



STUDY PROTOCOL

REVISED **Metformin as adjunctive therapy for dengue in overweight and obese patients: a protocol for an open-label clinical trial (MeDO) [version 2; peer review: 3 approved]**

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V2 First published: 06 Jul 2020, 5:160
<https://doi.org/10.12688/wellcomeopenres.16053.1>
 Latest published: 08 Feb 2021, 5:160
<https://doi.org/10.12688/wellcomeopenres.16053.2>

Abstract

Background: Dengue is a disease of major global importance. While most symptomatic infections are mild, a small proportion of patients progress to severe disease with risk of hypovolaemic shock, organ dysfunction and death. In the absence of effective antiviral or disease modifying drugs, clinical management is solely reliant on supportive measures. Obesity is a growing problem among young people in Vietnam and is increasingly recognised as an important risk factor for severe dengue, likely due to alterations in host immune and inflammatory pathways. Metformin, a widely used anti-hyperglycaemic agent with excellent safety profile, has demonstrated potential as a dengue therapeutic *in vitro* and in a retrospective observational study of adult dengue patients with type 2 diabetes. This study aims to assess the safety and tolerability of metformin treatment in overweight and obese dengue patients, and investigate its effects on several clinical, immunological and virological markers of disease severity.

Methods: This open label trial of 120 obese/overweight dengue patients will be performed in two phases, with a metformin dose

Open Peer Review

Reviewer Status

	Invited Reviewers		
	1	2	3
version 2 (revision) 08 Feb 2021			 report
version 1 06 Jul 2020	 report	 report	 report

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escalation if no safety concerns arise in phase one. The primary endpoint is identification of clinical and laboratory adverse events. Sixty overweight and obese dengue patients aged 10-30 years will be enrolled at the Hospital for Tropical Diseases in Ho Chi Minh City, Vietnam. Participants will complete a 5-day course of metformin therapy and be compared to a non-treated group of 60 age-matched overweight and obese dengue patients.

Discussion: Previously observed antiviral and immunomodulatory effects of metformin make it a promising dengue therapeutic candidate in appropriately selected patients. This study will assess the safety and tolerability of adjunctive metformin in the management of overweight and obese young dengue patients, as well as its effects on markers of viral replication, endothelial dysfunction and host immune responses.


Trial registration: ClinicalTrials.gov: [NCT04377451](https://clinicaltrials.gov/ct2/show/study/NCT04377451) (May 6th 2020).


Keywords

dengue, metformin, obesity, clinical trial, therapeutic, Vietnam



This article is included in the [Oxford University Clinical Research Unit \(OUCRU\) gateway](#).

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Competing interests: No competing interests were disclosed.

Grant information: This work is supported by the Wellcome Trust [106680]. The trial sponsor is the University of Oxford, Oxford, United Kingdom.

The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

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How to cite this article: Nguyen NM, Chanh HQ, Tam DTH *et al.* **Metformin as adjunctive therapy for dengue in overweight and obese patients: a protocol for an open-label clinical trial (MeDO) [version 2; peer review: 3 approved]** Wellcome Open Research 2021, 5:160 <https://doi.org/10.12688/wellcomeopenres.16053.2>

First published: 06 Jul 2020, 5:160 <https://doi.org/10.12688/wellcomeopenres.16053.1>

REVISED Amendments from Version 1

In response to the reviewer's comments, we have added the following sentences: We have included further discussion on the speed of the pharmacological action of metformin under the sub-heading "Metformin and dengue" in the background section. Additional information has been included on the absorption of metformin and time to peak concentration as well as data from two in-vitro and mouse studies demonstrating rapid activation of AMPK and viral suppression. A paragraph was added to the sub-heading "Screening, enrollment and retention", outlining our methods for reducing selection bias in the trial, including enrolment strategies as well as blinded outcome assessments by study investigators and an intention to treat analysis. At the end of the discussion heading, we have added that if the safety of this drug is established and this pilot study demonstrates improvement in any of the secondary endpoints, a larger randomized-controlled trial of metformin in dengue would be warranted.

Any further responses from the reviewers can be found at the end of the article

Background

Dengue is a major threat to global public health, with an estimated annual incidence of 390 million infections worldwide, leading to approximately 96 million symptomatic cases and 22,000 deaths in more than 120 countries¹. Despite increasing understanding of dengue pathogenesis, effective anti-viral or disease modifying agents are yet to be identified, and patient management remains reliant on judicious fluid replacement in response to plasma leakage during the critical phase of illness (usually days 4–6)^{2,3}.

While the vast majority of symptomatic infections give rise to the milder form of disease, dengue fever, those that progress to severe disease carry significant risk of vital organ dysfunction and death. Several patient groups have been identified as being at particular risk of severe dengue, including the elderly, pregnant women, those with comorbid illness, and those with prior exposure to alternative dengue serotypes⁴. An important and increasingly recognised severe dengue risk factor is obesity, a condition which has had a dramatic increase in prevalence among Vietnamese children and adolescents over the last two decades⁵. Several observational studies have reported an association between obesity and severe dengue^{6–9}. A recent systematic review and meta-analysis reported the odds of developing severe dengue to be significantly higher in obese children in comparison to their non-obese counterparts (OR = 1.38; 95%CI 1.10 - 1.73)¹⁰.

The underlying mechanism remains unknown, but could possibly be explained by chronic low-grade inflammation that is induced by being overweight or obese^{11,12}. Adipocytes are known to produce pro-inflammatory cytokines and adipokines, such as adiponectin, resistin and leptin, each of which have a role in immunomodulation. Obesity-induced leptin and resistin are known to be associated with many of the physiological abnormalities seen in dengue, including platelet

dysfunction, vascular inflammation, myocardial injury, endothelial dysfunction^{13–16}, and capillary hyper-permeability^{17–19}. Additionally, it is possible that more severe infection is permitted by altered CD8⁺ T cell immunity and impairment of natural killer (NK) cell activity in obese hosts^{20,21}.

