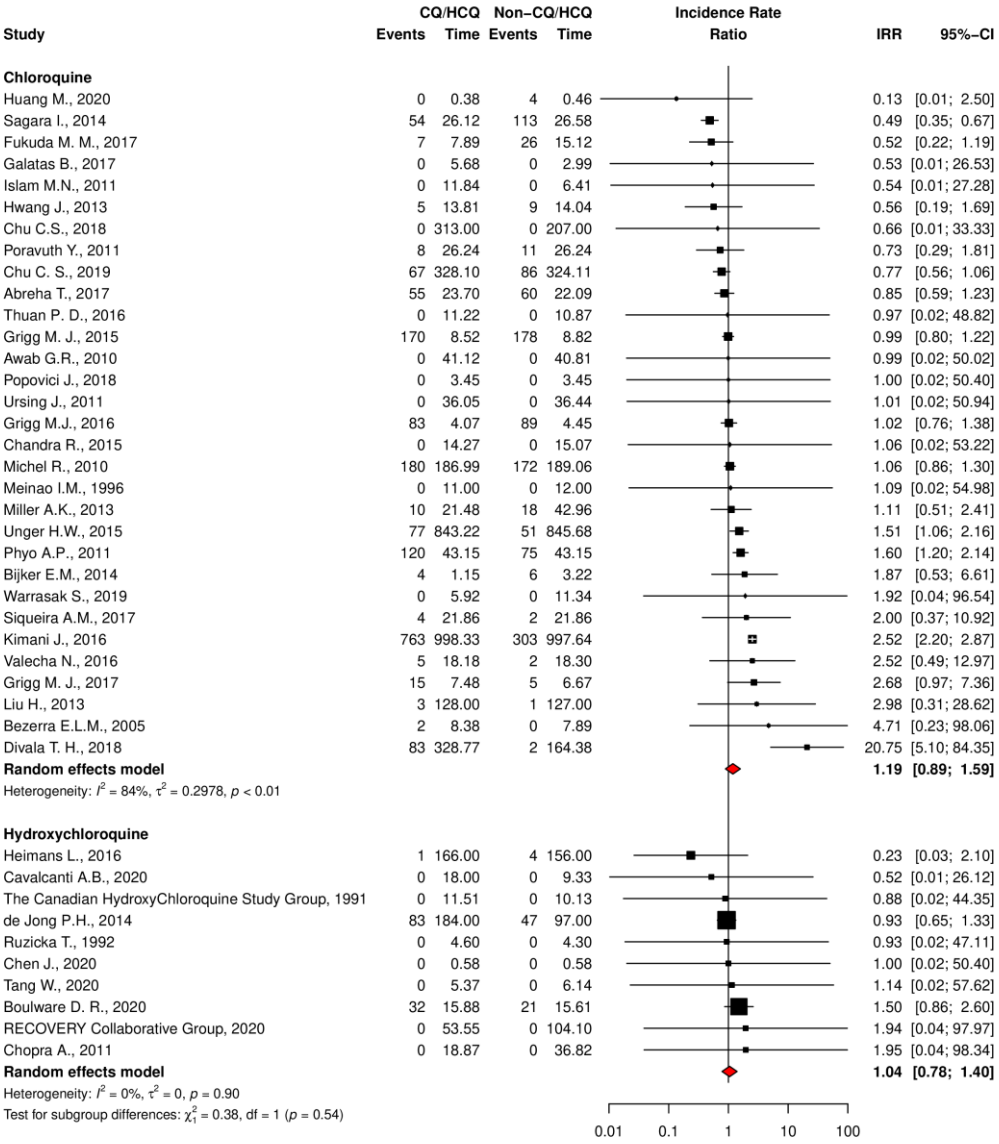

Figure S21. Forest-plot of neurological adverse events grouped by antimalarial in chloroquine/hydroxychloroquine users and non-users.

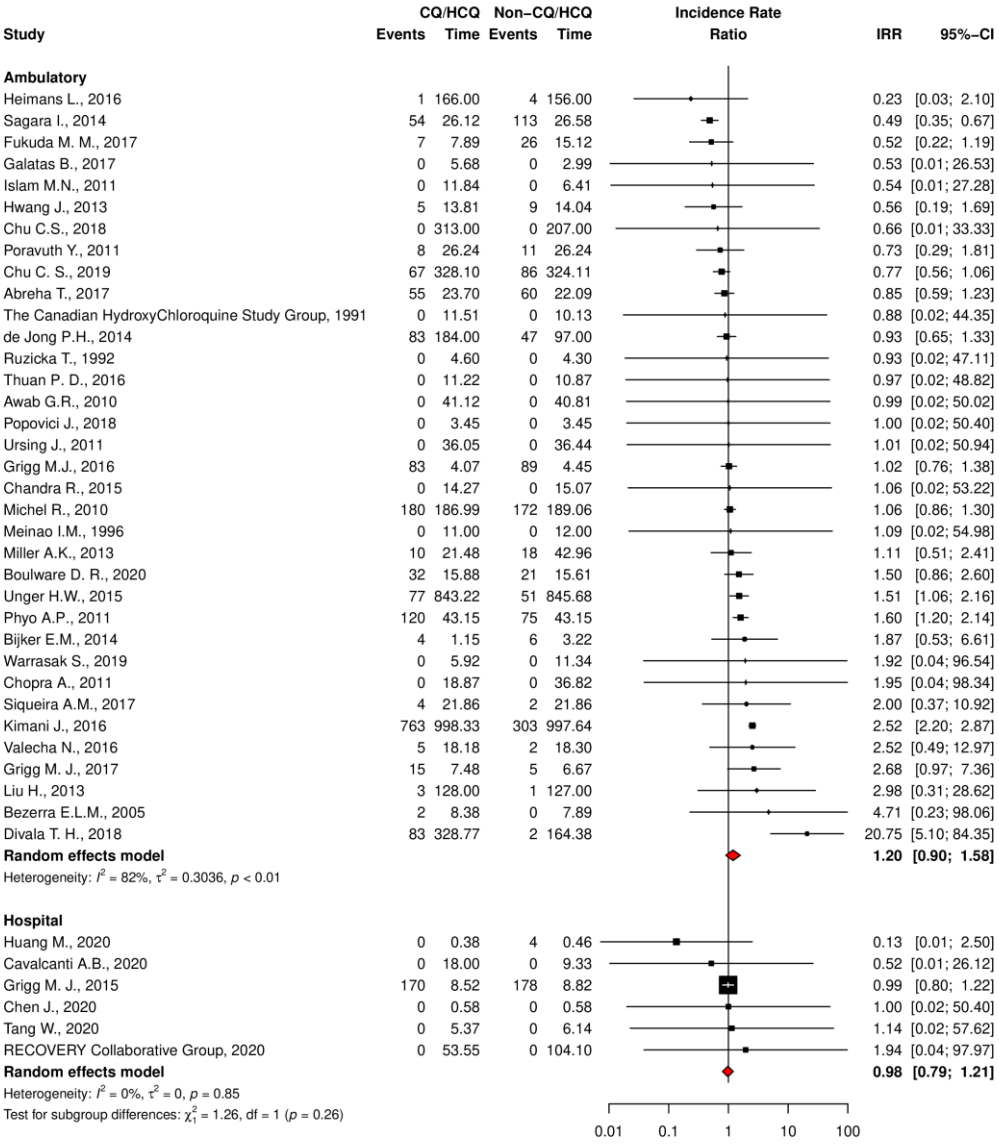

Figure S22. Forest-plot of neurological adverse events grouped by setting in chloroquine/hydroxychloroquine users and non-users.

