

CHAIN-COVID protocol

Investigators and Institutional Affiliations

Prof. James Berkley (Professor of Paediatric Infectious Diseases)^{1,2}, jberkley@kilifi.kemri-wellcome.org

Dr. Ambrose Agweyu (Head of Epidemiology & Demography)¹, aagweyu@kemri-wellcome.org

Dr. Judd Walson (Professor of Global Health, Pediatrics and Epidemiology)³, walson@uw.edu

Dr Benson Singa (Senior Research Scientist)⁴, singabo2008@gmail.com

Dr Kirkby Tickell (Senior Research Fellow)³, kirkbt@uw.edu

Dr. Robert Bandsma (Assistant Professor of Paediatric Gastroenterology)⁵, robert.bandsma@sickkids.ca

Mrs Caroline Tigoi (Laboratory Manager)¹ ctigoi@kemri-wellcome.org

1: KEMRI-Wellcome Trust Research Programme

2: Oxford University

3: University of Washington

4: KEMRI.

5: University of Toronto.

Objectives

These objectives will be met by a partnership of the Clinical Information Network (CIN) and the Childhood Acute Illness & Nutrition (CHAIN) Network. CIN will use their broad surveillance platform to focus on objectives 1, 4, 5, while also conduct secondary analyses investigating objectives 2 and 3. CHAIN will focus on address objectives 2, 3 & 6 through an in-depth cohort study, but will also offer secondary analyses to support objectives 1, 4 and 5.

1. To describe the clinical profile of children and adults admitted to CHAIN- and CIN-affiliated sites and estimate the incidence of COVID-19 within this population.
2. To detail the clinical presentation, course and outcomes of highly vulnerable patients with SARS-CoV-2, including those with HIV and malnutrition.
3. To determine risk of death in the inpatient and post-discharge periods among adults and children with SARS-CoV-2 and underlying vulnerabilities.
4. To examine utilization of respiratory care interventions (oxygen, high flow oxygen, CPAP, ventilation) as well as up-referral (transfer to higher level facilities), down-referral (transfer from higher level facilities) and criteria for discharge in order to develop clinical protocols.
5. To determine the impact of local SARS-CoV-2 response on the ability of the Kenyan health system to continue to deliver other health services, including management of acute illness (diarrhea, pneumonia, malaria, HIV), nutritional rehabilitation, follow-up care, prevention of mother to child HIV infection, and immunization services.
6. To determine the quantity, duration and contribution to transmission of fecal shedding of SARS-CoV-2 in LMIC settings where risk of fecal-oral contamination is high.

Study Design

Prospective cohort study

Study sites

CHAIN

Coast General Hospital, Mombasa

Homa Bay County Hospital, Homa Bay

Kilifi County Hospital, Kilifi

Kisii County Hospital, Kisii

Mbagathi County Hospital, Nairobi

Migori County Hospital and Nyatike Sub-County Hospital (Macalder) Hospital, Migori

CIN

Work will be conducted in collaboration with the Ministry of Health Emergency Operations Centre and County or Referral hospitals and with the agreement of all relevant authorities and facility management teams. As this work is to support national level sentinel surveillance, sites will be included based on feasibility and need as expressed by the Ministry of Health. A list of hospitals that are currently engaged in the CIN is provided in Appendix 1. Additional facilities will be invited to take part if they are recommended by the Ministry of Health or counties and if they have an interest in engaging and there is capacity to include them.

1. Study population

For the clinical surveillance de-identified data will be used to describe patient populations and the process and outcomes of care. We will therefore seek to include the whole population receiving inpatient medical services (paediatric and adult) at the participating hospitals.

Criteria for inclusion of hospitalised subjects

- Children and adults who meet the Kenyan National COVID-19 case definition at presentation to hospital or during an inpatient stay.

Additional inclusion criteria for CHAIN COVID study (objectives 2 and 3)

- Planning to remain within the hospital catchment area and willing to come for specified visits during the 6-month follow up period
- Patient willing to consent, or for children a parent or guardian who consents on a child's behalf (where possible assent will also be sought from children over the age 13).