Adenosine Monophosphate (AMP)-Activated protein kinase (AMPK) is an enzyme involved in maintaining cellular energy homeostasis, in particular lipid metabolism, protein synthesis and glucose metabolism, and has been linked to anti-inflammatory processes²². Obesity has been shown to down-regulate AMPK, either as a result of fatty acid, amino acid and glucose accumulation²², or due to an increase in circulating pro-inflammatory cytokines^{22,23}. One consequence of AMPK down-regulation is the inhibition of lipid catabolism and subsequent boosting of *de novo* lipid synthesis, creating conditions that favour viral replication and survival within host cells^{24,25}.

With the majority of symptomatic dengue infections leading to mild, self-limiting illness, development of novel treatment strategies should focus on patient groups at the highest risk of severe disease²⁶. This study aims to assess the effect of metformin, a drug known to attenuate inflammatory processes particularly in the context of obesity, on the disease course of dengue in overweight and obese children and young adults.

Metformin

Metformin (dimethylbiguanide) is an oral anti-hyperglycaemic agent. Its mechanism of action is complex and only partially understood, but AMPK activation is recognised as one of a number of key processes in modulating glucose metabolism²⁷. After sixty years of use as the first-line drug to manage type 2 diabetes mellitus (T2DM), metformin has proven to have an excellent safety and tolerability profile, while also demonstrating potential to have further therapeutic applications through its broad range of pleiotropic effects. These include antioxidant, anti-inflammatory and immunomodulatory properties, as well as aiding endothelial vascular reactivity and preservation of the glycocalyx layer^{28–30}.

Evidence regarding the effectiveness of metformin in treating obesity is well established. The drug has demonstrated utility in inducing both significant weight loss and a reduction in the inflammatory biomarkers interferon- γ , total plasminogen activator inhibitor-1 (PAI-1) and the adiponectin–leptin ratio in obese adults with or without T2DM^{31–33}.

Other studies have shown improved clinical outcomes with metformin use in a number of infectious diseases, including hepatitis C (HCV), tuberculosis and dengue^{34–39}. Notably, the use of metformin as an adjunct in the treatment of patients with HCV and insulin resistance has been shown to improve rates of sustained virological response in comparison to standard anti-viral treatment alone⁴⁰. Furthermore, two prospective trials of metformin use in patients with HCV and T2DM have demonstrated significant reductions in liver-related complications^{35,36}.

Metformin and dengue

Several of the aforementioned effects of metformin treatment have prompted consideration of its use as a potential dengue therapeutic. Firstly, the replication of dengue virus has been shown to be restricted by inhibition of lipid synthesis through AMPK activation^{24,41}. Furthermore, metformin's immunomodulatory effect, which facilitates NK cell activation, reduces T cell exhaustion profiles and enhances CD8⁺ T cell memory, could help reduce levels of peak dengue viremia and shorten viral clearance times^{39,41,42}.

Metformin is rapidly absorbed and eliminated as an oral formulation and steady state is usually achieved within 24 hours. Metformin has been shown to decrease dengue viral genome copies in a dose-dependent manner *in vitro* via activation of AMPK, with 90% reduction in NS1 secretion in infected cells after 24 hours of treatment⁴¹. In obese/overweight adolescents, time to peak concentration and absorption, appears to be faster than non-obese children⁴³. In addition, anti-viral effects using a 5 day course of metformin has also been demonstrated in a mouse model of coxsackievirus B3 (CVB3) infection, through rapid action on AMPK activation⁴⁴.

Prevention of progression to severe dengue by attenuating the inflammatory mediators leptin and resistin is also plausible⁴⁵. Additionally, metformin could help reverse endothelial dysfunction, a hallmark feature of dengue pathogenesis, by improving endothelial-dependent vasodilation and reducing expression of endothelial vascular cell adhesion molecule (VCAM) and intercellular adhesion molecule 1 (ICAM1)²⁴. Recent observational evidence suggests metformin use in adult dengue patients with T2DM is associated with reduced risk of developing severe dengue (adjusted RR = 0.60, 95%CI 0.37-0.98)³⁷. However, the mechanisms underlying this association, and any relationship they have to obesity, require further investigation.

Aims of the trial

We hypothesize that metformin therapy given early in the course of disease will attenuate obesity-induced lipid-inflammatory mediators and improve clinical parameters in dengue patients with obesity. In addition, the therapy could reduce viral replication through AMPK activation and immunomodulation in such patients.

This study aims to formally assess metformin as adjunctive therapy for dengue in overweight and obese patients.

Methods/design

This is protocol version 4.0, dated February 10th 2020.

Design

This is an open-label study investigating metformin therapy in 60 overweight and obese Vietnamese adults (30 patients) and children (30 patients). Age range of 10 to 30 years, who are admitted to the Hospital for Tropical Diseases (HTD) in Ho Chi Minh City (HCMC), Vietnam, with positive non-structural

protein 1 (NS1) rapid test and onset of fever <72 hours prior to admission.

We will investigate the effects of five days of metformin treatment on the grounds that the critical phase of disease usually occurs around the fifth day of illness.

The trial will be conducted in two phases, with a dose escalation between phases (Figure 1). In the initial phase (cohort 1), five young adults (age ≥ 16) and five children (age <16) with body mass index (BMI) >25 kg/m² (BMI-for-age >1 standard deviation – SD) will be provided with a low dose of metformin once daily at 850mg and 500mg, respectively. If the five-day safety and clinical data of cohort 1 show no safety concerns, the study will progress to the second phase (cohort 2). This will include 25 adults and 25 children, who will be given a weight-based dose of metformin; 1000mg (500mg twice daily) for participants with weight <60kg, and 1500mg (1000mg mane, 500mg nocte) for those ≥ 60 kg. Of note, the actual number of patients in each cohort will depend on the results of safety reviews. Recruitment is expected to happen over two dengue seasons, and discharged patients will be asked to return for a single outpatient follow-up (FU) visit around day 28 of illness.

The 60 patients receiving treatment will be compared to 60 overweight or obese patients with standard of care (without treatment) recruited contemporaneously from the same wards, with the same enrolment criteria, in an ongoing observational study as the control arm.

