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# HIV and risk of cardiovascular disease in sub-Saharan Africa: Rationale and design of the Ndlovu Cohort Study

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## Abstract

**Background:** The largest proportion of people living with HIV resides in sub-Saharan Africa (SSA). Evidence from developed countries suggests that HIV infection increases the relative risk of cardiovascular disease (CVD) by up to 50%. Differences in lifestyle, gender distribution, routes of HIV transmission and HIV subtype preclude generalisation of data from Western countries to the SSA situation. The Ndlovu Cohort Study aims to provide insight into the burden of cardiovascular risk factors and disease, the mechanisms driving CVD risk and the contribution of HIV infection and its treatment to the development of CVD in a rural area of SSA.

**Design:** The Ndlovu Cohort Study is a prospective study in the Moutse area, Limpopo Province, South Africa.

**Methods:** A total of 1000 HIV-positive and 1000 HIV-negative participants aged 18 years and older with a male to female ratio of 1:1 will be recruited. Measurements of CVD risk factors and HIV-related characteristics will be performed at baseline, and participants will be followed-up over time at 6-month intervals. The burden of CVD will be assessed with repeated carotid intima-media thickness and pulse wave velocity measurements, as well as by recording clinical cardiovascular events that occur during the follow-up period.

**Conclusion:** This project will contribute to the understanding of the epidemiology and pathogenesis of CVD in the context of HIV infection in a rural area of SSA. The ultimate goal is to improve cardiovascular risk prediction and to indicate preventive approaches in the HIV-infected population and, potentially, for non-infected high-risk populations in a low-resource setting.

## Keywords

Human immunodeficiency virus, cardiovascular risk factors, cardiovascular disease, carotid intima-media thickness measurement, pulse wave velocity, cohort study

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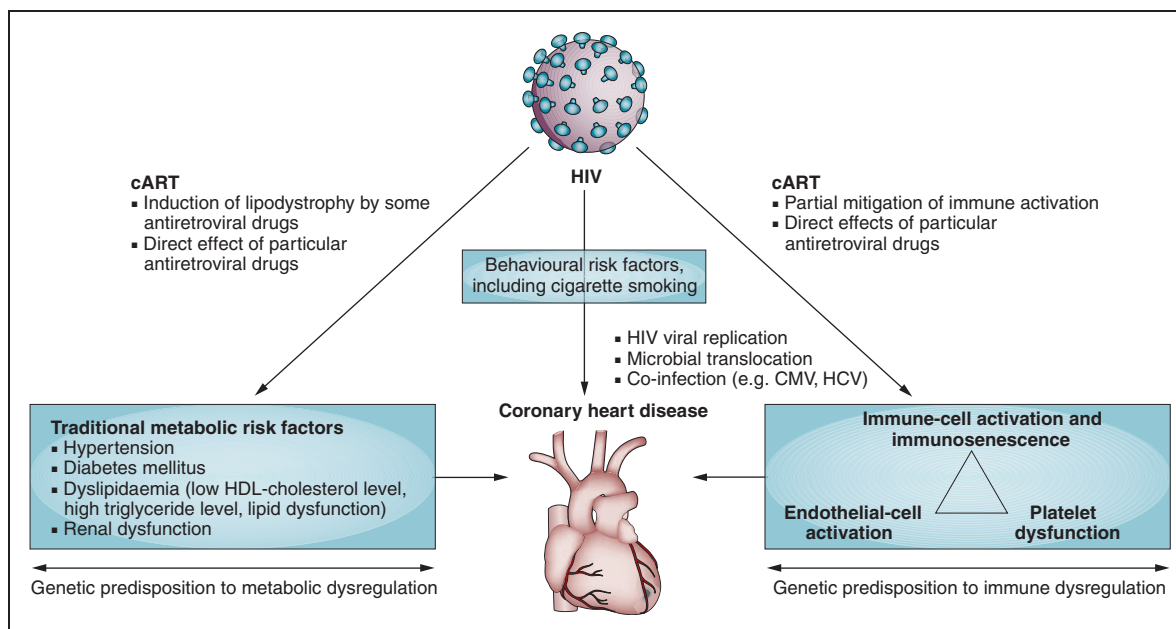
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**Figure 1.** Mechanisms and risk factors postulated to be involved with an increased risk of coronary heart disease risk in patients with HIV.

cART: combination antiretroviral therapy; CMV: cytomegalovirus; HCV: hepatitis C virus; HDL: high-density lipoprotein.