Criteria for exclusion of CHAIN COVID study (objectives 2 and 3)

- Previously enrolled in this study
- Referred from another facility and having been a suspected case for longer 24 hours.
- Suspected cases who have been at the study hospital for more than 24 hours.

Enrolment Stratification (objectives 2 and 3):

The study aims to recruit separate paediatric (2 month-15 years of age) and adult (>15 years of age) cohorts. The paediatric cohort aims include 300 children with WHO defined severe acute malnutrition and 300 children without severe acute malnutrition. Similarly, the adult cohort aims to recruit 300 HIV-infected and 300 HIV uninfected individuals. This stratification is used to optimize statistical power, while minimizing the number of COVID-19 patients exposed to the research protocol.

Sampling

i. Sample size determination (objectives 2 and 3)

The sample size calculations are based on the ability estimate the effect of host vulnerabilities on COVID-19 associated mortality, as this aim is likely requires the most participants to achieve statistical power.

For the paediatric cohort, the sample size calculation is conducted for a comparison between children with and without severe acute malnutrition. We assume 80% power, and alpha of 0.05 and that children without malnutrition will have mortality rate of 5% during the 30-days follow-up. Data from the CHAIN Network suggest that children presenting with severe malnutrition typically experience a 3-fold increased risk in mortality compared children without the condition across a broad range of acute illnesses. Therefore, 226 severely malnourished and 226 not severely malnourished children will allow us to detect a slightly conservative estimate of a 2.7-fold increase in risk associated with malnutrition. Accounting for a 20% loss to follow-up, this would yield a total sample size of 600 children, approximately 6-per week per-site.

For the adult cohort, the sample size calculation is conducted for a comparison between adults with and without HIV infection. We assume 80% power, and alpha of 0.05 and that adults without HIV

will have mortality rate of 3% during the 30-days follow-up. It is reasonable to also expect adults with HIV to experience a 3-fold increased risk in mortality compared adults without HIV. Again, suggesting that a total sample size of 600 adults should be recruited.

Procedures

ii. Screening & Enrolment

All included sites will participate in CIN surveillance. The CHAIN research staff will work with the MOH staff to identify suspected cases and collect nasopharyngeal swabs and surveillance in accordance with MOH and CIN protocol. These suspected cases will then be screened for eligibility to the CHAIN-COVID cohort, and full written consent will be taken from adults. The parents or legal guardians of eligible children will be asked to provide consent, and where appropriate the children themselves may be asked to provide documented verbal assent. The timing of consent, and subsequent data collection will be coordinated with MOH staff to ensure that research procedures do not delay, disrupt or replicate emergent care. The number of suspected cases that will be recruited each week will be subject to a dynamic restriction coordinated by the central CHAIN team in Nairobi and based on the anticipated ratio of positive to negative test results. Data from other settings suggest, and early data from CHAIN suggests that approximately 1 in 10 tests are likely to be positive.

After consent has been taken, enrolment data collection and sample will be collected. All enrolled participants will be followed until their test result is returned. Enrolled participants who test negative will exit the study at this point.

iii. Assessment

Baseline data of prognostic importance, including demographic and social information, a detailed clinical examination, and measurement of vital signs, including pulse oximetry, will be collected using a standard proforma. These standardized forms will be harmonized with the CIN surveillance tools to eliminate repetition. Anthropometry will be performed (MUAC, subscapular skin fold thickness, weight and length).

In addition to the nasopharyngeal swab collected for SARS CoV-2 testing, a research blood sample will be collected together with the routine clinical blood draw to minimize the patient's discomfort. The volume of blood taken will be up to a maximum of 5ml total. Rectal swabs and faecal sample will also be obtained from all participants. At admission, results of investigations performed for clinical care (CBC, biochemistry, glucose, provider-initiated HIV testing and counselling according to national guidelines, or any other routine laboratory investigations) will be utilised by the study. When the results of any of the clinically indicated tests are available, they will be returned to site clinicians caring for the patient. Patients and their families with newly diagnosed infections will be referred to their local HIV Care Clinic for follow-up. See Table 1 below for specimen collection timepoints.