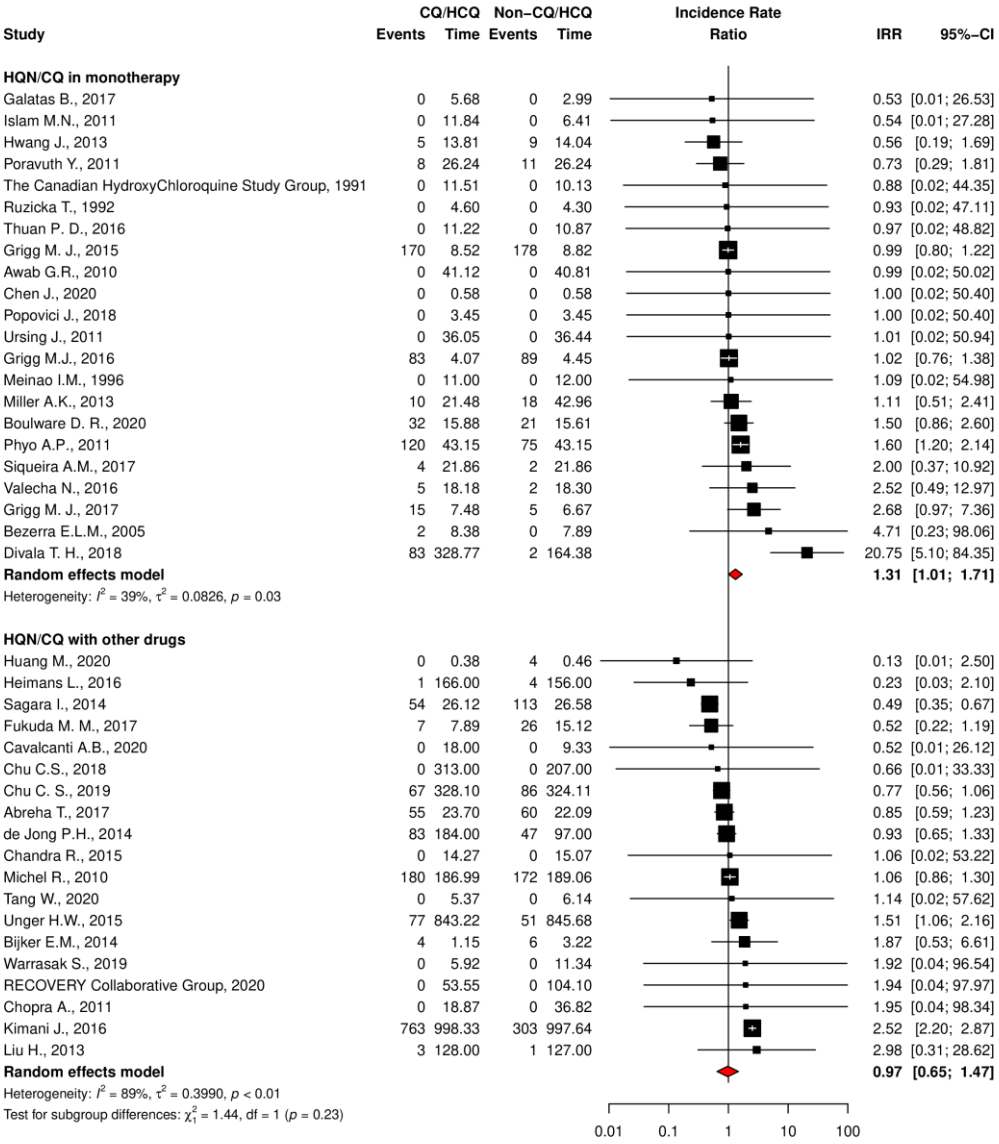

Figure S23. Forest-plot of neurological adverse events grouped by antimalarial used alone or in combination with other drugs in chloroquine/hydroxychloroquine users and non-users.

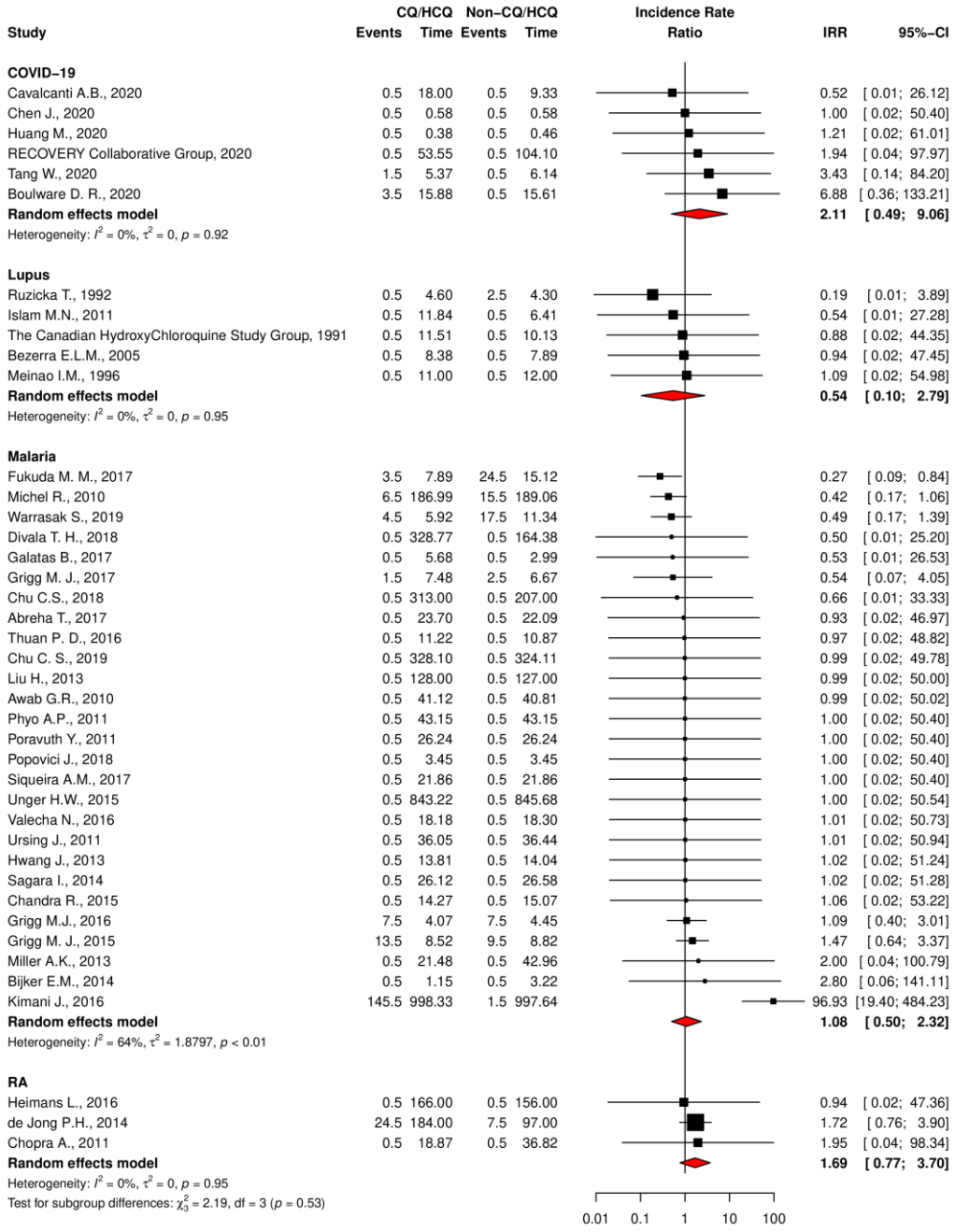

Figure S24. Forest-plot of ophthalmological adverse events grouped by disease in chloroquine/hydroxychloroquine users and non-users.