(Reproduced with permission from Zanni MV, Schouten J, Grinspoon SK, et al. Risk of coronary heart disease in patients with HIV infection. *Nat Rev Cardiol* 2014; 11(12): 728–741. doi:10.1038/nrcardio.2014.167.

## Background and rationale

Currently, more than 35 million people are estimated to be living with HIV. Nearly 71% of the global total – 24.7 million people – reside in sub-Saharan Africa (SSA), where the majority of people living with HIV are women (58% of all people living with an HIV infection).<sup>1</sup> Globally, combination antiretroviral therapy (cART) has been initiated in approximately 16 million people living with HIV<sup>2</sup> and, as a result, HIV has become a chronic disease that for many patients will span several decades of their lives.<sup>3</sup> Persistent immune activation and inflammation in treated HIV infection, however, appear to be associated with an ‘accentuated’ increased relative risk of cardiovascular disease (CVD) of up to 50% and an earlier ‘accelerated’ onset of CVD compared to HIV-negative individuals.<sup>4–7</sup>

Yet our understanding of the mechanisms driving CVD in HIV infection remains limited. The exact role of HIV infection, HIV treatment and their relationships with conventional risk factors still have to be determined.<sup>8–10</sup> In addition, it is unclear whether and how differential levels of viremia, immune activation and CVD are related to the HIV-1 subtype.<sup>11</sup> Several factors are hypothesised to contribute to an accelerated development of CVD amongst HIV-infected individuals. These include: (1) the host response to HIV

infection, which results in chronic immune activation and inflammation; (2) conventional CVD risk factors, potentially amplified by adverse effects of HIV treatment; (3) HIV treatment, with some antiretroviral drugs being particularly linked to an increased risk of CVD; and (4) HIV-induced metabolic effects. A summary of potential mechanisms is given in Figure 1.<sup>6</sup>

As data from SSA are scarce, especially longitudinal data, it is currently not clear whether an amplification of the CVD epidemic due to HIV infection is to be expected. Several cross-sectional studies in SSA addressing cardiovascular (CV) risk in HIV-infected individuals did not indicate an increase in CV risk profile as a result of HIV,<sup>12,13</sup> although available studies have mainly included HIV-positive patients and not added an HIV-negative control group for proper comparison.<sup>4</sup>

The Ndllovu Cohort Study (NCS) has been set up in a rural area in South Africa to address the aforementioned issues for the SSA region. This prospective study will include both HIV-positive and HIV-negative individuals. As the aim is to provide a comprehensive understanding of the interaction between HIV and CVD, the problem will be approached in a multidisciplinary manner. The impact of an HIV infection on social and mental health in both HIV-infected participants and the community will be evaluated, and virological characteristics such as resistance and

therapy failure will be identified. The ultimate aim is to improve CV risk prediction and identify potential novel, cost effective, preventive approaches in the HIV-infected population. These may also be applicable for non-infected, high-risk populations in a low-resource setting.

## Methods

The NCS is an initiative of the Ndlovu Research Consortium, formed by the Wits Reproductive Health and HIV Institute (Wits RHI), University of the Witwatersrand (South Africa), Utrecht University (The Netherlands), including the Faculty of Social Sciences and the University Medical Center Utrecht (departments of Infectious Diseases, Immunology, Clinical Epidemiology and Public Health), and the Ndlovu Care Group (South Africa).

### Study setting

The NCS is conducted in Elandsdoorn, a rural township in the Moutse area, Limpopo Province, South Africa. Dedicated research facilities are based at the Ndlovu Care Group ([www.ndlovucaregroup.co.za](http://www.ndlovucaregroup.co.za)), a non-governmental organisation advancing rural communities with healthcare, childcare, community development and research programmes.

