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Characterisation of HIV-1 reservoirs in paediatric populations: protocol for a systematic review and meta-analysis

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ABSTRACT

Introduction The success of antiretroviral therapy (ART) has changed HIV from a deadly to a chronic infection, thus increasing the transitioning from infancy toward adulthood. However, the virostatic nature of antiretrovirals maintains viruses in sanctuaries, with reactivation potentials. Because current ARTs are very limited for children, the emergence of new HIV epidemics driven by HIV drug-resistance mutations is favoured. Our systematic review aims to estimate the global burden of archived drug-resistance mutations (ADRMs) and the size of reservoir (HIV-1 DNA load), and their associated factors in children and adolescents.

Methods and analysis Papers from the PubMed/MEDLINE, Google Scholar, ScienceDirect, African Journals Online and Academic Medical Education Databases will be systematically identified using the keywords: "HIV-1 reservoirs", "viral reservoirs", "HIV-1 DNA", infants, adolescents, child and children, linked by the following Boolean operators: 'OR' and 'AND'. Randomised and non-randomised trials, cohort studies and cross-sectional studies published in French or English from January 2002 will be included, while case reports, letters, comments, reviews, systematic reviews and meta-analyses, and editorials will be excluded. All studies describing data on ADRMs, HIV-1 DNA load and/or immunological markers among children/adolescents will be eligible. A random-effects model will be used to calculate the pooled prevalence of ADRMs. Data will be reported according to type of viral reservoir (peripheral blood mononuclear cells, CD4 cells), geographical location (country/continent), ethnicity/race, age (infants vs adolescents), gender, HIV-1 clades, ART exposure (naïve vs treated, drug class, type of regimen, age at ART initiation and treatment duration), WHO clinical staging (I, II, III, IV), immune status (immune compromised vs immune competent) and virological response (viraemic vs non-viraemic). Multivariate logistic regression will be performed to determine predictors of HIV reservoir profile in paediatric populations. The primary outcome will be to assess the genotypical and quantitative profile of HIV reservoirs, while the secondary outcomes will be to identify factors associated with ADRMs and reservoir size in paediatric populations.

Ethics and dissemination Ethical approval is not applicable for this study as it will be based on published data. Results will be disseminated via a peer-reviewed scientific journal and relevant conferences.

PROSPERO registration number CRD42022327625.

STRENGTHS AND LIMITATIONS OF THIS STUDY

⇒ This systematic review and meta-analysis will use multiple databases (PubMed/MEDLINE, EMBASE, Google Scholar, ScienceDirect, African Journals Online, Academic Medical Education) to identify relevant literature.
⇒ The study proposes a thorough quality assessment process using well-established scales.
⇒ The non-availability of corresponding authors to provide complete data where reporting is incomplete could be a limitation of this study.
⇒ The review may encounter heterogeneity in terms of study designs, populations and methodologies across the included studies; while subgroup analyses are planned to address this, significant heterogeneity may still impact the validity of the pooled estimates.

INTRODUCTION

In spite of the reduction in HIV mother-to-child transmission (MTCT), new paediatric infections are occurring each year with more than 150 000 new cases reported among children.[1 2] Even though the benefits of antiretroviral therapy (ART) through prevention of MTCT programmes have been fundamental in achieving reduced rates of MTCT,[3–5] the virostatic nature of existing ART in children living with HIV goes with challenges of life-long treatment such as suboptimal adherence, ART-attributed toxicities, persisting immune dysfunction and HIV drug resistance (HIVDR) emergence.[6] In fact, in the frame of persisting viral replication with suboptimal ART pressure, there is a threat of an emerging new HIV epidemic, driven by HIVDR to existing antiretrovirals. This

threat is particularly true for paediatric populations due to limited ART options, poor drug formulations and increasing events of non-adherence as they grow from childhood toward adolescence.[7 8] Taking into account the rising call for long-term viral control among patients even off therapy,[9 10] strategies contributing to the better understanding of HIV (functional) cure or remission are of paramount importance, especially for vulnerable populations such as children and adolescents living with HIV in resource-limited settings (RLSs).[11–13]

The main barrier to reach HIV cure is the existence of stable viral reservoirs. Of note, HIV reservoir cell types are diverse including astrocytes, macrophages, dendritic cells, lymphocytes, among others, with HIV generally integrated in the form of proviral DNA. Proviruses are stable in resting memory CD4+ T cells,[14 15] which are established early during primary HIV infection.[16] Of note, CD4+ T cells (central memory CD4 T cells, transitional memory CD4 T cells and stem cell memory CD4 T cells) are the primary viral targets for persistent and replication-competent HIV and constitute only a small fraction of the overall latent reservoirs.[15] According to age, the profile of viral reservoir in children is different from those of adults, as during HIV perinatal infection, the virus is transmitted at a very early stage of immune system development with suboptimal efficiency in clearing infected cells.[11] Additionally, immune system of children is characterised by predominantly naive CD4+ T cells in children as compared with adults.[17] Moreover, these cells are known to be non-sensitive or less permissive to ART, and this non-permissiveness to drugs favours persistent viral replication and the selection of mutant strains, further conserved in the form of archived drug-resistance mutations (ADRMs) within the host proviral DNA.[8 18] HIV DNA load has been proven to be clinically relevant in predicting the progression toward AIDS/death, due in part to existing ADRMs, also known as occult DRMs within the viral populations.[19 20]