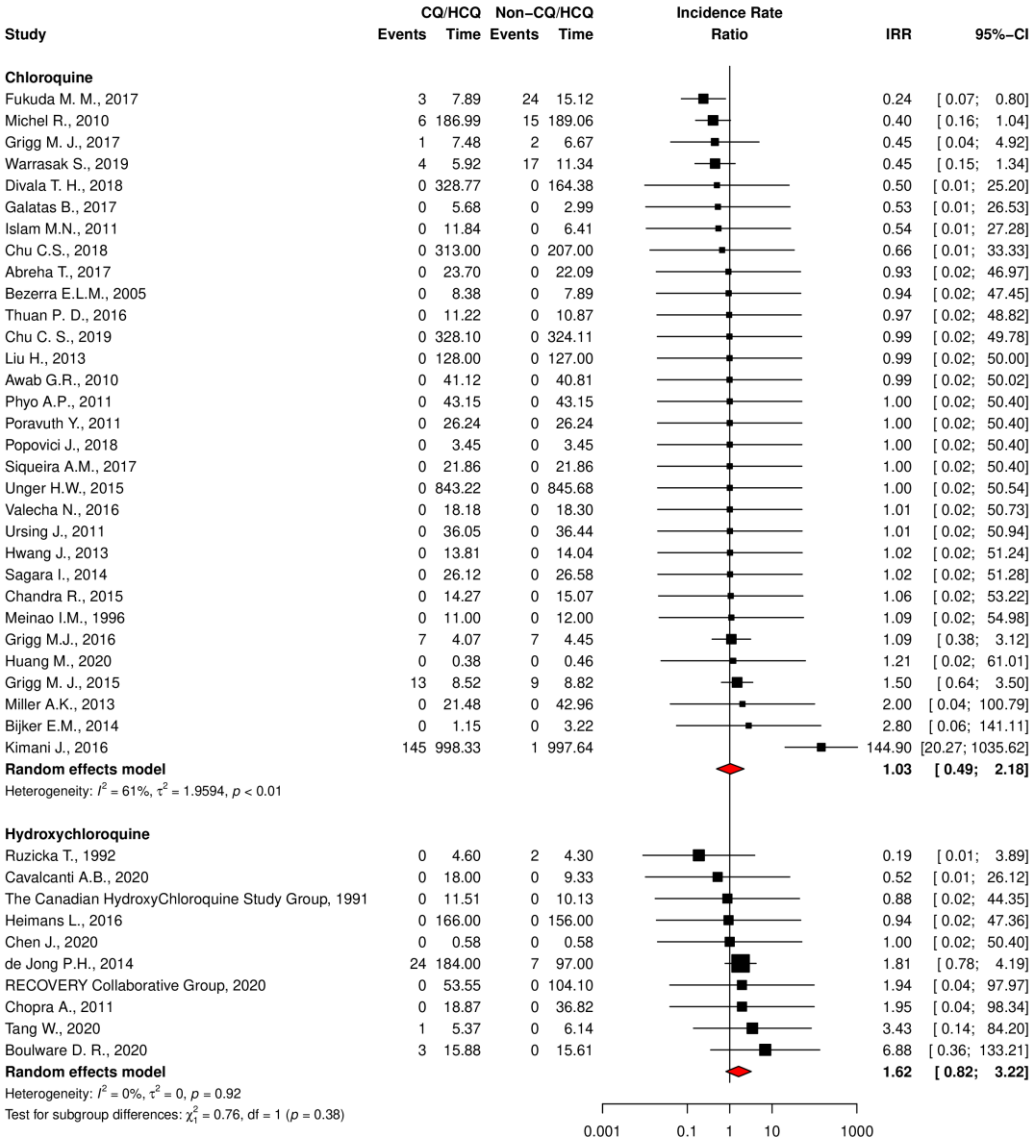

Figure S25. Forest-plot of ophthalmological adverse events grouped by antimalarial in chloroquine/hydroxychloroquine users and non-users.

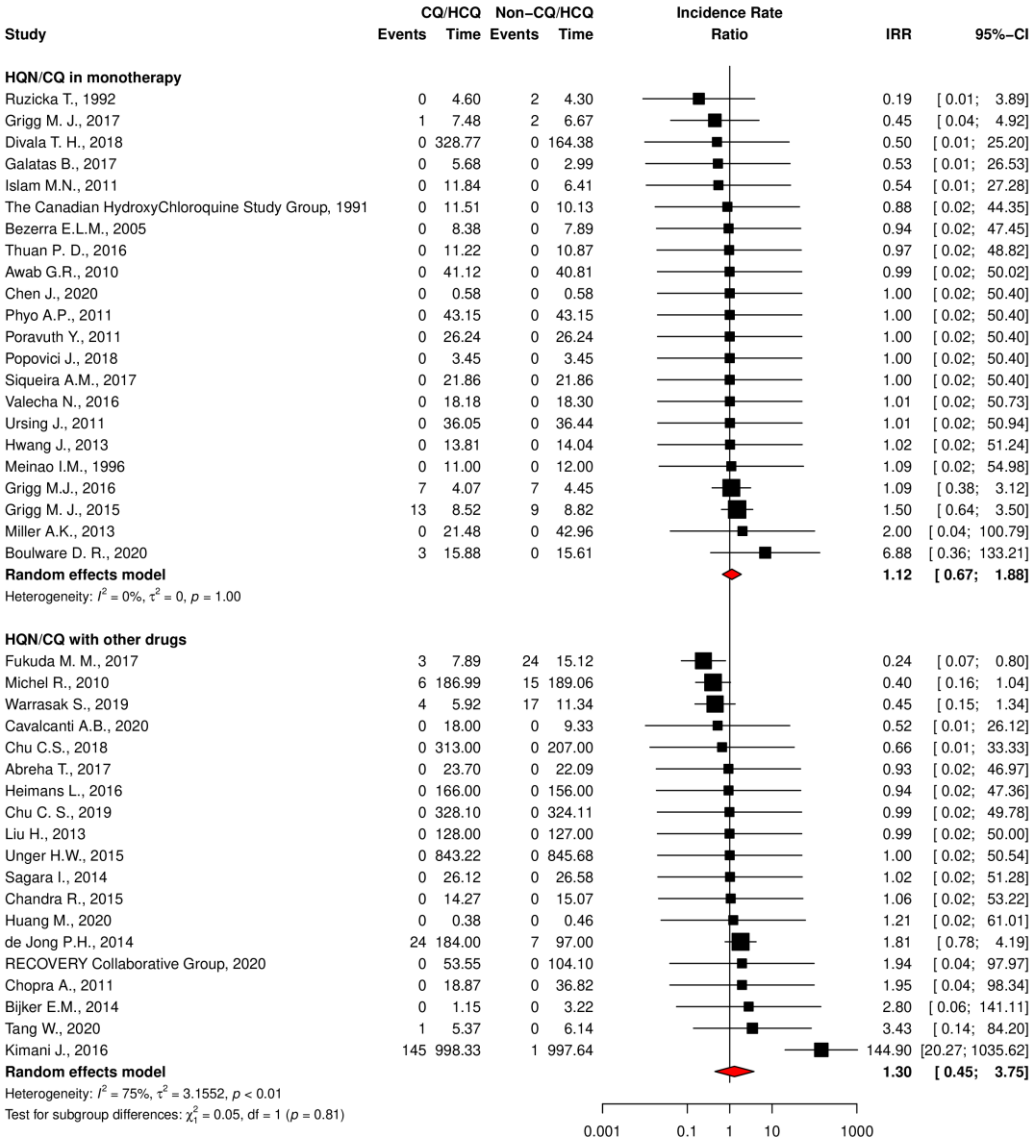

Figure S26. Forest-plot of ophthalmological adverse events grouped by antimalarial used alone or in combination with other drugs in chloroquine/hydroxychloroquine users and non-users.