Table 1: Study course, data collection and sample collection for children and adults admitted to hospital

| | SCREENING & ELIGIBILITY | ENROLMENT | DAILY INPATIENT REVIEW | DAY 2 | DAY 5 | MONTH 1,2,3,4,5,6 | DISCHARGE | DEATH |
|--------------------------|-------------------------|-----------|------------------------|-------|-------|-------------------|-----------|-------|
| MOH/CIN SURVIELLANCE | X | | | | | | | |
| NP SWAB | X | | | X | X | X | X | |
| STANDARD CASE MANAGEMENT | X | X | X | X | X | X | X | |
| GIVE STUDY INFORMATION | X | | | | | | | |
| INFORMED CONSENT | X | | | | | | | |
| ANTHROPOMETRY | | X | X | X | X | X | X | |
| DATA COLLECTION | | X | X | X | X | X | X | X |
| FAECAL SAMPLE | | X | | X | X | X | X | |
| BLOOD SAMPLE | | X | | X | X | X | X | |

During admission, participants will be reviewed daily and clinical features, progress and treatment received recorded on a structured case report form. In the event of death in hospital, a standard cause of death (verbal autopsy) tool will be completed using the medical notes. Any additional tests performed for clinical management (for example, blood culture, blood gases, renal and liver function), will be recorded by the study. When clinical blood draws are scheduled for the same day, research samples will be obtained from the same draw. If no clinical blood draw is anticipated the research blood draw will be performed, and results will be made available to the patient's clinical care team. At discharge, anthropometry, a clinical assessment and blood, rectal swab and whole stool will be collected.

All participants will have a standardized vital status, clinical data and sample set collection at 2, 5, and at discharge; follow-ups and sample collection will follow monthly for 6 months (1 month, 2, months, 3, months, 4 months, 5 months, and 6 months from enrolment. It is anticipated that the 2- and 5-day assessment, and the discharge visit will be completed in the facility. All monthly follow-up visits will be conducted as an outpatient. If any of these visits become contraindicated under Kenyan national COVID policy, they will be converted to telephone interviews where vital status is collected but in person contact is made. If the participant dies in the community a WHO standardized verbal autopsy will be completed by a trained staff member.

Health system capacity monitoring

During the study period, research staff at each hospital will conduct a health care capacity monitoring audit each Wednesday. This audit will record a systemic observation of current capacity

on standardized forms, and will include, essential medication availability, essential equipment availability and utilization (including oxygen, ventilators, intravenous access tools, imaging capacity), human resources for health capacity, and the distribution/awareness of updated COVID-19 guidance when it becomes available. Essential medication will be assessed at the hospital level (i.e. in the pharmacy), all other assessments will be done on in the paediatric and adult medical care wards, the admitting facility (emergency room) and any additional ward delivering care to suspected or confirmed cases of COVID-19.

Clinical Care

Sites will be encouraged to follow national treatment guidelines for managing the participants suspected/confirmed COVID-19 and any other comorbidities. Whilst in hospital, participants will be reviewed daily by study clinicians, working together with the hospital team. This will ensure that at least a minimum standard of care, based on Kenyan National guidelines, is provided to all study participants. Any clinically relevant laboratory test (per guidelines) will be made available. At discharge or at follow up, participants will be referred to existing nutrition services for outpatient therapeutic or supplementary feeding according to national policy, WHO guidelines and other clinics for additional conditions identified.

Data collection

Data will be recorded on a standardised CRF in use at every site by trained study staff. The data from all sites will be held locally and uploaded to the secure central CHAIN/CIN Network server in Nairobi. This will be overseen by the central Data Manager who will run regular reconciliation to derive the final study database. Access to the study database will be restricted and password protected.

Provisions for data verification, and validation

The data management system will generate automated queries and the data manager will generate manual queries. All queries will be passed through the study coordinator and clarified by the investigators and field staff with clear documentation. The database will maintain an audit trail.

Reporting

The data management team will adapt analytic methods developed to support routine data management processes including near real-time dashboards currently in use within the CIN to report morbidity, mortality and quality of care indicators for use at facility, county, and national level for monitoring the epidemic and planning for resource needs

Laboratory procedures

Laboratory assays will be a panel of tests done in real-time locally at a designated accredited laboratory, and stored for pooled analyses for the cohort.