### Study population

This prospective cohort study will recruit 1000 HIV-uninfected and 1000 HIV-infected participants and intends to include an equal proportion of men and women. Within the HIV-infected group, the aim is to include 20% antiretroviral therapy-naïve individuals with a CD4 count of  $>100$  cells/mm<sup>3</sup>. Eligible individuals are: (1) aged 18 years or over; (2) living within a range of 30 km around the Ndlovu Medical Center (NMC); (3) able to provide written, informed consent; and (4) committed to long-term follow-up. Individuals who are unable to undergo the study procedures for any reason will be excluded.

### Inclusion

The study aims to include a population that represents a typical rural South African district. Participants will be recruited through a community liaison officer and a team of counsellors at the Ndlovu Medical Centre HIV clinic, at local events, in shopping areas and through community campaigns. Based on the literature, it is estimated that 3.9% of HIV-negative participants will become infected with HIV and start cART within a follow-up period of 3 years.<sup>14</sup>

### HIV testing

HIV-negative participants or participants with an unknown status will undergo HIV testing at enrolment in the study and at yearly follow-up visits. Testing is performed with an antibody-based point-of-care test (ADVANCED QUALITY™ Rapid HIV Test [InTec Products, China]), which has a sensitivity of 98.8% and a specificity of 100%.<sup>15</sup> Specimens that test positive will be retested with a second point-of-care test (ABON™ HIV 1/2/O Tri-Line HIV Rapid Test Device [ABON Biopharm Hangzhou, China]), which has a sensitivity of 100% and a specificity of 97.7%.<sup>16</sup> An enzyme-linked immunosorbent assay will be performed in order to clarify any indeterminate results and to confirm positive results.

Participants with a confirmed HIV-positive status will be recruited from the Ndlovu Medical Centre or from outreach testing programmes. Documentation of a positive HIV test result is needed for all patients to be eligible for enrolment as HIV-positive participants in the study. HIV testing will be conducted in order to confirm HIV status according to the procedure described above in case of any doubt.

### Ethics approval

Study approval was obtained from the Human Research Ethics Committee at the University of Pretoria, Pretoria, South Africa, and the Limpopo Department of Health Ethics Committee, and written informed consent will be obtained from all participants prior to study participation.

### Sample size

Calculations are based on carotid intima-media thickness (CIMT) using a mixed model approach. A simplified approach was used in order to evaluate the power to detect very small differences between groups, as we did not have reasonable estimations for CIMT progression over time. Power was evaluated for a given sample size of 1000 HIV-positive and 1000 HIV-negative patients for a significance level of 0.05 and a constant difference in mean CIMT over time of 0.006, 0.012, 0.018 and 0.024 mm for an increasing correlation between measurements with time from 0.00 to 0.75 and a standard deviation of 0.09 mm. A minor difference of 0.012 mm is still detectable with 95% power and a correlation of 0.60. For larger differences, the power exceeds 0.95, even with a correlation of 0.75. This sample size can also detect meaningful differences in other outcomes, such as prevalence of CV risk factors and pulse wave velocity (PWV).

### Data analysis

Prevalence of the CV risk factors will be described and compared between various (sub)groups within the study population (HIV infected versus uninfected; whether or not on ART; and various CD4 levels and ART regimens). Continuous variables will be summarised by medians with interquartile ranges, or means with standard deviations. Categorical variables will be summarised by frequency counts and percentages. The 95% confidence intervals will be calculated for incidence rates as applicable. Continuous data will be compared with the Student's *t*-test, the Wilcoxon rank sum test or the Mann–Whitney U test. The distribution differences of categorical variables will be compared with the  $\chi^2$  test. The CVD risk factor burden will be calculated using the Framingham risk equation, D:A:D score and Reynold score. The prognosis (expressed as a percentage) of having a CVD event in the next 10 years will be reported. The distribution of CIMT and PWV among the whole population will be determined. The progression of CIMT and PWV over time will be analysed with a linear mixed model with a random intercept, a random effect for time and other covariates such as age, gender, CV risk factors and baseline CIMT or PWV. Multiple imputations will be used for missing data, presuming a random distribution.