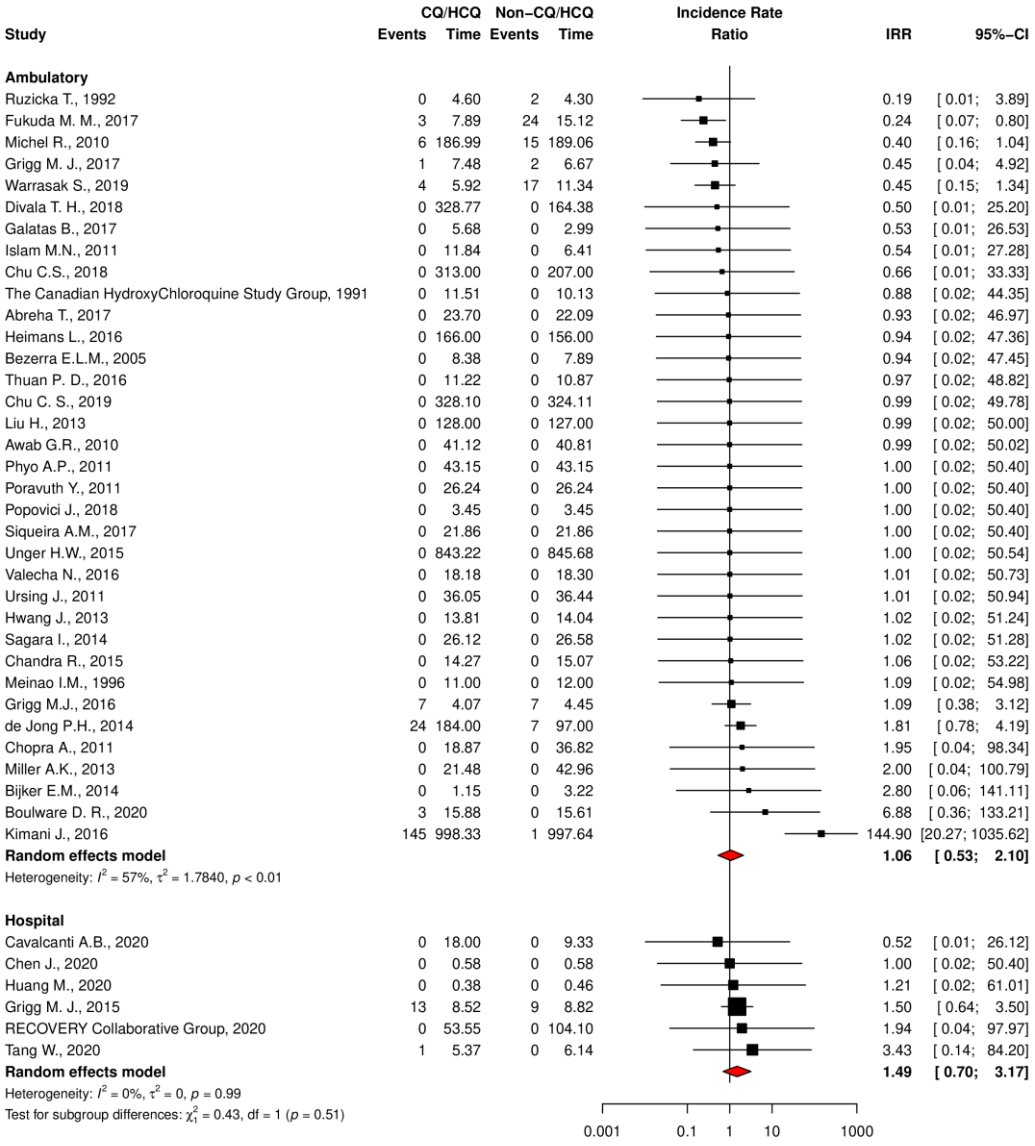

Figure S27. Forest-plot of ophthalmological adverse events grouped by setting in chloroquine/hydroxychloroquine users and non-users.

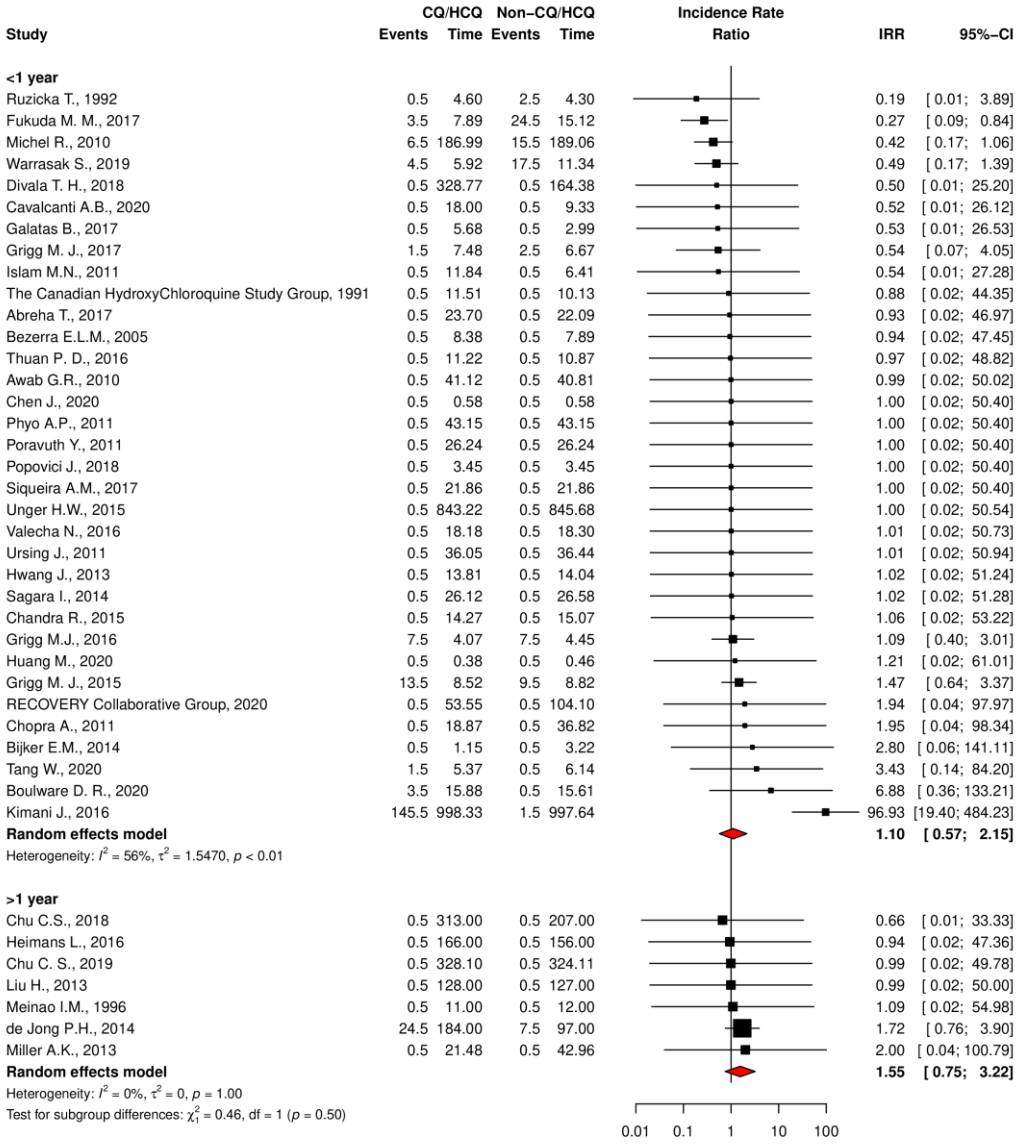

Figure S28. Forest-plot of ophthalmological adverse events grouped by follow-up in chloroquine/hydroxychloroquine users and non-users (follow-up ≥ 1 year versus <1 year).