Inclusion and exclusion criteria

Patients will be eligible for study enrolment if they: (i) are aged between 10 and 30 years; (ii) have a clinical diagnosis of dengue (based on World Health Organization (WHO) – Dengue guidelines)²; (iii) are NS1 rapid antigen test positive; (iv) have BMI >25kg/m² (or BMI-for-age >1 SD); (v) are admitted within 72 hours of fever onset; (vi) agree to come back for FU visit between days 21–28 of illness; and (vii) provide written informed consent.

Exclusion criteria include: (i) pregnancy (confirmed by either clinical examination or urine dipstick for human chorionic gonadotrophin hormone); (ii) localising features suggestive of an alternative diagnosis; (iii) significant diarrhoea and/or vomiting (>3 episodes/24 hours); (iv) history of hypersensitivity to metformin; (v) current use of metformin or any other regular hypoglycaemic agent, including insulin; (vi) current use of any drug with significant metformin interaction (*Extended data*⁴⁶); (vii) current treatment for heart failure or recent history of myocardial infarction (in the last year); (viii) presence of severe infection at enrolment, including severe dengue, central nervous system infection or septicaemia; (ix) baseline lactate level >2.0 mmol/L; (x) baseline blood glucose level <3.9 mmol/l (or <70 mg/dL); (xi) baseline liver impairment (alanine transaminase (ALT) and/or aspartate transaminase (AST) >250 U/L); and (xii) chronic renal impairment (glomerular

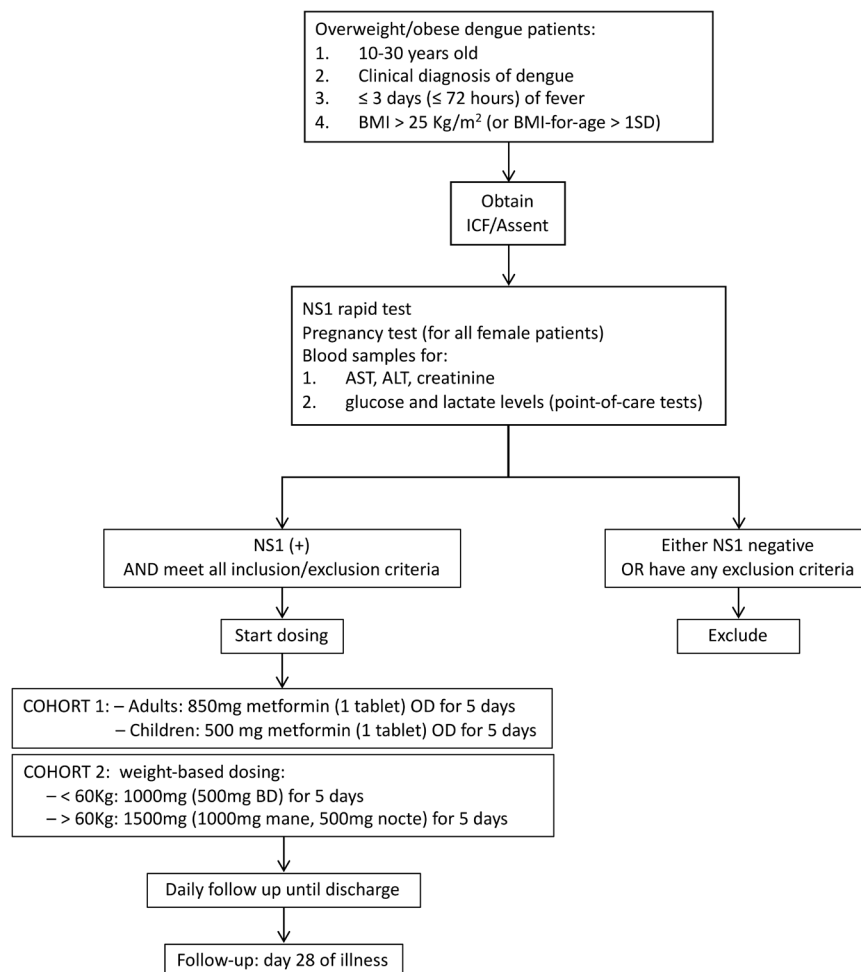


Figure 1. Trial flow chart.

filtration rate (GFR) <30 mL/min). Patients that are deemed unlikely to attend FU at 28 days from fever onset because of long travelling distance to the clinic will also be excluded.

Primary endpoint

The primary endpoint of this study is an evaluation of the safety and tolerability of metformin therapy in overweight and obese young adults and children with dengue. The number of clinical and laboratory adverse events (AEs) occurring during the five study days (Table 1), will be compared between the treatment population and the non-treated controls.

Secondary endpoints

The secondary endpoints are: fever clearance time (the time from enrolment to sustained body temperature <37.5°C for 48 hours); platelet nadir and highest AST/ALT between days 3 and 8 of illness; percentage increase in haematocrit (HCT) from baseline (using HCT obtained at the 28 day FU visit to reflect

baseline); plasma viraemia (area under the \log_{10} -transformed viraemia curve from study days 3 to 6); time from enrolment to first undetectable viraemia and first negative NS1 measurement; trend over time (5 days) in VCAM1, CRP, leptin, adiponectin, low density lipoprotein (LDL), and AMPK phosphorylation; and magnitude of activated/proliferating T and NK cells as well as frequency and phenotype of dengue-specific CD4+ and CD8+ T cells, quantified by flow cytometry at enrolment and hospital discharge.

Screening, enrolment and retention

Patients aged between 10 and 30 years that are admitted to one of the three study wards of the HTD, HCMC, Vietnam, with clinical suspicion of dengue and less than 72 hours of fever are invited to participate in the trial. After giving consent, patients are screened for their eligibility to commence treatment with the trial drug by the study physicians. Blood samples are collected to test for NS1, and AST, ALT and creatinine levels

Table 1. Adverse events.