### Data collection

**General characteristics.** Inclusion/exclusion criteria will be checked and signed informed consent will be obtained. Information will be collected on age, gender, demographics, general health and HIV testing behaviour. A full medical history, current medical condition(s) and chronic medication use will be obtained. Detailed information on HIV treatment (time between diagnosis and treatment initiation and specific medication prescribed) and responses to treatment (latest plasma HIV-1 viremia and latest CD4<sup>+</sup> T-cell count) will be recorded. Information on CV risk factors, family history, smoking and alcohol use will be obtained with a modified version of the World Health Organization (WHO) STEPS instrument.<sup>17</sup> Physical activity will be assessed with the International Physical Activity Questionnaire (IPAQ).<sup>18</sup> The Patient Health Questionnaire-9 (PHQ-9) will be used as a screening tool for depression and anxiety (Table 1).<sup>19</sup>

**Social and psychological aspects.** The following topics will be covered in (validated) questionnaires: employment, income position and household support (National Income Dynamics Study [NIDS] Wave 3 2012 Adults Questionnaire), food security and diet South African National Health and Nutrition Examination Survey

(SANHANES), impact of stigma,<sup>20</sup> opinions about HIV testing,<sup>21</sup> sexual history in the past 12 months and social support and quality of life (WHO Quality of Life, Brief Version). Data on adherence will be collected with a structured questionnaire in case of HIV infection.

**Physical measurements.** Information will be collected on height, weight and hip and waist circumference. Hip circumference will be measured in centimetres at maximum posterior extension of the buttocks and waist circumference will be measured halfway between the lower rib and the iliac crest during expiration, both in the standing position. Blood pressure will be measured in the seated position after 5 minutes of rest with a sphygmomanometric device on both arms, and repeated on the side with the highest values.

### Biological material

**Blood and urine samples.** Approximately 50 mL of blood will be collected during the baseline visit. This will comprise two ethylenediaminetetraacetic acid (EDTA) tubes, one serum separator tube (SST), one fluoride oxalate tube and one heparin tube. Full blood count (white cell count, red cell count, haemoglobin, haematocrit, mean cell volume and platelets), total cholesterol, high-density lipoprotein cholesterol, low-density lipoprotein cholesterol, triglycerides, random glucose, HbA1c and C-reactive protein will be measured in all individuals. Viral load and CD4 cell count will be measured in case of HIV infection. In a subgroup consisting of all newly diagnosed HIV-positive, ART-naïve patients ( $n=200$ ), EDTA plasma and peripheral blood mononuclear cells (PBMCs) will be stored for ultrasensitive HIV RNA and DNA analysis, co-receptor expression, drug resistance analysis and drug level measurements. A urine sample will be collected during the baseline visit for measurement of urine creatinine, microalbumin and the albumin/creatinine ratio.

**Blood storage and biobanking.** Plasma from blood collected in one of the EDTA tubes and in the heparin tube will be stored in a  $-80^{\circ}\text{C}$  freezer at the NMC for future research purposes. From the subgroup of 200 HIV-positive but therapy-naïve patients at baseline, two extra sodium heparin tubes (10 mL each) will be drawn for isolation of PBMCs for viro-immunological studies. PBMCs will be isolated in the NMC laboratory and stored at  $-120^{\circ}\text{C}$ .

### CV measurements

**Carotid intima–media thickness.** In order to estimate the presence of subclinical atherosclerotic disease, the

**Table 1.** Schedule of assessments at baseline and at follow-up.

Baseline	Every 6-month follow-up	Every 12-month follow-up
Baseline questionnaire:	Telephone questionnaire	Follow-up questionnaire
– Demographics	– Civil status	– Civil status
– Income	– Employment	– Employment
– Civil status	– Mental health	– Mental health
– Mental health	– Smoking, alcohol	– Smoking, alcohol
– Physical activity		
– Smoking, alcohol		
– Food security		
Physical examination:	–	Physical examination
– Blood pressure		– Blood pressure
– Weight, height		– Weight, height
– Waist and hip circumference		– Waist and hip circumference
– CIMT ultrasound		– PWV or CIMT ultrasound (alternating)
Medical history:	Medical history:	Medical history:
– Physical health	– Physical health	– Physical health
– Family history	– Chronic medication	– Chronic medication
– Chronic medication		– Tuberculosis questionnaire
– Tuberculosis questionnaire		– HIV status and cART
– HIV status and cART		
Biological examination:	–	Biological examination:
– Full blood count		– HIV test (in case of a HIV-negative participant)
– CD4 cells, viral load		– Full blood count
– Lipids		– CD4 cells, viral load
– Blood glucose, HbA1C		– Lipids
– CRP		– Blood glucose, HbA1C
– Urine microalbumin and creatinine		– CRP
		– Urine microalbumin and creatinine
Blood sample collection for storage:	Blood sample collection for storage only	Blood sample collection for storage:
– 1 EDTA tube and 1 heparin tube (plasma stored at $-80^{\circ}\text{C}$ )	for HIV-positive participants not on cART	– 1 EDTA tube and 1 heparin tube (plasma stored at $-80^{\circ}\text{C}$ )
– 2 extra heparin tubes for PBMC isolation (only for HIV-positive participants not on cART; every 3 months for 2 years; PBMCs are stored at $-120^{\circ}\text{C}$ )	– 1 EDTA tube (plasma stored at $-80^{\circ}\text{C}$ )	– 2 extra heparin tubes for PBMC isolation (only for HIV-positive participants not on cART; every 3 months for 2 years; PBMCs are stored at $-120^{\circ}\text{C}$ )
	– 2 extra heparin tubes for PBMC isolation (every 3 months for 2 years; PBMCs are stored at $-120^{\circ}\text{C}$ )	