Because of increasing numbers of infants initiating ART early and the gradual implementation of highly sensitive techniques to quantify viral reservoirs among people living with HIV (especially the vulnerable population of children and adolescents with perinatal infection), we here propose addressing one of the key research goals of the International AIDS Society Global Scientific Strategy 2021, by shedding light on HIV-1 reservoirs in paediatric populations through a systematic review and meta-analysis of available data. Moreover, it is important to note that health-related research plays a crucial role in achieving global development goals, and the field of paediatric HIV/AIDS is no exception. By shedding light on the factors influencing the size and genotypical profile of HIV-1 reservoirs in children and adolescents, our review will directly contribute to the efforts towards elimination of HIV/AIDS by 2030, a part of the Sustainable Development Goal 3's objectives. Specifically, we will estimate the pooled burden of ADRMs, the quantitative profile of HIV-1 DNA and/or immunological markers with their respective determinants in paediatric population. The finding of this review may help to better understand the dynamics and implications of viral reservoirs in the prognosis of HIV-1 infection among children and adolescents.

METHODS AND ANALYSIS

Design, reporting and registration

This systematic review and meta-analysis protocol was developed following the Preferred Reporting Items for Systematic Reviews and Meta-Analysis guidelines for protocol.[21] The present protocol is registered in the Prospective Register of systematic Reviews (CRD42022327625).

Inclusion and exclusion criteria

Inclusion criteria

1. **Type of studies:** randomised and non-randomised trials, cohort and cross-sectional studies assessing data on HIV-1 reservoirs in paediatric populations will be included.
2. **Type of participants:** we will consider studies conducted among infected children, aged between 0 and 19 years from all geographical locations worldwide. We will therefore include studies focusing on children/adolescents living with HIV as defined by the WHO available from: www.who.int/news-room/fact-sheets/detail/hiv-aids.
3. **Intervention:** this consisted of all ART-experienced children and adolescents living with HIV-1 infection.
4. **Comparator:** this will consist of all ART-naïve children and adolescents living with HIV-1 infection, further stratified according to geographical locations (industrialised vs RLS as defined by the World Bank Organisation[22]; country/continent[23]), ethnicity/race, type of viral reservoirs (peripheral blood mononuclear cells (PBMCs), CD4 cells), target HIV-1 gene as well as detection method, HIV clades, WHO clinical staging (I, II, III and IV), immune status (immune compromised vs immune competent), level of viraemia (viraemic vs non-viraemic), gender and age ranges.
5. **Types of outcomes:** primary outcomes will be the genotypical profiling (ADRMs) and quantitation of HIV-1 (proviral DNA load) in cellular reservoirs (PBMCs, CD4 cells) of children and adolescents. Secondary outcomes will be advanced clinical staging (WHO III/IV), immunity (CD4 <200, 200–349, 350–499 and ≥500 cells/mm^3; CD8 <150 and ≥150 cells/mm^3; CD4:CD8 ratio <1 and ≥1), high plasma viral load (>1000 copies/mL), circulating DRMs (in plasma) and/or death.
6. **Report characteristics:** we will include studies that have been published in English or in French from the last 20 years (2002 onwards).

Exclusion criteria

Case reports, letters, comments, reviews, systematic reviews and meta-analyses, and editorials will be excluded.

Search strategy

A systematic search will be performed using PubMed/MEDLINE, Google Scholar, ScienceDirect, African Journals Online and Academic Medical Education Databases using the keywords: "HIV-1 reservoirs", "viral reservoirs", "HIV-1 DNA", infants, adolescents, child and children, linked by the following Boolean operators: 'OR' and 'AND' (online supplemental file 1 shows the detailed search strategy for all the databases). A filter will be performed starting from January 2002. Additional studies will be retrieved manually from the references of included studies.

Study selection

An Excel spreadsheet will be used to combine records from the various sources included in our search strategy. Therefore, duplicate studies will be identified and removed. The titles and abstracts of the eligible studies will be independently examined by two study authors (ACK and ADN) for the selection of relevant studies. The discordant ideas of the investigators regarding the selection of the studies before data extraction will be resolved by discussion, consensus or intervention of a third person (AN or M-MS or BY or JF) when necessary.

Data extraction and management

Data from the included studies will be extracted using a Google Form by four study authors (ACK, ADN and ENJS) and verified by ACK. The extracted data will be: name of the first author, year of publication, study design, inclusion criteria, sampling method, sample type, sampling period, age (infants vs adolescents), gender, sample size, presence of ADRMs, prevalence of ADRMs, type of ADRMs, HIV-1 DNA load, HIV-1 clades, ART exposure (naive vs treated, drug class, ART regimen and treatment duration), WHO clinical staging (I, II, III, IV), immune status (CD4 count), virological response (for ART-experienced) or viraemia level (for ART-naïve), profile of the most prevalent cytokine and geographical location (country/continent). Potential disagreements observed by different data extractors during data extraction will be resolved by discussion and/or consensus. Whenever necessary and possible, the corresponding authors of the selected studies will be contacted for further information whenever pertinent data for the analysis were missing.