1- Worsening level of consciousness (fall in GCS \geq 2 points)
2- Change in blood pressure (PP < 20 mmHg, SBP < 90 mmHg or unmeasurable BP)
3- Vomiting (\geq 3 episodes separated by 15 minutes/ 24 hours)
4- Epigastric abdominal pain
5- Diarrhoea (\geq 5 episodes/24 hours)
6- Mucosal bleeding
7- Rash, urticaria
8- Increased lactate (\geq 3 mmol/L)
9- Episode of hypoglycaemia (glucose < 3.9 mmol/L or < 70 mg/dL)
10- Increased liver enzyme (AST or ALT > 400 U/L)
11- Acute kidney injury (eGFR reduction of >50% or < 30 ml/mn/1,73m ² or plasma creatinine >2 fold increase from baseline)
12- Thrombocytopenia (Platelet < 20.000/uL)
13- Other event, specify: [_____]
Grade of the event
Mild: Discomfort noticed but no disruption of normal daily activities
Moderate: Discomfort sufficient to reduce or affect daily activity
Severe: Inability to perform daily activity

and in female patients, a urine sample is collected for a pregnancy test. Glucose and lactate levels are measured using point-of-care (POC) tests.

All patients will be asked to come back for a final FU visit at 28 days after the onset of fever. Patients will be contacted 3–5 days prior to the FU visit to arrange an appropriate appointment date and time. Any case who misses the FU visit will be contacted by phone either to reschedule the visit or to collect information if patients could not arrange their time to come back. If the patients stay in the hospital for longer than 5 days, due to AEs or any other reason, the treatment for these cases will be at discretion of the attending physicians. No further blood samples or study procedures will be performed from this point onward. However, the final outcome at discharge will be collected in the case report form (CRF; *Extended data*⁴⁶).

At discharge, the participants will be offered a referral to nutrition specialists for a consultation of overweight/obesity management. Patients will also be provided with leaflets on healthy eating and lifestyle behaviours.

While not an RCT, this trial is designed to enroll patients contemporaneously with an observational obesity trial, where patients received standard care, to minimize systematic differences between the intervention (metformin) and control group, i.e. patients have the same inclusion /exclusion criteria, enrolled on same wards, with the same standard of care during the same dengue season (to avoid the issue of viral serotype shifts).

To minimize selection bias, we have set up a system on the dengue wards, to ensure eligible patients are recruited into both studies in a systematic way. This involves only one study enrolling for a 2-week time block. During that time, if a patient fails screening assessment or declines enrollment into one of the trials, they are not eligible for the other.

In addition, we will ensure the predefined inclusion/exclusion criteria for all patients enrolled are strictly adhered to reduce investigator subjective assessment and will carefully document screen failures and all exclusions and withdrawals in the trial. And lastly, we plan to have an intention-to-treat analysis where independent study investigators will perform a blinded outcome assessment for all patients.

Safety reviews

The trial will be conducted in two phases, with a dose escalation if no safety concerns arise during the low dose stage. Metformin dosing will begin at 500mg (children) and 850mg (adults) once daily for 5 days in cohort 1. Daily patient safety reviews will be performed by study doctors using clinical and laboratory data to decide whether patients are safe to continue taking the study drug.

The study drug will be stopped if participants: (1) request withdrawal from the study; (2) develop any AEs, including severe renal impairment (GFR below 30mL/min/1.73m²), elevated lactate to \geq 3 mmol/L, severe liver involvement (AST/ALT > 400U/L), severe diarrhoea (\geq 5 episodes of loose

stool/day), persistent vomiting (≥ 3 episodes/hour or $\geq 4 / 6$ hours) and hypoglycaemia (blood glucose < 3.9 mmol/l or < 70 mg/dL); (3) develop severe dengue (defined as admission to intensive care unit); or (4) are intolerant of metformin.

All AEs occurring during the trial/or until the FU visit at around day 28 of illness, will be recorded in the CRF, and whether or not attributed to trial medication. Specific AEs are shown in [Table 1](#). Laboratory events will be graded according to Common Terminology Criteria for Adverse Events (CTCAE) definitions. Clinical events will be assessed as mild, moderate, or severe. If the event is serious and not only related to the progression to severe dengue, or is fatal, then a serious AE form must be completed and the OUCRU CTU notified within 24 hours.

In cases of discontinuation due to AEs, participants will be followed up until the events have resolved or stabilised. A Data Monitoring Committee (DMC) review will take place after day 5 data is fully available for the first ten patients enrolled in cohort 1. Following satisfactory safety review of the tenth patient, metformin doses for all remaining patients (cohort 2) will be weight-based; 5 days of 1000mg (500mg twice daily) for those weighing < 60 kg and 5 days of 1500mg (1000mg mane, 500mg nocte) for those weighing ≥ 60 kg. All AEs and serious adverse events (SAEs) will be recorded and reported to the Ethics Committees (ECs) for review. Further DMC meetings will take place 6 monthly to review enrolment, AEs, treatment received and FU information, and any other requested data.

Treatment and drug dispensation

Metformin will be kept in a dry location below 30°C, in a secure area. A study pharmacist will make up and label individual-participant treatment packs with a trial number. Each pack contains sufficient study drug for 5 days of treatment and will be distributed to study wards as required. All medication storage and administration will be regulated through the central pharmacy to ensure good quality and control of medication handling.

Patients will be recruited into either cohort 1 (low dose) or cohort 2 (high dose). Study drugs will be administered as directly-observed-therapy. Patients will take the first dose of study drug with a light snack as soon as possible after enrolment. The remaining doses will be taken after meals. In case of vomiting within 30 minutes of taking the treatment, one replacement dose will be given. However, if patients continue vomiting after taking the replacement dose, no extra drug will be given thereafter.

Data collection

Clinical evaluation. Patients will be reviewed daily until discharge. All clinical symptoms and signs will be recorded in the CRF. An ultrasound scan will be performed on day 5–6 of illness to detect evidence of fluid accumulation. General management decisions will be at the discretion of the attending ward doctors. Details of all AEs and SAEs will be recorded on specific forms, together with an assessment as to whether the events are likely to have been related to any treatment

received. All SAEs will be reported promptly to the DMC and ECs according to policy.

