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# Pilot study of antibodies against varicella zoster virus and human immunodeficiency virus in relation to the risk of developing stroke, nested within a rural cohort in Uganda

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**Abstract**

OBJECTIVE The risk of stroke rises after episodes of herpes zoster and chickenpox, which are caused by varicella zoster virus (VZV). We conducted a pilot case–control study of stroke, nested within a long-standing cohort in Uganda (the General Population Cohort), to examine antibodies against VZV prior to diagnosis.

METHODS We used stored sera to examine the evolution of IgG and IgM antibodies against VZV among 31 clinically confirmed cases of stroke and 132 matched controls. For each participant, three samples of sera were identified: one each, taken at or near the time of (pseudo)diagnosis, between 5 and 10 years prior to diagnosis and at 15 years prior to diagnosis.

RESULTS All participants had detectable antibodies against VZV, but there were no significant differences between cases and controls in the 15 years prior to diagnosis. As a secondary finding, 16% (5/31) of cases and 6% (8/132) of controls had HIV (OR 3.0; 95% CI 0.8–10.1; $P = 0.06$).

CONCLUSIONS This is the first prospective study to examine a biological measure of exposure to VZV prior to diagnosis of stroke and although we identified no significant association, in this small pilot, with limited characterisation of cases, we cannot exclude the possibility that the virus is causal for a subset. The impact of HIV on risk of stroke has not been well characterised and warrants further study.

**keywords** varicella zoster virus, human immunodeficiency virus, stroke, cohort, prospective, Uganda

## Introduction

Varicella zoster virus (VZV) is a ubiquitous human herpes virus that, after primary infection (usually in childhood), causes chickenpox. It then persists asymptomatically (latently) in the sensory ganglia of the nervous system. Reactivation later in life and translocation via sensory nerve endings to the skin where it replicates is associated with the skin lesion, herpes zoster (shingles). VZV is the only human herpes virus that is transmitted via the respiratory route, and the prevalence in African populations exceeds 90% [1].

Both ischaemic and haemorrhagic strokes have been described at autopsy after herpes zoster. Among these cases, viral inclusions, DNA and antigen are present in cerebral arteries, confirming the association between VZV vasculopathy and stroke [2, 3]. Furthermore, population-based studies indicate substantial increases in risk of stroke, especially among younger people, after reports of both herpes zoster and chickenpox [4–8]. This raises the possibility that VZV is more widely implicated in the pathogenesis of cerebrovascular disease, although precise biological mechanisms remain unclear.

In general, there has been very little research aimed at characterising the major risk factors for stroke in Africa, but the burden of disease appears to be similar to that in the UK [9–12]. We therefore conducted a pilot study nested within a long-standing rural population cohort in Uganda, to investigate whether the pattern of VZV antibodies differed over time in individuals who subsequently develop stroke *vs.* those who did not.

## Methods

This pilot case–control study of stroke in relation to VZV was conducted within the context of an existing

 

population-based cohort study – the General Population Cohort (GPC). The GPC was originally established in 1989, by the UK Medical Research Council (MRC) and the Uganda Virus Research Institute (UVRI), in Kalungu District (at that time in Masaka District), south-western Uganda, to examine prevalence, incidence, risk factors and trends of infection with the human immunodeficiency virus (HIV) in a rural African population [13]. More recently, research activity has broadened to include the epidemiology and genetics of other communicable and of non-communicable diseases (NCDs), including cancer, cardiovascular disease and diabetes [14].

In brief, the GPC is a community-based open cohort study of residents of neighbouring villages within one half of a subcounty, lying about 40 km from the shores of Lake Victoria. The population is scattered across the countryside in villages defined by administrative boundaries with a few concentrated in small trading centres. Agriculture is the main economic activity with rain-fed, small-holder farms. A population of approximately 10 000 people in a cluster of 15 villages was studied from 1989 to 1999. In 2000, the GPC was expanded to cover a further 10 villages. The cohort is dynamic; new births, deaths and migration are reported at each round of follow-up, and the population under survey currently includes approximately 22 000 people. Data are collected through an annual census, questionnaire and serological survey. Details of sexual behaviour, medical, socio-demographic and geographic factors are recorded. Blood specimens are obtained at each survey. Serum is tested for HIV-1, and the remainder is stored at −80 °C in freezers in Entebbe. Seroprevalence of HIV initially declined from 8.9% in 1991 to 6.2% in 2001 and later increased to 10% in 2012 [14].

For the purposes of this pilot study, cases of stroke were identified in two ways. Firstly, prevalent cases were identified by the MRC/UVRI GPC Social Science Team from among GPC participants in and around a single village who showed signs of having had a stroke (face dropped on one side, paralysis, slurred speech). Each individual was then visited by a clinical team and the diagnosis of stroke confirmed on the basis of clinical signs and symptoms; in each case, the stroke had occurred within the previous 3 years. Thirty-one people were identified in this way, and of those, 19 had blood samples available for analysis from prior to the diagnosis. Secondly, between 2006 and 2008, all deaths occurring within the GPC were subject to verbal autopsy [15]; for 14 people, the cause of death was listed as stroke, of whom 12 had available blood samples. No detailed characterisation of the underlying pathology was possible as CT scan is not available near the study site. From this point on, data were de-identified and study numbers were used in all analyses.

