PEER REVIEW HISTORY

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ARTICLE DETAILS

TITLE (PROVISIONAL)	Establishment of a multi-site umbrella cohort study protocol to describe the epidemiology and etiologies of acute undifferentiated febrile illness in Latin America
AUTHORS	Cabada , Miguel; Aguilar, Patricia; Rodas, Juan; Hidalgo, Marylin; Mozo, Karen; Gonzalez-Diaz, Eugenia; Jimenez-Coello, Matilde; Diaz, Francisco; Dacso, Mathew; Ortega-Pacheco, Antonio; Arboleda, Margarita; Walker, David H.; Weaver, Scott; Melby, Peter C.

VERSION 1 – REVIEW

REVIEWER	Pokharel, Sunil University of Oxford Nuffield Department of Medicine, Centre for Tropical Medicine and Global Health
REVIEW RETURNED	05-Feb-2024

GENERAL COMMENTS	The study attempts to address the knowledge gap in understanding etiological classification of AUFI patients, which is of great relevance and interest. It is commendable that the study attempts to undertake an standardized approach in enrolling patients and conducting laboratory testing from across various countries and sites in LA. I suggest authors justify why they opt to choose 6 year cut-off as a inclusion criteria. The IMCI guidance uses 5 years cut-off to define its target population. It is important that younger children are included in such studies given the documented heterogeneity of infections between adults and children. It is suggested to clarify for not including younger children. Also '>6 years' is indicated as a criteria in the abstract. Double checking the figures to ensure uniformity is suggested. The list of component AUFI aetiologies for proposed diagnostic testing is non-exhaustive. I suggest authors to provide a justification on selection of few etiological agents and the choice of diagnostic tests against them. The bacterial cultures to identify common infections is not included in the testing list. Salmonella spp, for example. is a common bloodstream infection presenting as AUFI. Lines 280: Please clarify the testing plan for SARS-CoV2 testing, in the post pandemic scenario. It would be interesting to know the plan for interpretation of diagnostics' results given the sensitivities and

	specificities of tests included. With the present approach of simultaneously testing patients for multiple pathogens, to what extent multiple positive results are anticipated and thus the interpretation plans for co-infections or potential false positives? How do authors plan to interpret and present result if, for example, an arbovirus is isolated in a patient with a positive malaria RDT? I suggest authors to provide timeline for the patient enrolment and the progress at the time of this protocol. A study duration of at least a year across each site is desired to document the seasonality associated with each target infections.

REVIEWER	Kourouma , Karifa Centre National de Formation et de Recherche en Sante Rurale de Maferinyah, Clinical Research
REVIEW RETURNED	07-Feb-2024

GENERAL COMMENTS	Thanks to the authors for this well written research protocol which will provide key information to present health challenge. However, I have some minor comments which I hope can be considered in improving the manuscript and recommend acceptance of the manuscript after the authors have addressed all comments. 1. Ethics considerations • Page 11, Line 188-189: the authors said, "The protocol, consent forms, and assent forms were approved by local IRBs at each site". However, that does not suffice. • I recommend them to provide the registration number of ethics approval from each of the IRBs. 2. Inclusion criteria • Page 11, line 195-197: it is said "At physician discretion, subjects without documented fever may be included in the study if the clinical presentation suggests an arbovirus infection". I foreseen a significant variation of this judgment as per the physician's level, experience and as per the site level which will impact the data. • To harmonize, I suggest defining a minimum criterion to be considered by physician when making those judgements. For example, reporting fever within 24h or order practical criteria. • Moreover, can you please give the rational for starting inclusion from 6 years? 3. Sample size (page 12, line 214-215) • It is not clear whether the sample size of 200 will recruited over a year period or a number of months. As well, provide the maximum number of recruitments per day or per week. 4. Subject-selection process (page 12, line 222) • My understanding of the ascent form was for children/adolescent (10 sometimes 12 to 17 years) who can read and understand the study implication and importance. But because they have not yet reached the age majority, they are asked to sign an ascent form. • Looking your ≥6 years, I doubt how fair this will this