Laboratory evaluation. Full blood count (FBC) and POC glucose and lactate tests will be undertaken daily until discharge. An additional POC glucose test will be performed before their evening meal.

In addition, levels of serum albumin, transaminases (ALT/AST), creatinine, total cholesterol, HDL-cholesterol, LDL-cholesterol, triglycerides, and C-reactive protein (CRP) will be measured on alternate days. All tests will be done more frequently when clinically indicated.

Research samples collected daily until discharge will be used for dengue diagnostics (NS1 antigen detection and PCR assays). Residual blood from FBC samples will be collected for daily plasma viraemia levels. Serology tests (Capture IgM/IgG ELISAs) will be performed on enrolment (acute) and discharge/FU (convalescent) samples. Other laboratory investigations will include endothelial, inflammatory and lipid biomarkers (VCAM1, syndecan-1, leptin, adiponectin), using commercially available ELISAs and Luminex kits, immunological tests (T cell phenotype and functional analysis), and immunometabolism (AMPK phosphorylation). RNA will be extracted from a blood sample collected on study day 2, and will be sequenced to investigate gene expression in the metformin treatment group in comparison to the non-treatment group.

At day 28 of illness, final routine (FBC, biochemistry) and research blood samples will be taken to check whether patients have fully recovered from the acute dengue episode.

Statistical considerations

Sample size. This is an exploratory study focusing primarily on safety, and there are no preliminary data regarding the effects of metformin in dengue on which to base a sample size calculation. A target sample size of 60 patients was chosen based on clinical judgement and feasibility considerations. Using the trend in VCAM over time, which is one of the secondary endpoints, by assuming a slope of change in concentration over time at 0.34 (\log_2 VCAM concentration per day, data based on biomarkers of endothelial function in dengue), we conducted a power calculation using a t-test to compare effects on endothelial function between two groups. It is suggested this study will have a power of 80% to detect a difference of at least 0.023 (\log_2 VCAM concentration per day) in the slope of change.

Statistical analysis. The primary analysis population will include all patients from cohort 1 and cohort 2, plus 60 controls as mentioned previously. Baseline characteristics and outcomes will be summarised for each treatment group. The cohort of patients enrolled in this study will form the active treatment group, while the control group will consist of overweight and obese dengue patients that participated in a matched cohort observational study, none of whom received metformin therapy.

Continuous variables will be described using medians and quartiles, while categorical variables will be described in terms of frequencies and percentages.

The primary endpoint (number of AEs), will be compared between the treatment groups using negative binomial regression model. The clinical, biomarker, immunological will be compared between the treatment arms using linear regression or random effects model for continuous endpoints, and Cox regression for time-to-event endpoints (fever clearance time, time to first undetectable viremia measurement, time to first negative NS1 measurement). All comparisons will be adjusted for age, sex, and day of illness, as patients will not be randomized. For percentage increase in HCT and area under the curve of viraemia, comparisons will be also adjusted for the pre-dose value of the respective marker.

Ethical considerations

Ethical approval

The protocol, informed consent forms, assent forms and patient information sheets have been reviewed and approved by the ECs of the Hospital for Tropical Diseases in Ho Chi Minh City (CS/BND/19/34), the Vietnamese Ministry of Health (24/CN-HĐĐĐ) and the Oxford Tropical Research Ethics Committee (OxTREC reference: 36-19). The Investigator will submit and, where necessary, obtain approval from the above committees for all amendments to the originally approved documents.

Informed consent and information sheet

All patients entering the study must give written informed consent. The study physician will describe the purpose of the study, the study procedures, possible risks/benefits, the rights and responsibilities of participants, and alternatives to enrolment. The patient or parent/guardian will be invited to ask questions which will be answered by study staff, and they will be provided with appropriate numbers to contact if they have any questions subsequently. If the patient or parent/guardian agrees to participate, they will be asked to sign informed consent/assent forms (ICFs; *Extended data*⁴⁶). A copy of the form, with signature, will be given to them to keep. In addition to the procedures above, illiterate signatories will have the ICF read to them in the presence of a witness who will sign to confirm this.

Patients, who are between 10 and 18 years of age, will be asked to sign the assent form in which, the study purpose and procedures will be explained to them in child friendly terms. Their parent/guardian will be asked to sign the consent form as well to give permission for their child to participate in the study.

Confidentiality

Patients who enter the trial will be given a unique identification number. This number will be used on both laboratory specimens and CRFs. The study wards and the research unit have the facilities to store study information securely. Only study staff will have access to the password-protected computer where entered data is stored. After conclusion of

the study, data will be stored in a safe place. Any scientific publications or reports will not identify any patient by name or initials. When the research team reviews the clinical notes, they are also bound by professional confidentiality.

Data monitoring and trial steering committees

A Trial Steering Committee (TSC) will be formed to provide overall supervision of the conduct of the trial and provide advice through its independent Chair. The ultimate decision for the continuation of the trial lies with the TSC. In particular, the TSC will concentrate on progress of the trial, adherence to the protocol, patient safety, and the consideration of new information.

An independent DMC will be set up consisting of qualified volunteers with the necessary knowledge of clinical trials and statistics. The DMC will review the protocol and agree to a data review schedule and reporting requirements before the study commences. All data reviewed by the DMC will be in the strictest confidence. A DMC charter will outline its responsibilities and operations (*Extended data*⁴⁶).

Monitoring will then be carried out approximately annually by OUCRU CTU staff. The frequency, type and intensity of routine monitoring and the requirements for triggered monitoring will be detailed in the monitoring plan, which will also detail the procedures for review and sign-off. The monitoring will adhere to the principles of ICH GCP and the monitoring plan.

Data collection and management

All study data will be recorded on standard CRFs and entered on the CliRes database. The participants will be identified by a unique study specific number and/or code in any database. The name and any other identifying detail will not be included in any stored electronic CRFs. Direct access will be granted to authorized representatives from the University of Oxford and any host institution for monitoring and/or audit of the study to ensure compliance with regulations. Original paper documents will be stored at the OUCRU-CTU for 15 years after which paper files will be scanned and archived electronically. Electronic data will be stored indefinitely on a secure OUCRU-VN server for a minimum period of 10 years and paper copies will be destroyed securely.