CIMT: carotid intima-media thickness; EDTA: ethylenediaminetetraacetic acid; PWV: pulse wave velocity; cART: combination antiretroviral therapy; CRP: C-reactive protein; PBMC: peripheral blood mononuclear cell.

thickness of the carotid artery intima-media will be measured using a standardised ultrasonographic protocol (details are given in Appendix 1). CIMT is a well-established, continuous, quantitative measure of atherosclerosis and is able to identify increased CV risk in both black and white populations.<sup>22-24</sup> Ultrasound measurements of the CIMT will be performed by trained nurses at the Ndlovu Research Unit. Quality assurance processes will include regular performance reviews and training.

All images will be read in a batch fashion after completion of scanning per study period (e.g. after completion of baseline scans and at the 2-year follow-up

scans). The reader will be blinded to the HIV status of the participant. Mean and maximum thicknesses will be measured semi-automatically with the Artery Measurement System software (Chalmers University, Sweden) with a uniform reading protocol that ensures standardised settings across reading stations.

**Pulse wave velocity.** In order to gain insight into central vascular function, a PWV measurement will be taken by trained nurses using a SphygmoCor<sup>®</sup> device (AtCor Medical, Australia), starting from the 1-year follow-up visit (details are given in Appendix 2). PWV is regarded as the gold standard for measuring aortic stiffness;

an increase was found to be an independent risk factor for CVD.<sup>25,26</sup> Carotid–femoral PWV is calculated by dividing travelled distance by transit time. The direct carotid–femoral distance will be multiplied by 0.8, and a cut-off value of 10 m/second will be used to define abnormal PWV.<sup>27</sup>

### *Repeat measurements and follow-up*

Data will be obtained on mortality, prevalent and incident cases of CVD morbidity and hospitalisations. The clinical manifestations of CVD that will be considered in the study will include acute myocardial infarction, revascularisations, symptomatic heart failure and stroke. Comorbidity and non-CV outcomes will be measured by recording non-CV-related hospitalisations and mortality. All participants with abnormal findings (e.g. hypertension) will be referred to the Ndlovu Medical Centre or to a primary healthcare clinic for further analysis. Information on treatment will be updated at every follow-up visit (Table 1).

*Telephone follow-up.* Six months after the baseline visit and yearly thereafter (month 6, month 18, month 30 and so on), information on the current/ongoing medical condition(s), medical conditions during the past 6 months and medication use, lifestyle factors (e.g. alcohol usage and smoking habits and cART adherence for HIV-positive participants on cART) will be collected during phone interviews with participants.

*Follow-up visit at the research centre.* Participants will be invited for annual (month 12, month 24, month 36 and yearly thereafter following baseline) visits to the research centre for follow-up questionnaires, measurements and blood sampling in line with baseline assessment (see Table 1 for a list of the data that will be collected during follow-up). Blood drawing will be intensified for the group of 200 HIV-positive, ART-naïve participants at baseline; one EDTA tube and two sodium heparin tubes (10 mL blood each) will be drawn quarterly during the first 2 years after enrolment for storage and isolation of PBMCs. Both CIMT measurements and PWV measurements will be performed bi-annually, in alternating sequence.