	process be. 5. Convalescent visit (page 12, line 229) • It is said "Subjects are evaluated 2-3 weeks after the acute illness visit." Why 2-3 weeks? I recommend standardizing this period for all participants to either 2 weeks or 3 weeks. 6. Specimen collection, processing, storage & diagnostic testing (page 14) • There is no information about the maximum volume of sample that will be collection to perform all the planned tests. • Please, provide information about the maximum volume of sample to be collected and how you will handle this in participants with anemia. 7. Data management (page 16, line 312) • Something is missing in the sentence making the sense incomplete. Please, check this.

VERSION 1 – AUTHOR RESPONSE

Reviewer 1:

The study attempts to address the knowledge gap in understanding etiological classification of AUFI patients, which is of great relevance and interest. It is commendable that the study attempts to undertake an standardized approach in enrolling patients and conducting laboratory testing from across various countries and sites in LA.

Authors: The authors thank the reviewer for the kind comments.

Reviewer 1:

I suggest authors justify why they opt to choose 6-year cut-off as a inclusion criteria. The IMCI guidance uses 5 years cut-off to define its target population. It is important that younger children are included in such studies given the documented heterogeneity of infections between adults and children. It is suggested to clarify for not including younger children.

Authors: The IMCI groups children between 2 months and 5 years of age for integrated management. The integrated management focuses on detecting the sickest children and providing early management. This approach is justified in part by the multiple confounders and simultaneous problems that children of those ages can present with to the healthcare system. Thus, defining AUFI in children 2 months to 5 years would be more challenging in our settings. In addition, obtaining the multiple sample types from younger children, particularly blood, was considered a significant logistic challenge and a potential barrier to recruitment.

Reviewer 1:

Also '>6 years' is indicated as a criteria in the abstract. Double checking the figures to ensure uniformity is suggested.

Authors: The authors have verified and corrected the mistake in age inclusion criteria

Reviewer 1:

The list of component AUFI aetiologies for proposed diagnostic testing is non-exhaustive. I suggest authors to provide a justification on selection of few etiological agents and the choice of diagnostic tests against them. The bacterial cultures to identify common infections is not included in the testing list. Salmonella spp, for example. is a common bloodstream infection presenting as AUFI.

Authors: The authors agree with the reviewer in that this study does not include an exhaustive list of potential etiologies of AUFI in Latin America. Out study performs targeted testing of common and emerging arbovirus and bacterial pathogens in the region. In addition, the study includes an unbiased

approach to samples sequencing that could allow the discovery of unexpected pathogens and expand the testing panel if needed. The non-exhaustive testing of potential AUFI causes is acknowledged in the Strengths and Limitations section of the manuscript. Our study population does not include infants, young children, or HIV infected populations which are at the highest risk of non-typhoidal Salmonella infections.

Reviewer 1:

Lines 280: Please clarify the testing plan for SARS-CoV2 testing, in the post pandemic scenario.

Authors: As stated in the diagnostics section of the protocol, we will continue testing for SARS-CoV2 until the end of this pilot protocol. If the health systems stop testing or the result from the subjects is not available, we will run the PCR in the pharyngeal swabs collected.

Reviewer 1:

It would be interesting to know the plan for interpretation of diagnostics' results given the sensitivities and specificities of tests included. With the present approach of simultaneously testing patients for multiple pathogens, to what extent multiple positive results are anticipated and thus the interpretation plans for co-infections or potential false positives? How do authors plan to interpret and present result if, for example, an arbovirus is isolated in a patient with a positive malaria RDT?

Authors: The laboratory testing algorithm described in the methods section tries to minimize the possibilities for false positive results. All multiplex and singleplex PCR tests have internal reaction controls and negative/positive controls to optimize sensitivity and specificity. Expanded testing for positive serological testing for potentially cross-reacting arbovirus including plaque reduction neutralization is included in the laboratory algorithm. The results will include the certainty of the diagnosis as described in the serological testing for Leptospira and Rickettsia in the methods section. For example, I high titer for Leptospira antibodies in the non- paired acute serum samples will be reported as “possible Leptospira infection” while a four-fold increase in antibody titers in paired samples will be reported as “confirmed Leptospira infection”. Dual infections are possible and subjects with malaria are not excluded from the study for that reason. Dual infections will be reported as such but considering the certainty of the diagnosis according to the test used. A section to clarify results reporting was added as suggested by the reviewer.