Spirit checklist

A SPIRIT checklist for this trial protocol is attached (*Reporting guidelines*⁴⁶).

Data sharing and dissemination

In line with research transparency and greater access to data from trials, OUCRU's clinical trials are registered at Clinical-Trials.gov and a data sharing policy is in place. Data exchange complies with Information Governance and Data Security policies in all of the relevant countries.

Participants will not be individually informed of results. Data from this study will be published in peer-reviewed journals. Authorship and reporting of this work will follow international

guidelines and all authors will have made a noteworthy contribution to the work.

Trial status

We expect that this trial will start recruiting patients in June 2020.

Discussion

Despite increasing investment in strategies to prevent infection, dengue remains a major global pathogen, with extremely high associated health, social and economic disease burden⁴⁷. Severe dengue can be rapidly life threatening as a result of plasma leakage, hypovolaemic shock and subsequent end organ damage, and is known to disproportionately affect overweight and obese individuals. Although optimal supportive care is largely successful in permitting recovery from most dengue infections, effective therapeutic agents are desperately needed to enable treatment of patients with the greatest disease severity. Metformin has previously demonstrated therapeutic potential due to observed anti-viral and immunomodulatory effects, and an association with reduced risk of severe dengue in adults with T2DM. Additionally, its low cost and excellent safety profile in the treatment of T2DM make metformin a prime candidate for use in appropriately selected patients on a mass scale. This study will assess the safety and tolerability of metformin in the context of dengue infection, and whether metformin treatment early in the course of disease is clinically beneficial for overweight and obese children and young adults. It will also provide a key insight into the effects of metformin on markers of viral replication, endothelial dysfunction and

the host immune response in this patient group. If the safety of this drug is established and this pilot study demonstrates improvement in any of the secondary endpoints, a larger randomized-controlled trial of metformin in dengue would be warranted

Data availability

Underlying data

No underlying data are associated with this article.

Extended data

Oxford University Research Archive: Oxford University Clinical Research Unit Metformin trial supporting documents, <https://doi.org/10.5287/bodleian:5Rk2p9EVK>⁴⁶.

- Consent form
- Participant information sheet
- DMC charter
- Case Report Form
- Drug interactions

Reporting guidelines

Oxford University Research Archive: SPIRIT checklist for 'Metformin as adjunctive therapy for dengue in overweight and obese patients: a protocol for an open-label clinical trial (MeDO)', <https://doi.org/10.5287/bodleian:5Rk2p9EVK>⁴⁶.

Data are available under the terms of the [Creative Commons Attribution 4.0 International license](#) (CC-BY 4.0).

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Open Peer Review

Current Peer Review Status:   

Version 2

Reviewer Report 24 February 2021

<https://doi.org/10.21956/wellcomeopenres.18074.r42516>

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 **Rosa María Del Ángel** 

Department of Infectomics and Molecular Pathogenesis, Center for Research and Advanced Studies (CINVESTAV-IPN), Mexico City, Mexico

This revised version of the manuscript is now suitable for indexing.

Competing Interests: No competing interests were disclosed.

I confirm that I have read this submission and believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard.

Version 1

Reviewer Report 08 October 2020

<https://doi.org/10.21956/wellcomeopenres.17609.r40457>

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This manuscript reports the rationale and design of a clinical trial to test the safety of a 5-day

course of metformin as treatment to reduce the likelihood of severe disease in obese dengue patients. The motivation of this study is the emerging epidemiological association between obesity and increased risk of severe dengue. The authors reasoned that activation of AMPK by metformin would counteract the reduced AMPK activity in obese patients; lowered AMPK is associated with altered lipid metabolism that increase dengue virus (DENV) replication, *in vitro*. Thus, the authors postulate that activation of AMPK by metformin would result in an increase in lipid catabolism inhibiting DENV replication and viremia, which has been found in several studies to be correlated with disease outcome. They also postulate that metformin would also exert effects on the immune response, including NK cell activation and reduction in T-cell exhaustion, that would collectively contribute to limiting DENV infection and replication. To test the hypothesis, the authors' first aims to show that high-dose metformin can be given safely to patients acutely ill with dengue; standard doses of metformin would be administered to ensure safety before dose escalation. The primary endpoint of the study is to observe the safety and tolerability of metformin in overweight and obese young adults and children with dengue.

This is a timely study as the prevalence of both obesity and dengue are increasing globally. There are several areas of consideration.

1. It has been described that viremia in most patients after illness onset decline rapidly. Thus impact of metformin on AMPK activation must be rapid. How much time is necessary to induce the activation of AMPK by metformin? Please include a discussion about this aspect.
2. If obesity is a risk factor for dengue infection. Why in this clinical trial enrollment of non-obese persons is not considered?
3. Why did the authors select 28 days?
4. Why is placebo not going to be included?
5. DENV infection will be determined by the detection of NS1. How frequent is the infection with other flavivirus in the region? Is the detection of NS1 specific to confirm DENV infection? The authors could use RT-PCR instead of NS1 to confirm DENV infection.
6. How are the authors going to measure VCAM1, CRP, leptin, adiponectin, low density lipoprotein (LDL), and AMPK phosphorylation?

Minor comment:

1. GRF on page 5 should be replaced with GFR.

Is the rationale for, and objectives of, the study clearly described?

Yes

Is the study design appropriate for the research question?

Yes

Are sufficient details of the methods provided to allow replication by others?

Partly

Are the datasets clearly presented in a useable and accessible format?

Yes

Competing Interests: No competing interests were disclosed.

Reviewer Expertise: Antiviral in flavivirus infection

We confirm that we have read this submission and believe that we have an appropriate level of expertise to confirm that it is of an acceptable scientific standard, however we have significant reservations, as outlined above.