Controls were selected from among adults in the same village without a diagnosis of stroke using the following procedure: each case was classified by sex and age at the time of their diagnosis. Potential controls were then examined to find the time points at which their characteristics (sex and age) matched those of the case, and this time point was then taken as the pseudodiagnosis date for that control. For each case, all matched controls were selected and included in a data set as matched to the corresponding case. At least four controls were then identified from this database and matched to the case on sex and age at the time of pseudodiagnosis/death and on availability of stored blood samples. In this way, 31 clinically confirmed cases were identified and 132 controls. Each participant had three stored serum samples identified from the archive: one from the period at or near the time of (pseudo)diagnosis, one 5–10 years and one at about 15 years before diagnosis.

The serial serum samples taken prior to the diagnosis/pseudodiagnosis dates for cases and controls were tested for antibodies against VZV using standard, well-validated and commercially available quantitative indirect chemiluminescent immunoassays against both IgM and IgG (Novatec Immundiagnostica GmbH). Testing was conducted at the Uganda Virus Research Institute in Entebbe, Uganda, by experienced individuals who were blind to whether the samples were from a case or a control. Briefly, serum samples were thawed and diluted 100-fold in sample diluent before dispensing 100 μl of samples and controls into each well of a 96-well plate coated with varicella zoster virus (VZV) antigen. Plates were covered and incubated for 1 h at 37 °C before - thorough washing. Hundred microlitre of VZV anti-IgM or IgG was then added and the plate incubated for 30 min at room temperature before washing again. Hundred microlitre of tetra-methyl-benzidine (TMB) substrate solution was then added, the plate was incubated for 15 min in the dark at room temperature, and 100 μl of 0.2 M sulphuric acid was added to stop the reaction. Plates were read at 450 nm to obtain optical density (OD) – a marker of antibody titre.

The characteristics of cases and of controls were initially compared using a Mann–Whitney two-sample test or chi-squared test as appropriate. The changes in antibodies for both IgG and IgM were analysed using linear regression adjusted for matching factors age and sex. Correlation between repeated measurements on individual patients was adjusted for using generalised estimating equations (GEE) with an exchangeable correlation structure. To graphically present trends in OD, the GEE

model was used to predict mean ODs, linear predictor and standard errors for both cases and controls from a population of individuals in a 1:1 sex ratio, with a median age of 60 years at (pseudo)diagnosis. The model allowed for interaction between case indicator and years preceding diagnosis of the case, to assess whether the effects vary with duration. Tests for statistical significance were derived from likelihood ratio test statistics. All *P*-values are two-sided, and significance was considered at the 5% level. Analyses were carried out using Stata 12 (StataCorp LP, College Station, TX, USA).

Ethical approval for this study was obtained from the Uganda Virus Research Institute Science and Ethics Committee and from the Uganda National Council for Science and Technology.

## Results

Table 1 shows the characteristics of study participants at the time point closest to (pseudo) diagnosis of stroke. Of the 31 cases and 132 controls, all had detectable IgG and IgM antibodies against VZV. There were no statistically significant differences observed in the optical densities between cases and controls (Mann–Whitney: IgG, $P = 0.47$; IgM, $P = 0.69$). In relation to HIV, 16% (5/31) of cases were seropositive compared to 6% (8/132) of controls (odds ratio (OR) = 3.0; 95% confidence interval (CI) 0.8–10.1; $P = 0.06$). Of those who were HIV seropositive, three cases and two controls were on antiretroviral therapy at the time of (pseudo)diagnosis.

**Table 1** Characteristics of study participants at the time point closest to (pseudo)diagnosis of stroke

| Factor | Cases $n = 31$ | Controls $n = 135$ | *P* value |
|---|---|---|---|
| Age in years, mean (standard deviation) | 59.0 (13.7) | 60.2 (13.7) | Matching factor |
| Sex | | | |
| Females | 15 (48%) | 67 (50%) | Matching factor |
| Males | 16 (52%) | 68 (50%) | |
| Median VZV IgG (Interquartile range – IQR) | 2.06 (1.45–2.42) | 1.91 (1.52–2.26) | 0.47* |
| Median VZV IgM (IQR) | 0.32 (0.19–0.43) | 0.29 (0.20–0.50) | 0.69* |
| HIV status | | | |
| Negative | 26 (84%) | 124 (94%) | 0.06† |
| Positive | 5 (16%) | 8 (6%) | |
| ART status among HIV+ | | | |
| Not on ART | 2 (40%) | 6 (75%) | 0.21† |
| On ART | 3 (60%) | 2 (25%) | |

*Mann–Whitney two-sample test, †chi-squared test; VZV, varicella zoster virus; ART, antiretroviral therapy; IQR, interquartile range.