### *Intended analysis of stored blood samples*

Future analysis will focus on the prospective analysis of immune markers representing different pathways of immune dysfunction and immune activation in relation to CIMT, PWV and overt CV outcomes. Examples are IL-6, CD163, D-dimer and sVCAM-1.<sup>28,29</sup>

### *Current state of the art*

Enrolment started in November 2014 and was finished in February 2017. The intended follow-up duration is 10 years.

### **Discussion**

Life expectancy is rising in the HIV-infected population as a result of increasing ART coverage. Consequently, the risk of experiencing non-communicable diseases such as CVD that lead to substantial morbidity and mortality amongst HIV-infected people increases.

So far, data on CVD risk in HIV-infected populations in SSA are scarce. Existing studies are limited by size, retrospective character or a single focus on risk factors, without any observations on the prevalence of CV end points or disease. Moreover, an HIV-negative control group is generally lacking from these studies. The current literature suggests a different CV risk profile in HIV-infected individuals compared to non-HIV-infected individuals in SSA, but it does not clarify to what extent HIV or HIV-related factors influence the CV risk profile and the occurrence of CVD.<sup>4</sup>

### *Strengths*

The NCS is designed to address CVD risk in relation to HIV infection in a rural SSA population. The Moutse area is an ideal setting for performing a cohort study, as it is a typical rural area. Lifestyle, environmental characteristics, gender distribution (more females than males infected) and the economic status of extreme poverty are representative of the situation in resource-limited settings, making the results generalisable to other rural districts in SSA.

The affiliation with the Ndlovu Care Group provides an excellent setting for establishing and following a cohort given its long-term relationship with the local community since 1994. A major advantage of the cohort is the inclusion of both HIV-positive and HIV-negative individuals, making it possible to investigate the CV risk profile of the rural population in general, as well as the additional effects of HIV and HIV treatment. As the intended duration of follow-up is several years, the study will not only provide data on the prevalence of CV risk factors, but also data on CV end points. In the meantime, CIMT and PWV will be used as surrogate markers in order to gain insight into the risk of CVD. The multidisciplinary approach of the NCS, integrating clinical and translational medicine, (clinical) epidemiology, infectious diseases, virology, immunology, pharmacology, social sciences, mathematical modelling and public health, will

combine efforts in order to gain new insights into pathogenesis, prevention and risk behaviour for both HIV and CVD.

### Limitations

Unemployment rates in the Moutse area are up to 50%, which may result in loss to follow-up, especially for men, due to labour migration. However, drop out is expected to be randomly distributed, and multiple imputation will be used to account for missing data. We used a simplified approach in order to estimate the power given the number of patients to be included. Even though the planned analysis will require additional degrees of freedom compared to the power analysis, we will offset this by analysing power for a very small difference for increasing correlations between time measurements of CIMT. Another limitation is the relatively young age of the population, limiting the number of expected CVD events. Moreover, survival of end points may hamper further examinations (e.g. disability after a stroke, which makes a visit to the clinic impossible). Effort will be made to trace all participants in order to keep follow-up rates as high as possible and to document reasons in case of loss to follow-up. This will be done by telephone calls, home visits when needed and community engagement.

### Conclusion

The NCS is designed to investigate CV risk profiles and the occurrence of CVD in a rural population in SSA where HIV infection is prevalent, more females than males are infected and the predominant HIV subtype is subtype C. The multidisciplinary approach will foster new insights into the prevalence of risk factors, risk behaviours, the role of HIV and ART in the pathogenesis of CVD and the occurrence of CV end points. The ultimate goal is improvement of risk prediction and development of targeted prevention and treatment strategies in order to provide integrated care for HIV and CVD.

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### Author contribution

HT, WD, RB, AW, MK, KK-G, AH, KT, CU, FV, RC and DEG contributed to the conception or design of the work.

AV, SA and MM contributed to the acquisition, analysis or interpretation of data for the work. AV, WD, KK-G and DEG drafted the manuscript. HT, RB, AW, MK, AH, FV and RC critically revised the manuscript. All gave final approval and agree to be accountable for all aspects of work ensuring integrity and accuracy.

### Declaration of conflicting interests

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