Author Response 02 Nov 2020

Nguyet Nguyen, Oxford University Clinical Research Unit, Ho Chi Minh City, Vietnam

Comment #1: Thank you for this suggestion, we also acknowledge that there is a narrow therapeutic window, in all dengue therapeutic trials. We are therefore aiming to give the drug within the first 48 hours of fever (latest 72 hours). Metformin is rapidly absorbed and eliminated as an oral formulation and steady state is usually achieved within 24 hours. In obese/overweight adolescents, time to peak concentration and absorption, appears to be faster than non-obese children {van Rongen A. et al. 2018. *Paediatr Drugs*}. An in-vitro study showed metformin increased AMPK activity after 24 hours and reduced of dengue viral replication by one logarithm and up to 90% of NS1 secretion {Soto-Acosta R. et al. 2017. *PLoS Pathog*}. In addition, anti-viral effects using a 5 day course of metformin has also been demonstrated in a mouse model of coxsackievirus B3 (CVB3) infection, through rapid action on AMPK activation {Xie W. et al. 2015. *J Mol Cell Cardio*}.

The effect of metformin on AMPK and reduction in viremia is just one mechanism why we hypothesize this drug will be beneficial in dengue patients, its other effects, including anti-inflammatory, immunomodulatory and endothelial stabilizing properties are also likely to be important mechanisms in attenuating the disease outcome.

A paragraph has been added to "Metformin and Dengue" section.

Comment #2: We chose to focus on obese patients initially, as these patients have a higher risk of disease progression and therefore warrant an intervention. In addition, we hypothesize many of metformin actions, including anti-inflammatory, immunomodulatory, endothelial stabilizer and AMPK activation are already chronically impaired at baseline in obese individuals, thus maximizing the chance of demonstrating efficacy in this population.

Comment #3: The follow-up time point of 28 days is to allow full recovery from the acute illness, and is in line with other clinical trials.

Comment #4: This is a pilot study investigating the safety and tolerability of dengue obese patients to metformin. If the safety of this drug is established, we plan to move to a phase 3 RCT, with larger sample size to allow investigation of clinical endpoints.

Comment #5: Other flaviviruses do circulate in Vietnam including Zika and Japanese encephalitis but these are rare and do not cause the same clinical syndrome as dengue {Quyen NTH et al. 2017. *Am J Trop Med Hyg*}. The sensitivity of NS1 tests for dengue varies by immune status, illness day and serotype ranging from 62-89% , but are highly specific (around 100%) {Tricou V. et al. 2011. *PLoS Negl Trop Dis*; Hermann LL et al. 2014. *PLoS Negl Trop Dis*}

As RT-PCR is costly and time consuming, it is not ideal as a screening test- we are however confirming diagnosis at a later stage with RT-PCR and paired serology.

Comment #6: VCAM1, CRP, leptin, adiponectin, low density lipoprotein (LDL), and AMPK phosphorylation will be measured using a luminex platform and commercially available ELISAs.

This has been added to the laboratory evaluation section.

Competing Interests: We have no competing interests with the reviewer

Reviewer Report 06 October 2020

<https://doi.org/10.21956/wellcomeopenres.17609.r40522>

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Quirijn de Mast 

Department of Internal Medicine, Radboudumc Center for Infectious Diseases, Radboud University Medical Centre, Nijmegen, The Netherlands

This manuscript describes the rationale and design of a trial to investigate the safety and tolerability of metformin as adjunctive therapie in obese children and adults with dengue. Secondary endpoints include dengue-specific (e.g. fever clearance time, viremia, blood markers of severity) and immune parameters

The manuscript is well written. The rationale and design are clear.

Areas for consideration are:

- the trial is not placebo controlled. The authors may consider explaining in more detail why they opted for an open label trial without randomisation
- dengue is an acute infection and any intervention should preferably have an immediate effect. The onset of action of metformin on blood sugars usually takes some days with a maximum effect of up to 2 weeks. The authors may consider arguing in more

detail that the onset of the expected pharmacological effect of metformin is sufficiently rapid to have an effect on the course of dengue

Is the rationale for, and objectives of, the study clearly described?

Yes

Is the study design appropriate for the research question?

Yes

Are sufficient details of the methods provided to allow replication by others?

Yes

Are the datasets clearly presented in a useable and accessible format?

Yes

Competing Interests: No competing interests were disclosed.

Reviewer Expertise: clinical infectious diseases; pathophysiology studies

I confirm that I have read this submission and believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard.

Author Response 02 Nov 2020

Nguyet Nguyen, Oxford University Clinical Research Unit, Ho Chi Minh City, Vietnam

Comment #1: This is a pilot study investigating the safety and tolerability of metformin in dengue patients. As metformin has not been used in acute dengue infections before, this trial was designed to establish safety first, as well as generate some clinical and viral efficacy data. A larger phase 3 RCT will be conducted if this trial shows a positive result.

Comment #2: Thank you for this suggestion, we also acknowledge that there is a narrow therapeutic window, in all dengue therapeutic trials. We are therefore aiming to give the drug within the first 48 hours of fever (latest 72 hours). Metformin is rapidly absorbed and eliminated as an oral formulation and steady state is usually achieved within 24 hours. In obese/overweight adolescents, time to peak concentration and absorption, appears to be faster than non-obese children {van Rongen A. et al. 2018. *Paediatr Drugs*}. An in-vitro study showed metformin increased AMPK activity after 24 hours and reduced of dengue viral replication by one logarithm and up to 90% of NS1 secretion {Soto-Acosta R. et al. 2017. *PLoS Pathog*}. In addition, anti-viral effects using a 5 day course of metformin has also been demonstrated in a mouse model of coxsackievirus B3 (CVB3) infection, through rapid action on AMPK activation {Xie W. et al. 2015. *J Mol Cell Cardiol*}.

The effect of metformin on AMPK and reduction in viremia is just one mechanism why we hypothesize this drug will be beneficial in dengue patients, its other effects, including anti-inflammatory, immunomodulatory and endothelial stabilizing properties are also likely to be important mechanisms in attenuating the disease outcome.

A paragraph has been added to "Metformin and Dengue" section.