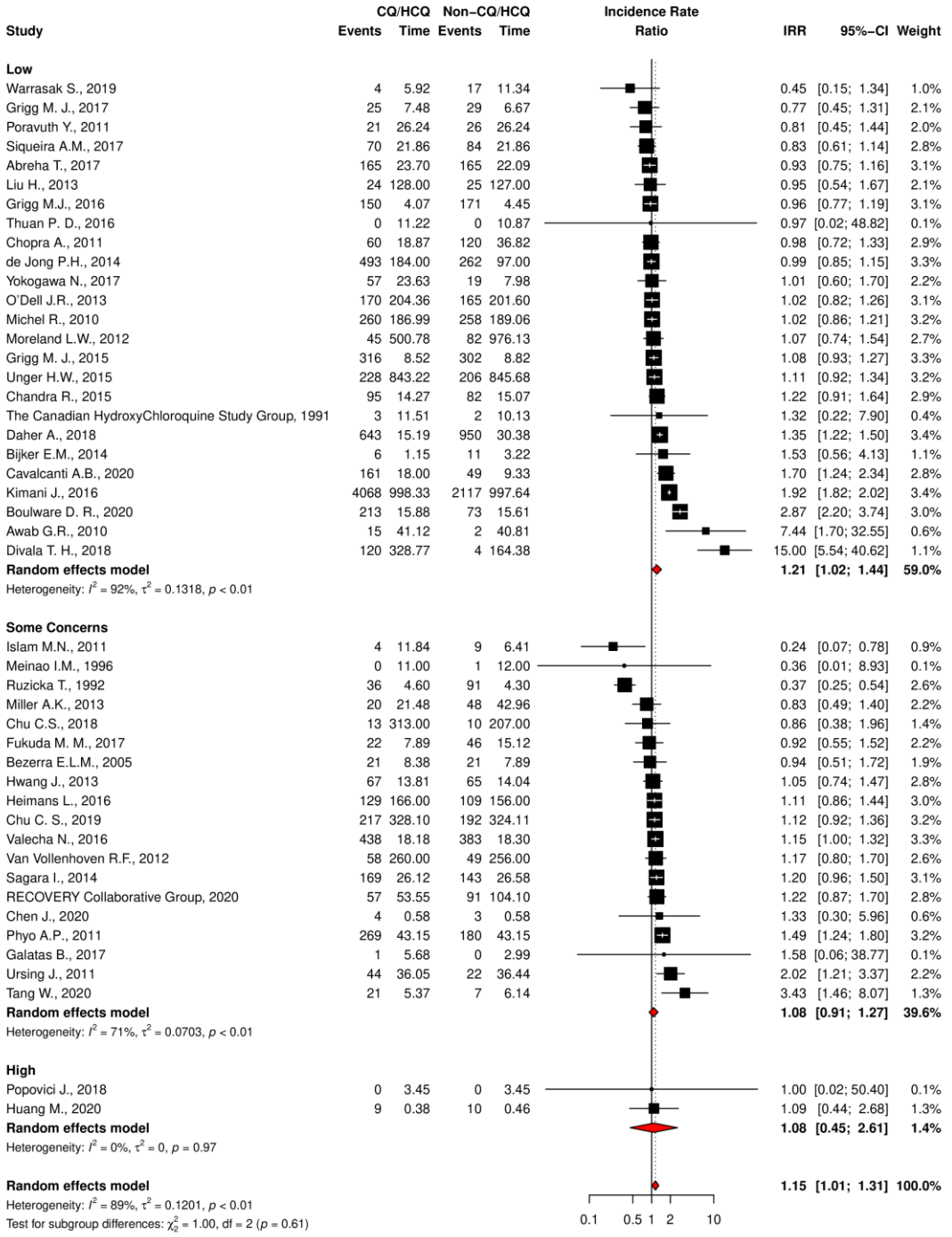

Figure S29. Forest-plot of the pooled adverse events grouped by Risk of Bias.