Reviewer 1:

I suggest authors to provide timeline for the patient enrolment and the progress at the time of this protocol. A study duration of at least a year across each site is desired to document the seasonality associated with each target infections.

Authors: Data collection spans more than a year in all the countries. A timeline has been added to the methods for clarity.

Reviewer: 2
Thanks to the authors for this well written research protocol which will provide key information to present health challenge. However, I have some minor comments which I hope can be considered in improving the manuscript and recommend acceptance of the manuscript after the authors have addressed all comments.

Authors: The author would like to thank the reviewer for his constructive criticism.

Reviewer 2:

1. Ethics considerations
Page 11, Line 188-189: the authors said, “The protocol, consent forms, and assent forms were approved by local IRBs at each site”. However, that does not suffice.
I recommend them to provide the registration number of ethics approval from each of the IRBs.

Authors: We have included the name of the IRBs and IRB approval numbers for each of the sites.

Reviewer 2

2. Inclusion criteria
• Page 11, line 195-197: it is said “At physician discretion, subjects without documented fever may be included in the study if the clinical presentation suggests an arbovirus infection”. I foreseen a significant variation of this judgment as per the physician’s level, experience and as per the site level which will impact the data.

• To harmonize, I suggest defining a minimum criterion to be considered by physician when making those judgements. For example, reporting fever within 24h or order practical criteria.

Authors: We have added text to the inclusion criteria section to clarify what findings would lead the study physician to include a subject without fever.

Reviewer 2

• Moreover, can you please give the rational for starting inclusion from 6 years?

Authors: Please see the response to a similar question made by reviewer 1.

Reviewer 2

3. Sample size (page 12, line 214-215)
• It is not clear whether the sample size of 200 will recruited over a year period or a number of months. As well, provide the maximum number of recruitments per day or per week.

Authors: The samples size of 200 subjects in this pilot/feasibility study was the minimal number expected and did not have a set duration goal. The pace of the enrollment depended on the epidemiological situation in each of the sites and the resources in each health center. For example, in Peru, recruitment took over two years, but in the last months the sample size was increased due to the dengue outbreaks in the region of Quillabamba.

Reviewer 2

4. Subject-selection process (page 12, line 222)
• My understanding of the ascent form was for children/adolescent (10 sometimes 12 to 17 years) who can read and understand the study implication and importance. But because they have not yet reached the age majority, they are asked to sign an ascent form.
• Looking your ≥6 years, I doubt how fair this will this process be.

Authors: Federal regulations in the United States requires documenting the assent of children before their participation in research but does not provide age guidance. The WHO International Ethical Guidelines for Health-Related Research Involving Humans requires the documentation of the assent of children and adolescents before participating in research. The Ethics Committees in each of the sites required the inclusion of an informed assent for children. The ethics committees did require different language in the assents for children between 6 and 12 years and children 13 and older.

Reviewer 2

5. Convalescent visit (page 12, line 229)
• It is said “Subjects are evaluated 2-3 weeks after the acute illness visit.” Why 2-3 weeks? I recommend standardizing this period for all participants to either 2 weeks or 3 weeks.

Authors: The evaluation after 2-3 weeks is to document a rise in antibody titers. In this case, an interval of a week does not make a big difference in levels. In the field, it is actually very difficult to follow up subjects to obtain convalescent samples and often subjects are followed several weeks after the acute episode, especially if they come from remote areas. We have eliminated the 2 weeks as suggested.

Reviewer 2

6. Specimen collection, processing, storage & diagnostic testing (page 14)
• There is no information about the maximum volume of sample that will be collection to perform all the planned tests.