Competing Interests: We have no competing interests with the reviewer

Reviewer Report 28 July 2020

<https://doi.org/10.21956/wellcomeopenres.17609.r39444>

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✓ **Eng Eong Ooi** 

Emerging Infectious Diseases Program, Duke NUS Graduate Medical School, Singapore, Singapore

This manuscript reports the rationale and design of a clinical trial to test the safety of a 5-day course of metformin as treatment to reduce the likelihood of severe disease in obese dengue patients. The motivation of this study is the emerging epidemiological association between obesity and increased risk of severe dengue. The authors reasoned that activation of AMPK by metformin would counteract the reduced AMPK activity in obese patients; lowered AMPK is associated with altered lipid metabolism that favoured dengue virus (DENV) replication, *in vitro*. They thus postulate that activation of AMPK by metformin would result in increased lipid catabolism that would inhibit DENV and thus lower viremia, which has been found in several studies to be correlated with disease outcome. They also postulate that metformin would also exert effects on the immune response, including NK cell activation and reduction in T cell exhaustion, that would collectively contribute to limiting DENV infection and replication. To test their hypothesis, the authors first aims to show that high-dose metformin can be given safely to patients acutely ill with dengue; standard doses of metformin would be administered to ensure safety before dose escalation. The primary endpoint of the study is to observe the safety and tolerability of metformin in overweight and obese young adults and children with dengue.

This is a timely study as the prevalence of both obesity and dengue are increasing globally. There are several areas of consideration.

1. The overview of metformin and its potential impact on dengue pathogenesis, through its activity on the AMPK pathway, has not considered how fast metformin would exert this pharmacologic effect. Viremia levels in most patients after illness onset would be declining rapidly. The impact of metformin on AMPK activation must thus be rapid. Likewise, the effect AMPK-induced lipid metabolism has on DENV replication must also be rapidly induced, if metformin is to have a chance to be repurposed as an anti-dengue drug. Perhaps the authors would consider adding a discussion on this challenge to the manuscript?
2. Phase 1 of the trial will observe the safety and clinical data in 10 dengue patients given a 5-day course of standard doses of metformin, over a 5-day period. Should the safety and clinical data not be observed over a longer period, at least until the drug has washed out before a conclusion of no safety concern is made?

3. This is not a placebo-controlled trial. Neither are the patients randomised into receiving metformin or standard of care. Instead, the authors planned to compare treated patients with contemporaneously enrolled patients. How would the authors address the issue of bias in their patient selection?

4. On a related note, how would the authors address the issue of observer bias without blinding?

Minor comment:

1. GRF on page 5 should be replaced with GFR.

Is the rationale for, and objectives of, the study clearly described?

Yes

Is the study design appropriate for the research question?

Yes

Are sufficient details of the methods provided to allow replication by others?

Yes

Are the datasets clearly presented in a useable and accessible format?

Not applicable

Competing Interests: I have co-authored papers in the past with several authors of this report. I do not feel this has affected my ability to review impartially.

Reviewer Expertise: Dengue virus-host interactions, pathogenesis and immunity.

I confirm that I have read this submission and believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard.

Author Response 02 Nov 2020

Nguyet Nguyen, Oxford University Clinical Research Unit, Ho Chi Minh City, Vietnam

Comment #1: Thank you for this suggestion, we also acknowledge that there is a narrow therapeutic window, in all dengue therapeutic trials. We are therefore aiming to give the drug within the first 48 hours of fever (latest 72 hours). Metformin is rapidly absorbed and eliminated as an oral formulation and steady state is usually achieved within 24 hours. In obese/overweight adolescents, time to peak concentration and absorption, appears to be faster than non-obese children {van Rongen A. et al. 2018. *Paediatr Drugs*}. An in-vitro study showed metformin increased AMPK activity after 24 hours and reduced of dengue viral replication by one logarithm and up to 90% of NS1 secretion {Soto-Acosta R. et al. 2017. *PLoS Pathog*}. In addition, anti-viral effects using a 5 day course of metformin has also been demonstrated in a mouse model of coxsackievirus B3 (CVB3) infection, through rapid action on AMPK activation {Xie W. et al. 2015. *J Mol Cell Cardiol*}.

The effect of metformin on AMPK and reduction in viremia is just one mechanism why we

hypothesize this drug will be beneficial in dengue patients, its other effects, including anti-inflammatory, immunomodulatory and endothelial stabilizing properties are also likely to be important mechanisms in attenuating the disease outcome.

A paragraph has been added to "Metformin and Dengue" section.

Comment #2: Thank you - all patients will be followed up at day 28 and will have safety data recorded at that follow-up time-point. For cohort 1 the DSMB have agreed to review day-5 data for the first safety review and all 28-day data for further reviews.

We do not anticipate any issues of drug accumulation, as in patients with normal renal function, the half life is short and the drug rapidly eliminated (a dose of 1000mg has a Tmax of 1.5 hours and a half-life of 2.3hrs {Chung H. et al. 2018. *PLoS One*}). The drug will be stopped in any patient developing acute renal failure.

Comment #3 and #4: While not an RCT, this trial was designed to enroll patients contemporaneously with an observational obesity trial, where patients received standard care, to minimize systematic differences between the intervention (metformin) and control group. I.e. patients have the same inclusion /exclusion criteria, enrolled on same wards, with the same standard of care during the same dengue season (to avoid the issue of viral serotype shifts).

To minimize selection bias, we have set up a system on the dengue wards, to ensure eligible patients are recruited into both studies in a systematic way. This involves only one study enrolling for a 2-week time block. During that time, if a patient fails screening assessment or declines enrollment into one of the trials, they are not eligible for the other.

In addition, we will ensure the predefined inclusion/exclusion criteria for all patients enrolled are strictly adhered to reduce investigator subjective assessment and will carefully document screen failures and all exclusions and withdrawals in the trial. And lastly, we plan to have an intention-to-treat analysis where independent study investigators will perform a blinded outcome assessment for all patients.

We have added this to the 'Screening, enrollment and retention paragraph.'

Comment #5: Thank you. The typo error has already been corrected

Competing Interests: We have no competing interests with the reviewer.