Data analysis

To estimate the heterogeneity among studies, I^2 and H statistics will be used.[24] The I^2 value will be an indication of the degree of heterogeneity, with values of 0%, 18%, 45% and 75% designating none, low, moderate and high heterogeneity, respectively.[25] Lack of evidence on heterogeneity among studies will be designated by obtaining an H statistic close to 1, which will be inversely proportional to the degree of heterogeneity. The results of these parameters will help in choosing what type of model (fixed-effects model or random-effects model) will be used during the meta-analysis process. Rate of ADRMs or data to estimate it will allow quantitative analysis. The pooled prevalence of ADRMs and 95% CIs will be estimated.[26] Subgroup analyses according to the study design, country, immune-virological profile, presence/absence of ADRMs and sample types will be employed to adjust for the variations in pooled estimations, and OR will be used to identify associated factors to HIV-1 reservoir profile. The statistically significant threshold will be fixed at $p<0.05$. The publication bias will be assessed by visual inspection of the asymmetry of the funnel plot and the Egger's test, with $p<0.1$ indicating a potential bias.[27] The R V.3.6.0 software (package 'meta' and 'metafor') will be used to perform all meta-analyses, through the RStudio interface.[28 29]

Quality of assessment and risk of bias

The quality of each study will be independently assessed by three study authors (ACK, ADN and ENJS) using a dedicated scale for prevalence studies that is based on 10 components divided into two groups: internal and external validity of the study (online supplemental file 2).[30] The scores of 0 or 1 will be assigned to each question in the assessment tool for a total score of 10 per study. The scores of 0–3, 4–6 and 7–10 represented a high, moderate and low risk of bias, respectively. For non-randomised studies, the evaluation of included ones for risk of bias will be done using Risk of Bias in Non-Randomized Studies of Intervention.[31] Regarding randomised controlled trial studies, we will used Risk of Bias in randomised controlled trial studies (RoB 2.0)[32] to evaluate risk of bias assessment (online supplemental file 3). Importantly, divergence in risk of bias assessment among the review authors will be solved through discussion and consensus, or by arbitration of a third review author.

Patient and public involvement

None.

Ethics and dissemination

Ethical approval is not applicable for this study as it will be based on published data. Results will be disseminated via a peer-reviewed scientific journal and relevant conferences.

DISCUSSION

This systematic review with meta-analysis may help to provide findings that will contribute to advancing HIV cure research in paediatric populations (vertically infected) by reviewing the global genotypical and the quantitative profile of HIV-1 DNA. More specifically, the study will estimate the pooled prevalence of ADRMs and estimate the HIV-1 reservoir size (HIV-1 DNA) with their determinants among children (infants vs adolescents), as well as potential clinically relevant outcomes. Disparities of reservoir cells in heterogeneous population between high versus low-income settings and ethnicity/race if available might further contribute to adapting cure

strategic agenda according to the realities and challenges of different geographical settings/populations.

Our results will be valuable to researchers and clinicians working in this field in understanding HIV-1 reservoirs in variable context and with diverse paediatric populations. More importantly, our findings may serve as a call for optimal approaches toward HIV eradication, functional cure or remission, especially for the most vulnerable populations of children/adolescents, who are faced with programmatic and psychosocial hurdles far different from those of the adult populations. As possible limitations of this review, we may be challenged with the potential non-distinction between replication competent cells and defective ones in included papers as most of the proviral DNA is defective for production of replication-competent virus. So, the measure of total HIV-DNA will be a proxy for reservoir size. Important study incompleteness and these will be considered in statistic models during analysis; if not performed, studies' incompleteness would therefore be solved by contacting corresponding study authors. Additional limitation may be at the level of reviewing and inclusion of studies, which will be mitigated by discussions to outweigh reviewing discrepancies between articles concerned, through a consensus decision. Another limitation might be the effects of community engagement on the study outcomes, which might be covered in subsequent studies focusing on qualitative determinants.[33] A notable limitation of this systematic review is the scarcity of contemporary therapy-naïve paediatric samples available for analysis. Most studies that focused on therapy-naïve subjects primarily used cryopreserved samples collected during the 1980s–early 2000s.

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Contributors ACK, AN and JF conceived the study. ACK, BY, ADN, ENJS, WP, DT and NS designed the methodology. ACK, ADN and BY planned formal analysis. ACK and JF drafted the initial manuscript. AN, M-MS, BY, GA, BS, ADN, ENJS, VC, C-FP, FC-S and CTT critically revised the manuscript. M-MS, FC-S, SRL, CTT and JF supervised the work. All the authors validated the final manuscript. ACK and JF are the guarantors of the protocol.

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Competing interests None declared.

Patient and public involvement Patients and/or the public were not involved in the design, or conduct, or reporting, or dissemination plans of this research.

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