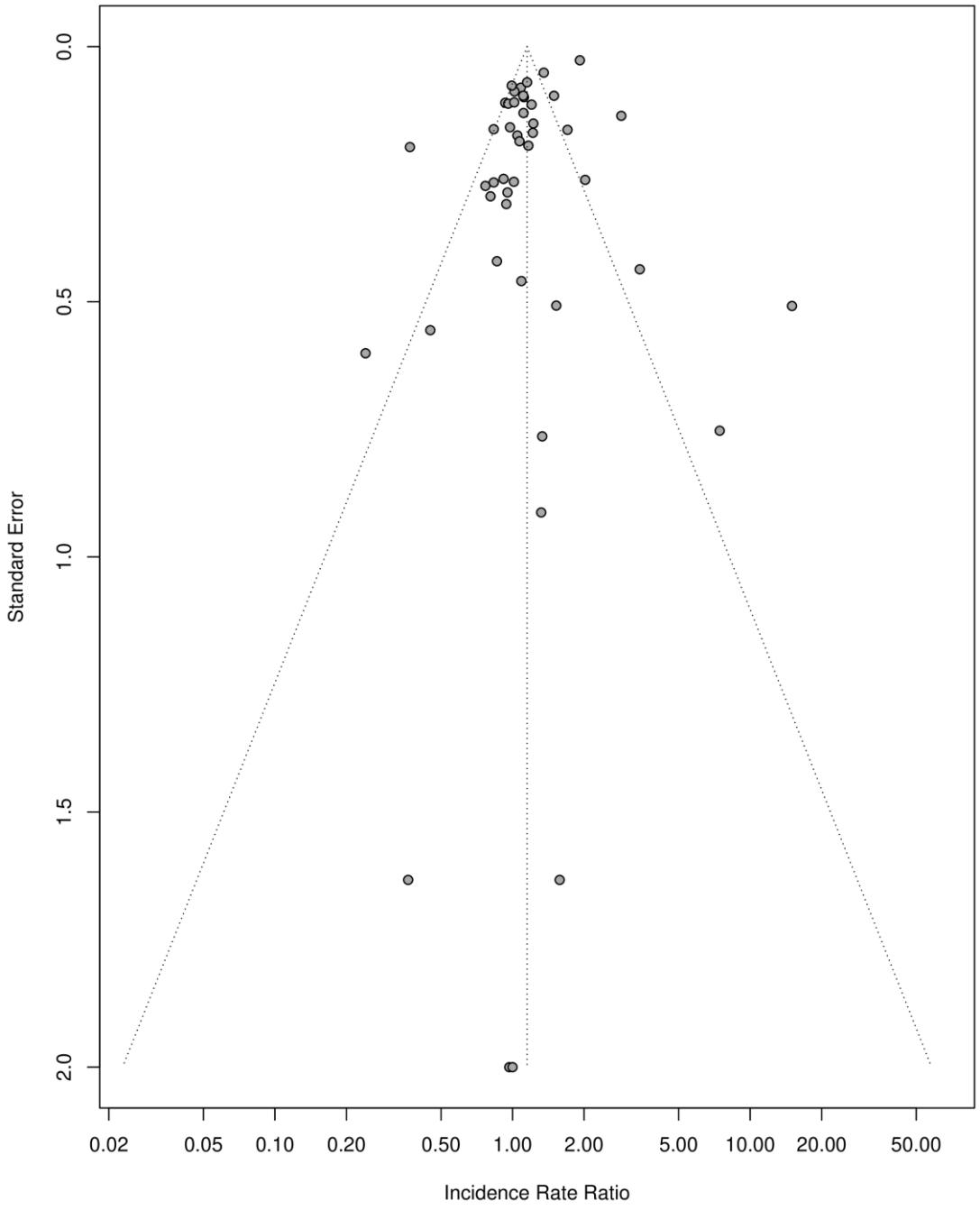

Figure S30. Funnel-plot

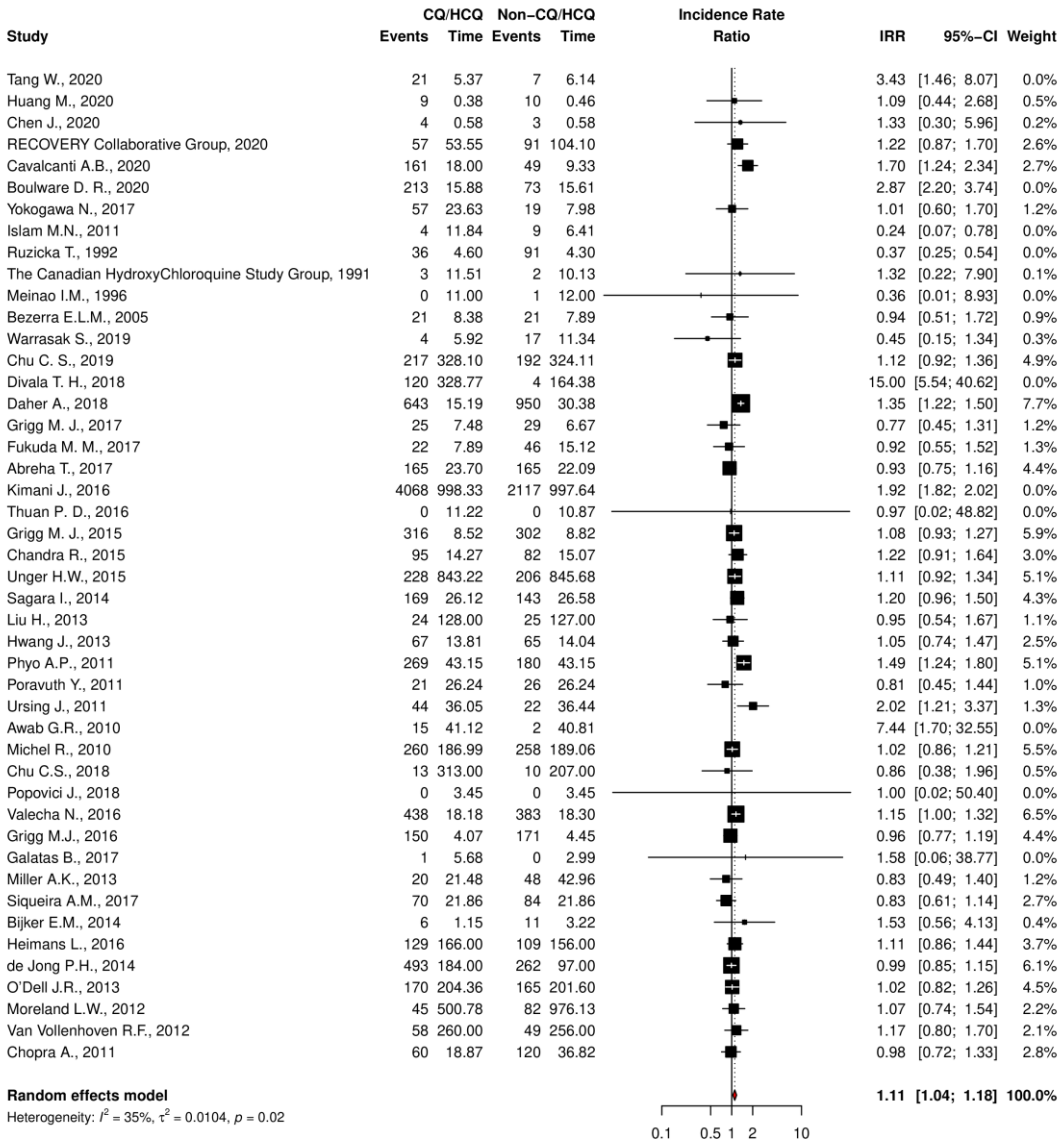

Figure S31. Forest-plot of the pooled adverse events after exclusion of outliers (weight = 0%).

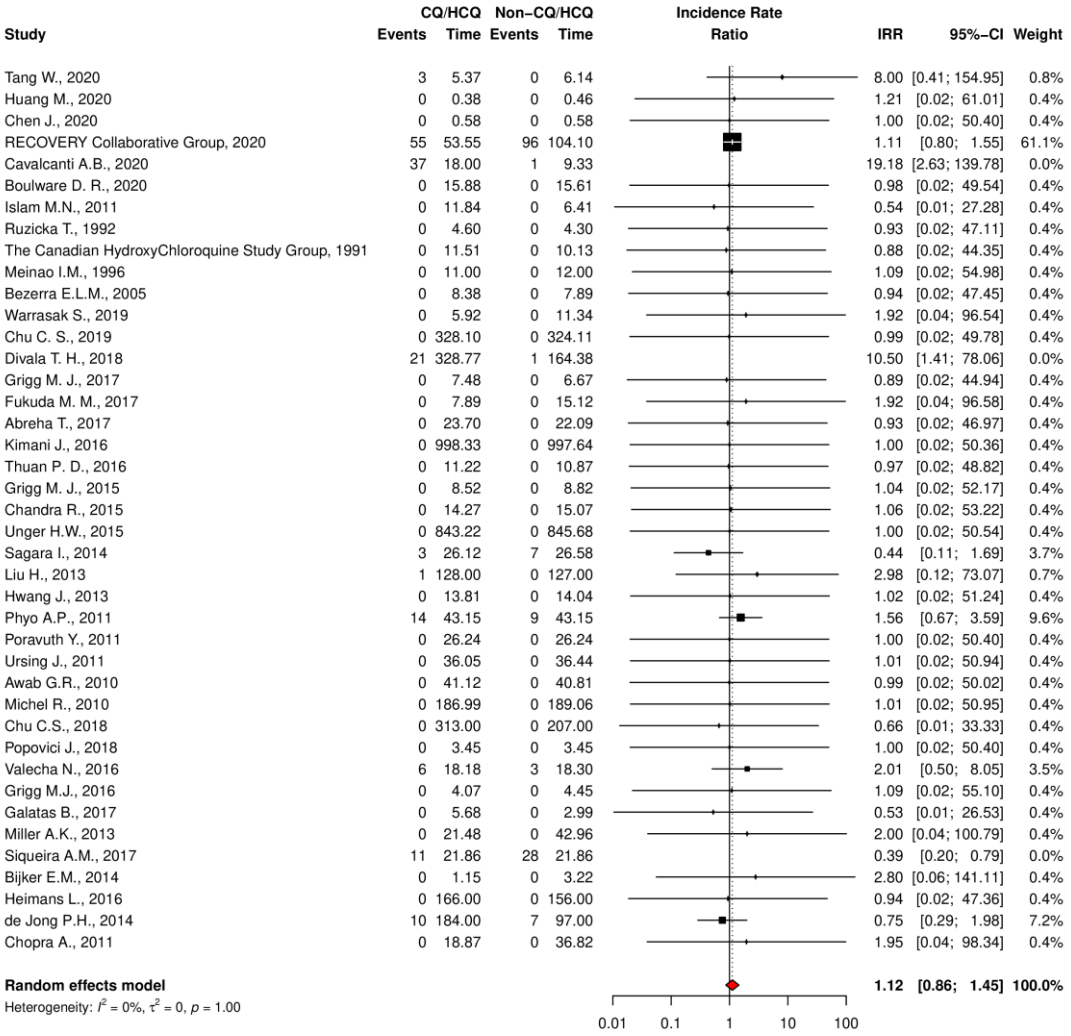

Figure S32. Forest-plot of the pooled cardiovascular adverse events after exclusion of outliers (weight = 0%).

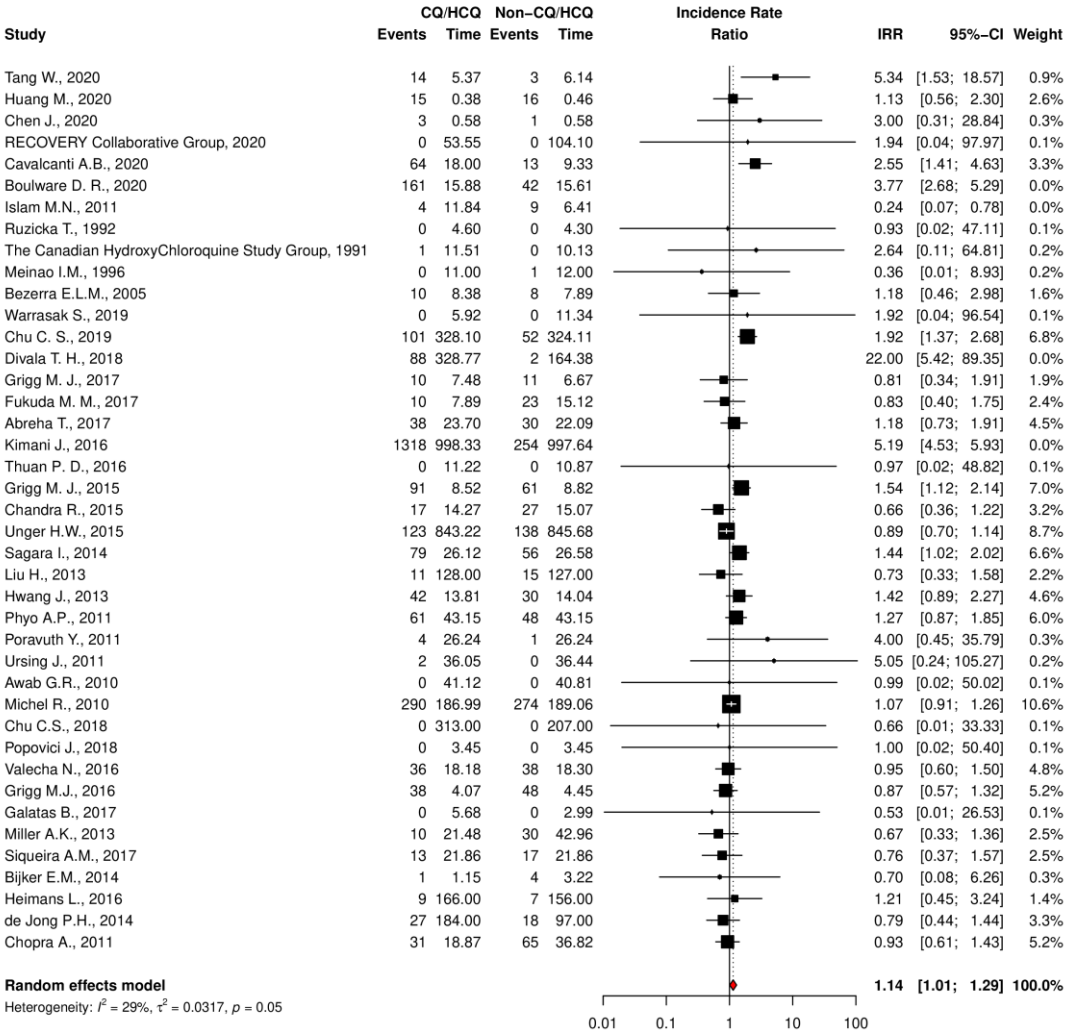

Figure S33. Forest-plot of the pooled gastrointestinal adverse events after exclusion of outliers (weight = 0%).