Blood samples

Standardised laboratory SOPs will be followed at each site to ensure comparability. The results of abnormal or relevant tests will be made available to clinicians managing the patients at all review time points. In accordance with the sites laboratory capacities and the specific sub-studies being run at each, the blood test that will be run may vary by site. The site-specific samples are outlined in the Table 2 below.

Storage aliquots will be processed by the site laboratory and the products will be stored at -80°C for shipping to the CHAIN repository in Kilifi, Kenya. The tests expected be run on stored blood samples

in a nested case control analysis are outlined in Table 2. Not all tests will be run on samples from all participants. Analyses will include molecular detection of pathogen nucleic acids, inflammatory markers and markers of organ disfunction.

Stored samples will be shipped to the CHAIN sample biorepository at the KEMRI/Wellcome Trust Research Programme in Kenya to be stored as permitted by local regulatory authorities in each country. These analyses will be undertaken in Kilifi, or where facilities or expertise is unavailable, shipped to network members and collaborators laboratories, including at the University of Oxford, UK, the University of Washington, USA and University of Toronto, Canada. Samples shipped overseas will contain no personally identifying information.

Table 2. Samples to be taken and immediately processed or stored.

| Sample | Test | Admiss-ion | Day 2 | Day 5 | Months 1,2,3,4,5,6 | Discharge |
|---------------------|----------------------------------|--------------|----------|----------|-----------------------|-----------|
| NP swab | SAR CoV-2 testing | Surveillance | Research | Research | Research | Research |
| Blood | CBC | Clinical | Research | Research | Research | Clinical |
| Blood | Biochem | Clinical | Research | Research | Research | Clinical |
| Blood | Glucose | Clinical | Research | Research | Research | Clinical |
| Blood | Malaria RDT | Clinical | | | | Clinical |
| Blood | HIV | Clinical | | | | |
| Blood | Plasma Storage | Research | Research | Research | Research | Research |
| Blood | Serum Storage | Research | Research | Research | Research | Research |
| Blood | Whole blood Storage | Research | Research | Research | Research | Research |
| Dried blood spot | Storage | Research | Research | Research | Research | Research |
| Rectal swab | Stored for SARS CoV-2 Culture | Research | Research | Research | Research | Research |
| Rectal Swab | Stored for COVID- 19 PCR | Research | Research | Research | Research | Research |
| Stool | Stored for SARS CoV-2 Culture | Research | Research | Research | Research | Research |

Table 3: Laboratory tests to be performed on stored blood samples taken at any site after selection as a case or control.

| TEST | SAMPLE TYPE |
|--|----------------------|
| BIOCHEMISTRY AND MARKERS OF ORGAN FUNCTION | Plasma |
| CYTOKINES & CHEMOKINES – MULTIPLEX BEAD ARRAY OR ELISA | Serum |
| PROTEOMICS – LCMS, HPLC, NMR | Serum |
| METABOLOMICS | Serum/DBS |
| VITAMINS, MICRONUTRIENTS, MACRONUTRIENTS (AMINO ACIDS ETC) | Serum/DBS |
| SARS COV-2 ANTIBODY TESTING | Serum/DBS |
| PBMCS – MULTIPARAMETER FLOW CYTOMETRY | EDTA |
| MOLECULAR PATHOGEN DETECTION – TAQMAN CARD ARRAY, SINGLEPLEX PCR AND 16S RRNA SEQUENCING | EDTA |
| SARS COV-2 PCR AND CULTURE | Stool & Rectal swabs |

To determine the viability of SARS CoV-2 in faecal samples, 100 enrolment and 100 30-day faecal samples, which have viral DNA detected by PCR, will be selected for viral culture in an appropriate level biosafety facility in Kenya. A similar selection and analysis will be performed on the rectal swabs collected from these participants.