• Please, provide information about the maximum volume of sample to be collected and how you will handle this in participants with anemia.

Authors: The maximum volume of blood collected is 10 mL in adult subjects and 5-7 mL in children. The maximum number of blood samples collected from each participant is two. This number and volume of blood will have very little impact of the subject’s iron status or hemoglobin levels.

Reviewer 2:

7. Data management (page 16, line 312)
• Something is missing in the sentence making the sense incomplete. Please, check this.

Authors: The sentence was revised for clarity as suggested by the reviewer.

VERSION 2 – REVIEW

REVIEWER	Pokharel, Sunil University of Oxford Nuffield Department of Medicine, Centre for Tropical Medicine and Global Health
REVIEW RETURNED	17-Apr-2024

GENERAL COMMENTS	I do not have further comments.

REVIEWER	Kourouma , Karifa Centre National de Formation et de Recherche en Sante Rurale de Maferinyah, Clinical Researchb
REVIEW RETURNED	15-Apr-2024

GENERAL COMMENTS	Thanks to the authors for this well written research protocol which will provide key information to present health challenge. However, I still have two minor comments that were not addressed, and I think can be considered in improving the manuscript and recommend acceptance of the manuscript after the authors have addressed all comments. 1. Inclusion criteria • I missed the rational for starting inclusion from 6 years. Can you please elaborate on this? 2. Specimen collection, processing, storage & diagnostic testing (page 14) • There is no information about the maximum volume of sample that will be collection from each participant to perform the planned tests. • Please, provide information about the maximum volume of sample to be collected and how you will handle this in participants with anemia. .

VERSION 2 – AUTHOR RESPONSE

Reviewer: 2
Dr. Karifa Kourouma , Centre National de Formation et de Recherche en Sante Rurale de Maferinyah
Comments to the Author:
Thanks to the authors for this well written research protocol which will provide key information
to present health challenge. However, I still have two minor comments that were not addressed,
and I think can be considered in improving the manuscript and recommend acceptance of the
manuscript after the authors have addressed all comments.
1. Inclusion criteria
• I missed the rational for starting inclusion from 6 years. Can you please elaborate on this?

Authors:

The authors acknowledge that children younger than 6 years old are affected by specific causes of AUFI. However, AUFI in children 2 months to 5 years would require a much more complex algorithm than what we are able to perform in this pilot study. Obtaining repeated blood samples (acute and

convalescent) would raise concerns for worsening iron deficiency in this group that is already vulnerable to this problem. In addition, obtaining the multiple sample types from young children would require field personnel with significant experience with this age group and those are usually employed by Ministry of Health. Hiring them for our studies would likely weaken the system in the long term. Text was added in the manuscript explaining the decision to only include children ≥ 6 years.

Reviewer 2

2. Specimen collection, processing, storage & diagnostic testing (page 14)
• There is no information about the maximum volume of sample that will be collection from each participant to perform the planned tests.
• Please, provide information about the maximum volume of sample to be collected and how you will handle this in participants with anemia.

Authors

The maximum volume of blood obtained is 10 mL for adults and 5 mL for children. We do not obtain blood for anemia testing and the health system does not routinely check for anemia in subject consulting with acute febrile illnesses. Ministry of Health school anemia programs are in place to address testing and iron supplementation. The volume of blood drawn from adults and children older than 5 years are standard, approved as not endangering subjects by the ethics committees in all sites, and are unlikely to cause an impact on iron stores in these age groups. Text has been added detailing the volume of blood drawn from each subject.

Reviewer: 1
Dr. Sunil Pokharel, University of Oxford Nuffield Department of Medicine
Comments to the Author:
I do not have further comments.

Authors

The authors appreciate the reviewer's input.

VERSION 3 – REVIEW

REVIEWER	Kourouma , Karifa Centre National de Formation et de Recherche en Sante Rurale de Maferinyah, Clinical Research
REVIEW RETURNED	23-May-2024

GENERAL COMMENTS	Thanks for integrating the comments and suggestions.