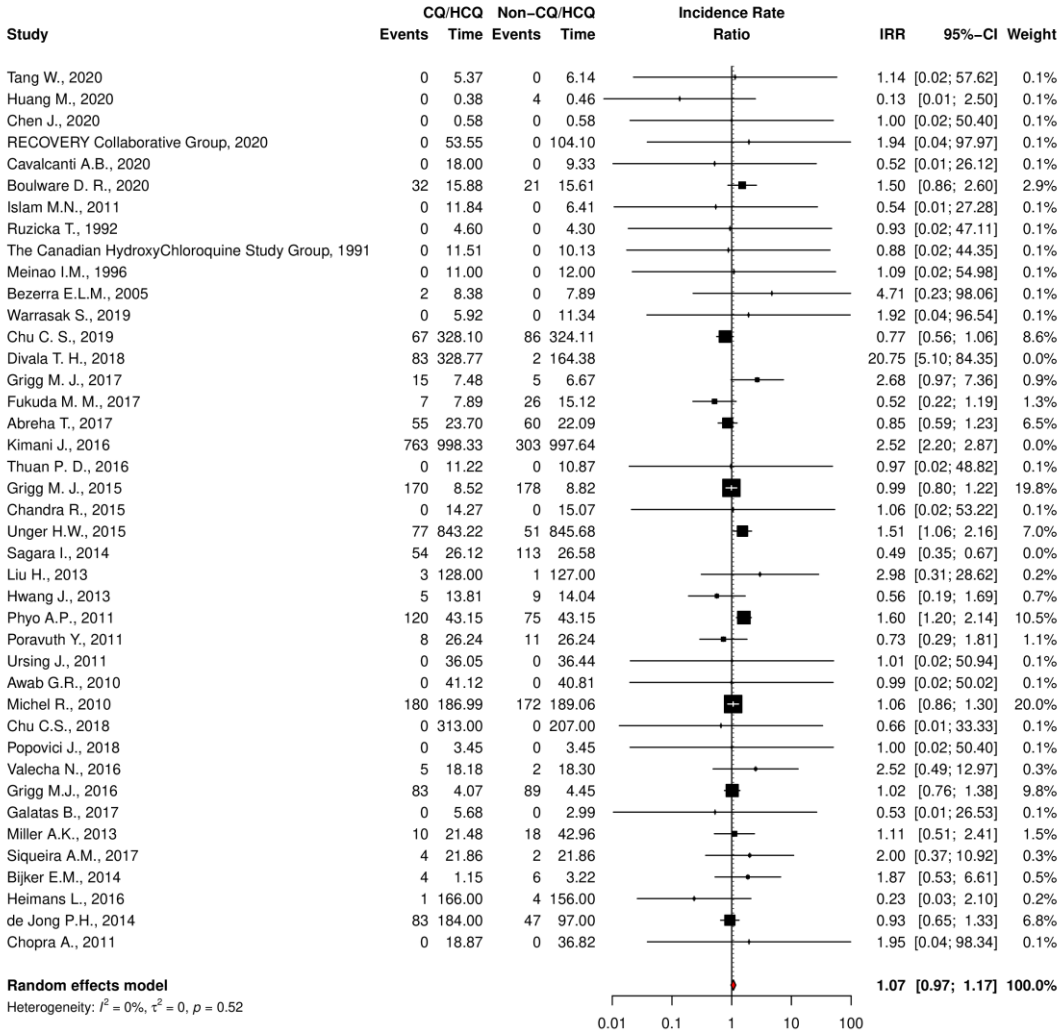

Figure S34. Forest-plot of the pooled neurological adverse events after exclusion of outliers (weight = 0%).

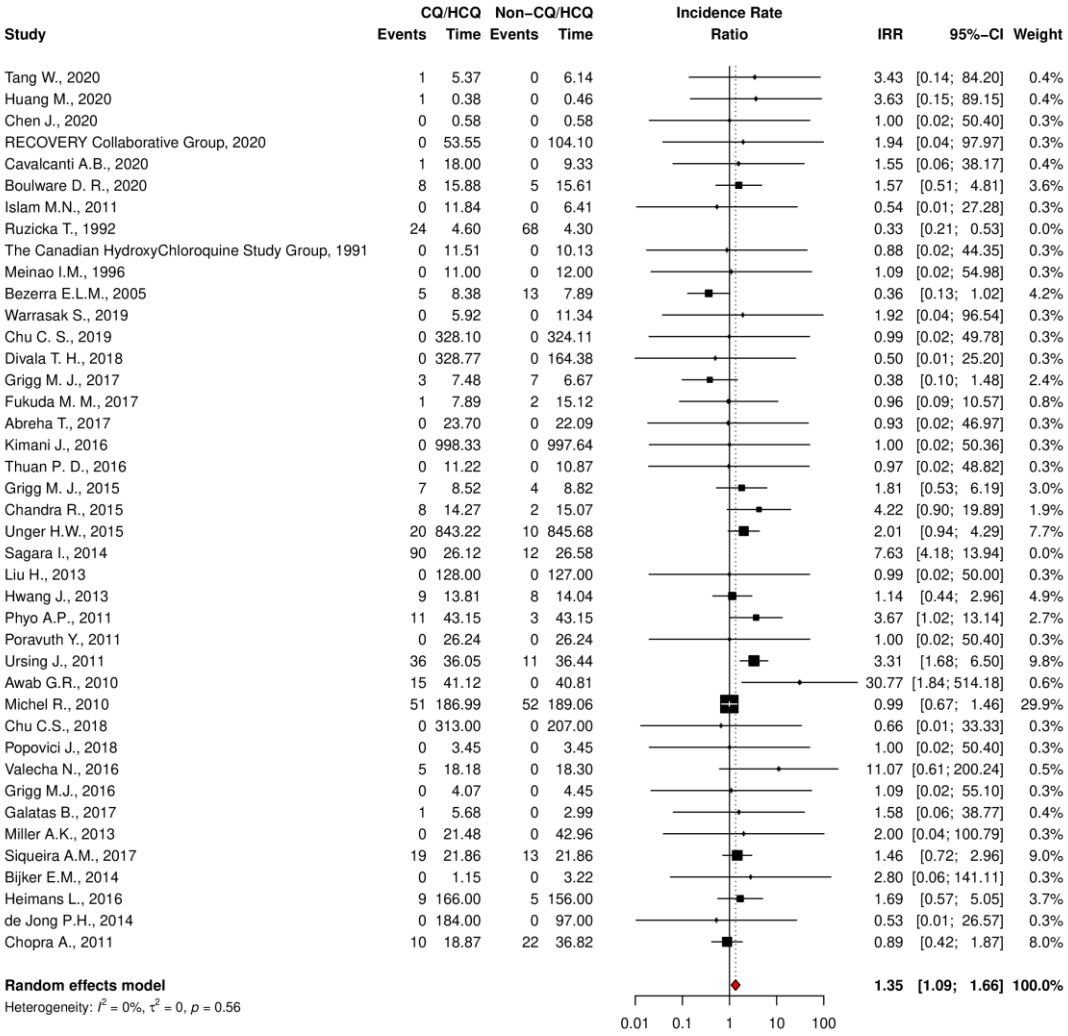

Figure S35. Forest-plot of the pooled dermatological adverse events after exclusion of outliers (weight = 0%).