Data Management

iv. Data Storage

All records will be kept in a locked filing cabinet at the appropriate site, which is accessed only by the investigators and the study staff. All local computer entry and networking programs will be done with coded numbers and initials only, and will be password protected. Only the investigators and the clinical monitors will have access to the records. The central database in Nairobi will be held on a dedicated secure server with secure off-site backup. To reduce risks of disclosure, participant's names will not be included in the database.

v. Data Management

The site data manager at each site will be responsible for receiving, entering, cleaning, and forwarding data to the secure central database at the KWTRP offices in Nairobi and responding to data queries. In Nairobi, the Network data manager will undertake checks, querying, analysing and storing all data that accrues from the study.

vi. Analysis

Primary cohort analyses - mortality

The data from all sites will be combined into a paediatric (under 15 years of age) and separate adult cohort. The primary analysis will compare the mortality in specific subgroups of interest (HIV infected adults, and severely malnourished children, to comparable individuals without these

conditions/comorbidities. Specifically, the relative risk of mortality during follow-up will be calculated in multivariable Cox proportional hazard regression

Secondary cohort analyses

Secondary endpoints will be the proportion of COVID-19 patients with SARS CoV-2 shedding in stool and nasopharyngeal swabs at 30-days after enrolment. These proportions will be calculated using generalized linear models. Risk factors for prolonged faecal shedding will be explored using multivariate generalized linear models. These risk factors will include baseline clinical status and demographic, clinical therapies administered, duration of illness and peak severity of illness.

Laboratory Analysis of SARS CoV-2 viability

To determine the importance of SARS CoV-2 faecal shedding, a subset of faecal samples, rectal swab and nasopharyngeal swabs will be selected from storage and viral culture will be performed. The proportion of enrolment and 30-day samples from which live virus can be cultured will be estimated with 95% confidence intervals calculated under a binomial distribution.

vii. Data Sharing

Data generated from this study will be shared between the research sites involved and the coordinating centre in Nairobi to support cross-site analysis. All systems for sharing data between sites will ensure data security and privacy. De-identified data from individual sites or across the collaboration may be shared with the funder and/or external institutions. Requests for access to study data will be reviewed by data governance bodies within institutions and across the CHAIN collaboration, with referral to national level where appropriate.

Intellectual property

Any intellectual property rights that arise from the work will be safeguarded according to current KEMRI guidelines and the Industrial Property Act of 2001, sections 32, 58 and 80. The scientific and intellectual contributions of all persons involved in the research will be appropriately acknowledged in all publications and presentations arising from the work.

2. Time Frame/Duration of the Project

The study will begin as soon as scientific and ethical approvals are granted, which is expected to be within Q2 2016. Each site will recruit for a period of up to 1 year, but may be halted when the epidemic in Kenya has ended. A further 30 days will be allowed to complete follow up.

3. Ethical Considerations

All CIN data are de-identified. The project does not involve active recruitment of patients or any additional questioning or examination. The project is approved under a series of annually renewed protocols submitted to KEMRI Scientific and Ethical Review Unit (SSC 1746 and SSC 2771).

Prof. Maureen Kelley at the Ethox Centre at the University of Oxford is a co-investigator and the CHAIN Network advisor on bioethics. This protocol will be subject to ethical approval from SERU, KEMRI, Nairobi; and the IRB at the University of Washington, Seattle, USA.

a) Human Subjects

First do no harm, the study will maintain ethical standard through internal and external training, monitoring and standardisation of procedures.

i. Risks

This study does not introduce risks to participants beyond those they would normally face in the context of illness. There is some risk that the additional follow-up at 30-days could risk further spreading SARS CoV-2. This visit maybe converted to a phone call to obtain vital status, without sample collection, if the visit appears to be in conflict with Kenyan National Guidelines at any point during the study. Some pain and discomfort will be experienced when samples are obtained. There is a risk of breaching medical confidentiality. Potential disadvantages for participants also include the time taken to attend follow ups and answer questions, and the potential for interviews to remind interviewees of difficult and disturbing incidents and situations in their lives. Training and standard SOPs will be implemented to help avoid these problems. Patients will have expenses related to travel and lost earnings to and from the hospital for study follow-up reimbursed by the study.