Figure 1a,b shows the evolution of optical density over time for IgG and IgM antibodies against VZV, respectively, in the 15-year period prior to (pseudo)diagnosis. For neither was there any significant difference between cases and controls or indeed any change over time prior to (pseudo)diagnosis.

## Discussion

This is the first study of VZV in relation to risk of stroke that assessed exposure using a biological measure – both

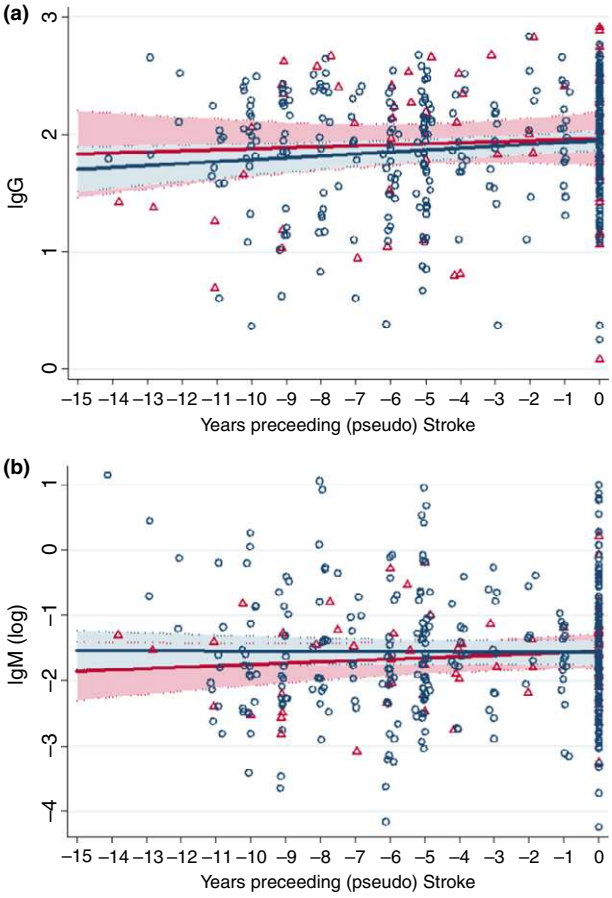


**Figure 1** IgG and IgM antibodies against Varicella Zoster Virus in the 15 years prior to diagnosis of stroke for cases, or pseudo-diagnosis (for controls). The red symbols and lines are the stroke cases and the blue symbols and lines are for controls.

IgG and IgM antibodies against VZV antigens – and also the first study from a resource-constrained setting. No associations were identified. However, as a secondary finding, the data suggest a threefold excess risk of stroke in association with HIV infection, based on five HIV-seropositive cases of 31 and eight of 132 controls; the finding was of borderline statistical significance ($P = 0.06$).

A possible association between VZV and stroke was first identified in clinical series from western countries, in which individuals with herpes zoster who subsequently died of stroke were found to have evidence of VZV vasculopathy in the brain, at post-mortem [2, 3]. In the light of this, a number of very large population-based studies were conducted in which records of prescription for herpes zoster antiviral drugs or of diagnosis of herpes zoster or chickenpox were linked to records of subsequent stroke, using national registers or clinical databases [4–8]. The risk of stroke was highest within 6 months of the diagnosis of VZV disease (or receipt of antiviral drugs), declining thereafter and was also highest among people under the age of 40 years compared to those who were older. It is difficult in such studies to be sure that any association is causal, when both diagnoses could be secondary to some other factor or factors.

A clear strength of this pilot study is the measurement of antibodies against VZV: IgG is a marker of past exposure and IgM is thought to indicate more recent exposure. All participants had antibodies against both antigens, confirming the ubiquitous nature of infection with VZV in Uganda. However, given the small size of the study and the lack of characterisation of stroke pathology, we cannot completely exclude the possibility that VZV is a cause of a subset of stroke cases. In addition, the median age of our participants was 60 years, so any effect on younger stroke cases could not be examined. However, given the prospective design of the study, it would seem unlikely that VZV is a major cause of stroke in rural Uganda.

The increased risk of stroke in association with HIV infection, although a secondary finding and based on small numbers, is of interest. Numerous hospital series of HIV-infected people and of stroke patients have suggested a link between HIV and stroke, but there have been no other prospective studies and few case–control studies to adequately quantify the excess risk or to characterise the specific pathologies of stroke in the context of HIV [16, 17]. There are a number of possible biological mechanisms whereby both HIV itself and the antiviral therapies used to treat it might increase the risk of stroke [18, 19]. The relative importance of these, however, is unclear.

In summary, in this small pilot study, we find no evidence to suggest that VZV is a major cause of stroke in rural Uganda. However, we do provide some evidence to suggest that infection with HIV may be an important risk factor. In the future, we plan more thorough identification of stroke cases within the entire cohort, with active case-finding, real-time reporting from local people who serve the GPC as village birth and death recorders and use of verbal autopsy to identify those who did not survive the initial event. It would seem that stroke is sufficiently common in this population and that over time, the number of cases we can identify could be more substantial than we report here.

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