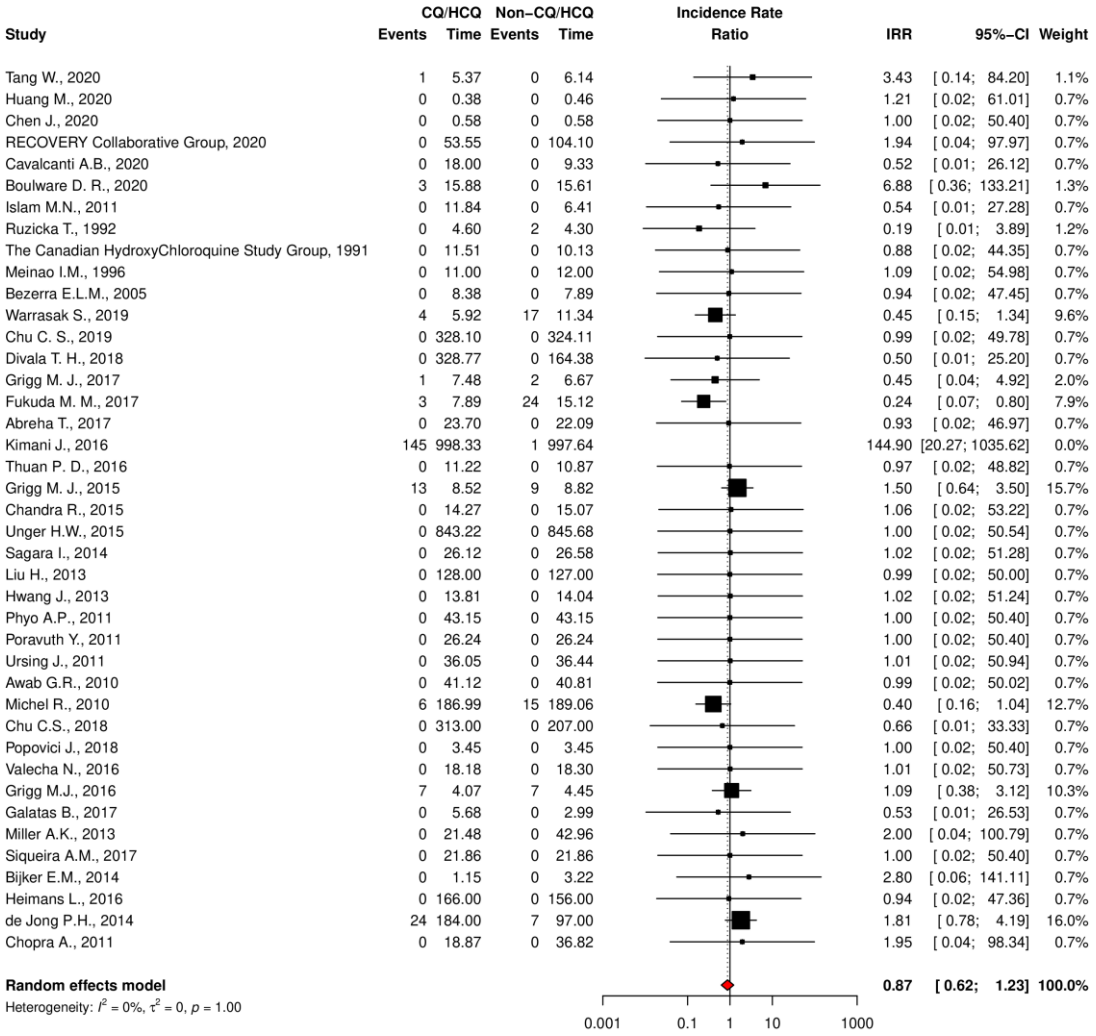

Figure S36. Forest-plot of the pooled ophthalmological adverse events after exclusion of outliers (weight = 0%).

Apêndice D – Tabelas suplementares do artigo 2

Author	Year	Country	N	Setting	Follow-up (days)	Dose	Disease	Risk of Bias
Carter, A.E.	1971	NA	617	Inpatients	NA	600 mg/daily	Postoperative thromboembolism prophylaxis	Some concerns
Carter,A.E.	1974	NA	204	Inpatients	NA1200 mg before surgery / 800 mg after surgery until discharge		Postoperative thromboembolism prophylaxis	Some concerns
Hansen, E.H.	1976	Denmark	153	Inpatients	21	600 mg/daily	Fractures	Some concerns
Chrisman,O.D.	1976	United States	100	Inpatients	21	400 mg on admission / 600 mg daily	Postoperative thromboembolism prophylaxis	Some concerns
Wu,T.K.	1977	United States	134	Inpatients	7	600 mg/daily	Postoperative thromboembolism prophylaxis	Some concerns
Hume,M.	1977	United States	81	Inpatients	NA	600 mg/daily	Postoperative thromboembolism prophylaxis	Some concerns
Cooke,E.D.	1977	England	50	Inpatients	NA	1200 mg preoperatively + 1000 mg/daily	Postoperative thromboembolism prophylaxis	Some concerns
Johansson,E.	1981	Sweden	35	Inpatients	12	1200 mg pre-operation + 800 mg	Postoperative thromboembolism prophylaxis	Some concerns
Snook,G.A.	1981	United States	75	Inpatients	NA	600 mg/daily	Postoperative thromboembolism prophylaxis	Some concerns
Erkan,D.	2017	United States	20	Outpatients	627	< 60Kg = 200 mg/daily > 60 kg = 400 mg/daily	Antiphospholipid Syndrome	Low
Self,W.H.	2020	United States	479	Inpatients	28	800 mg/day for 2 days + 400 mg/day for 8 days	COVID-19	Low
Cavalcanti,A.B	2020	Brazil	665	Inpatients	15	600 mg/daily	COVID-19	Low
Kravvariti, E.	2020	Greece	50	Outpatients	936	< 60Kg = 200 mg/daily > 60 kg = 400 mg/daily	Antiphospholipid Syndrome	Low

Table 1. Main characteristics of the studies included in the meta-analysis.

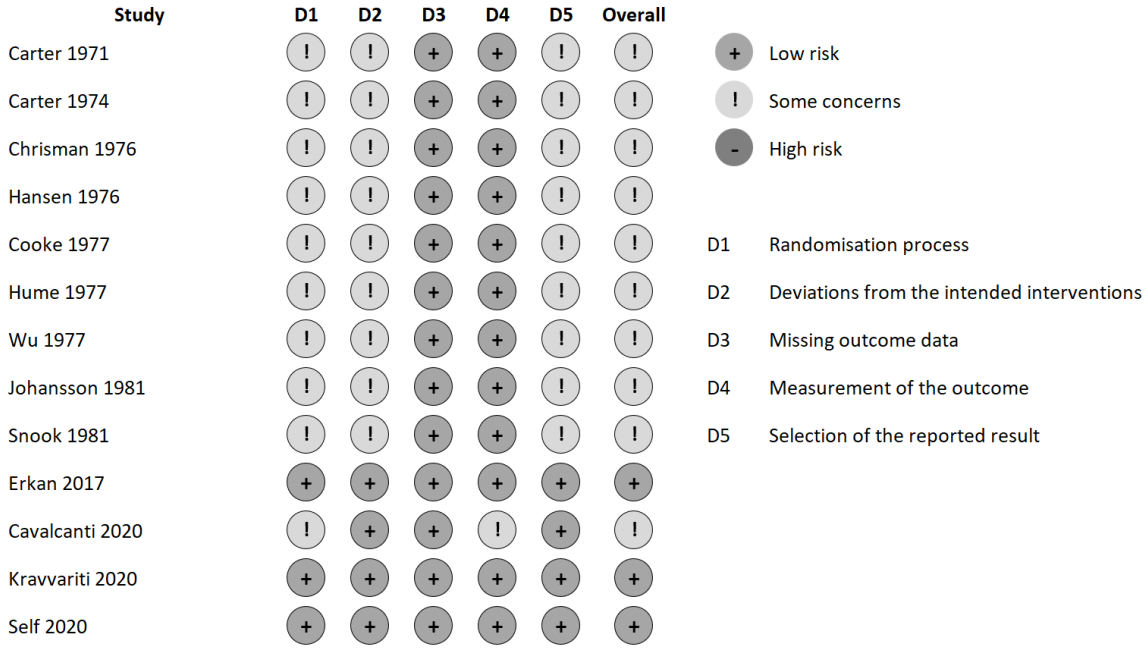

Table 2. Risk of bias analysis