In the event a significant event caused as a direct result of participation in the study, the study staff will document the incident, inform the site PI, and local medical costs incurred, outside those associated with the child's underlying condition, will be covered by i) local medical malpractice insurance; and ii) overall study liability by the sponsor, the University of Washington.

ii. Benefits to the Patients and Community as a whole

All patients will benefit from institutional training, standardisation of procedures and additional staffing. Where health or social problems are identified, participants will be referred to appropriate services. All the communities in this study have a high prevalence of undernutrition. Findings from this study are expected lead to improvements in the national response to COVID-19 by generating novel data about the viruses effects on vulnerable populations, and the health systems challenges being faced by facilities mounting a response to the disease.

iii. Confidentiality

Every effort will be taken to maintain normal medical confidentiality. All records and transcripts will be kept in a locked filing cabinet at the appropriate site, which is accessed only by the investigators and the study staff. All computer entry and networking programs will be done with coded numbers and participant's initials only. Only the investigators and the clinical monitor will have access to these records. Only de-identified data will be shared with the funder or other investigators, as specified under the section on data sharing.

iv. Community Engagement Strategy

Community engagement will primarily be through the inpatient wards and follow-up clinics at the hospital study sites and through the County Directors offices. Referral clinics and hospitals will be informed through these means. At this time further Community sensitisation appears inappropriate as it would break with national social distancing recommendations.

Stakeholder information giving

The key stakeholders in this study are the hospitals hosting the study, the community engaged in the research, the ministries of health, the investigators and the funders. Written and oral (virtual/phone) information regarding the study's purpose and processes will be given to each of these parties. The funders will be engaged through regular progress reports given by the scientific leads.

v. Individual informed consent process

The study will be carried out in conformity to the ICH-GCP principles for informed consent. These principles will be stated and explained clearly in an informed consent SOP. This will be the basis for training staff involved in obtaining informed consent. Patients, parents or guardian will receive an

explanation of the study by a member of the study team in private and in an appropriate language (see informed consent form) during the stabilisation period after admission to hospital. They will be given a chance to ask questions before written permission for them, or their child, to be included in the study is sought. Participants or their caregivers who are unable to write will be asked to provide a witnessed thumbprint. The quality assurance mechanisms of the CHAIN cohort will ensure that the procedures described in the informed consent SOP are adhered to by checking during the monitoring process and ensuring that each study participant has duly completed consent form.

vi. *Training/ support for those involved in community engagement and administering consent.*

Virtual training in community engagement and consent will be given to all study staff prior to enrolment beginning. All persons administering consent will have research ethics and GCP training. Staff performing verbal autopsy will be trained in accordance with WHO recommendations by staff engaged with an existing verbal autopsy study.

vii. *Feedback of information*

Results of the study will be fed back to the study communities through each research institution's community representatives or community liaison group, public meetings, hospital and counties involved and the follow up clinics in each site. Results will be shared through presentation at local and international scientific meetings.

b) ***Animal Subjects***

Not applicable

4. Expected Application of the Results

This study will rapidly deepen our understanding of how COVID-19 affects vulnerable population, the toll which the COVID-19 response has on the Kenyan healthcare system. This information may directly inform the Kenyan and sub-Saharan African Response to SARS CoV-2. Secondly, it will identify the viability of SARS CoV-2 in faecal samples, which will inform how research laboratories should handle these samples during the pandemic, or during a future epidemic or endemic phase of this disease. This research will be vital to ensuring health science research after the pandemic is able to continue using faecal samples as a relatively low-risk, non-invasive sample type.

5. Insurance Statement

The University of Washington has a specialist insurance policy in place which would operate in the event of any participant suffering harm as a result of their involvement in the research.

Appendix 1: CIN Hospitals

1. Busia County Referral Hospital.
2. Bungoma County Referral Hospital.
3. Jaramogi Oginga Odinga Teaching and Referral Hospital
4. Homabay County Referral Hospital
5. Vihiga County Referral Hospital
6. Kakamega County General Teaching & Referral Hospital
7. Kitale County Hospital
8. Kiambu Level 5 Hospital
9. Mama Lucy Kibaki Hospital
10. Machakos Level 5 Hospital
11. Kisumu County Hospital
12. Embu Level 5 Teaching & Referral Hospital

13. Naivasha County Referral Hospital
14. Mbagathi County Hospital
15. Nyeri County Referral Hospital
16. Kerugoya County Referral Hospital
17. Pumwani Maternity Hospital
18. Thika Level 5 Hospital
19. Nakuru Level 5 Hospital
20. Migori County Hospital