Cytomegalovirus-Specific Immunoglobulin G is associated with chronic lung disease in children and adolescents from sub-Saharan Africa with perinatal HIV infection

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Abstract

In a cross-sectional study of 296 perinatally HIV-infected children and adolescents from Zimbabwe, individuals with the top tertile of CMV-Specific Immunoglobulin G titre had an increased odds of chronic lung disease (odds ratio 3.33; 95% confidence interval 1.37-8.85; p = 0.010).

Keywords: Cytomegalovirus, HIV, Immunoglobulin-G, Lung Disease

Introduction

Widespread use of combination antiretroviral therapy (cART) has increased the number of children with perinatal HIV infection (PHIV) surviving into adolescence. However, there is growing evidence that despite cART, HIV infection in children is associated with multisystem chronic comorbidities and concomitant disability (1). This is likely driven by chronic systemic immune activation, which CMV co-infection can exacerbate.

Studies from sub-Saharan Africa have shown that chronic lung disease (CLD) is a common comorbidity affecting about a third of PHIV children aged 10 years or more (2). Radiological findings are consistent with constrictive obliterative bronchiolitis (COB, as the main cause (3). COB is typically characterised by ongoing airway inflammation, resulting in progressive tissue remodelling, fibrosis of the small airways and lung function decline (4). Pediatric COB in the southern hemisphere mostly occurs as a sequela of severe lower respiratory tract infections (LRTIs) (4). As a common LRTI in infants with HIV, the association of cytomegalovirus (CMV) with chronic lung disease is of particular interest. CMV is a common cause of pneumonitis in infants with PHIV and has been shown to exacerbate experimental pulmonary fibrosis in murine models (5). In individuals with HIV, CMV co-infection contributes to immune activation and inflammation-related morbidities, even in the context of virological suppression of HIV by cART (6).

Recent studies from our group described a high prevalence of CMV DNA in the plasma of children and adolescents with PHIV from Zimbabwe (7). In this population, CMV viral load above 1000 copies/ml was associated with reduced forced vital capacity (FVC), lower CD4 T-cell counts and stunting. In the present cross-sectional study, we sought to determine the associations between both CMV-specific immunoglobulin G (IgG) titre alongside CMV plasma viremia with CLD as defined by airflow obstruction in a cross -sample of the participants from the BREATHE clinical trial (8).

Methods

We conducted a cross-sectional case-control study nested within the Bronchopulmonary Function in Response to Azithromycin Treatment for Chronic Lung Disease in HIV-infected Children (BREATHE) trial (8) (ClinicalTrials.gov, NCT02426112). The trial recruited PHIV children and adolescents aged between 6-19 years old from Malawi and Zimbabwe, who had been taking cART for at least six months, with a diagnosis of chronic lung disease, defined as forced expiratory volume in one second (FEV_1) z-score <-1 with lack of reversibility with salbutamol. Z-scores were generated using Global Lung Function Initiative reference standards. Individuals with tuberculosis (TB), acute respiratory tract infections or potentially fatal conditions at time of screening were excluded. A comparison group matched for age (6-12 and 13-19 years) and duration on cART (6 months to <2 years and >2 years) was recruited from HIV clinic attendees with FEV_1 z-score >0 and no chronic cough in the past 3 months. First thaw cryopreserved baseline plasma samples for participants recruited in Harare were used for this study.

Laboratory Methods

CMV-specific IgG levels were measured using the Abcam Anti-Cytomegalovirus (CMV) IgG Human ELISA kit as per manufacturer's instructions. Samples were run in duplicate and mean values per participant are reported in International Units (IU) per ml. CMV-specific IgG levels were split into tertiles for the entire cohort and the CLD group.

Total viral nucleic acids were extracted from 200 µL plasma using the QIAamp MinElute Virus Spin Kit (Qiagen, Hilden, Germany). 100µL of total viral nucleic acids were eluted and immediately stored at −80°C for subsequent testing. CMV detection was performed by quantitative polymerase chain reaction (PCR) using the RealStar CMV PCR kit v1.0 (Altona Diagnostics, Hamburg, Germany) as per the manufacturer's instructions. Samples were run on the QuantStudio 3 Real-Time PCR system in duplicate (Applied Biosystems, CA). Samples were repeated when technical replicas were not concordant for CMV presence. CMV viral load is reported in international units (IU)/ml.

Statistical Methods

Data were analysed in R Studio (Version 1.1.383). The mean and standard deviation was used to describe continuous variables and categorical variables were described with proportions. Differences between study groups were assessed by Mann-Witney U Tests or Chi-square tests as appropriate. Weight-for-age and height-for-age z-scores were calculated using British 1990 Growth Reference Curves. Z-scores less than -2 represented wasting and stunting respectively. Chronic lung disease was defined as per the BREATHE protocol (FEV_1 z-score <-1) (8).

The association of CMV-specific IgG tertile and CLD was assessed by logistic regression. Linear regression was used to assess the association between both CMV measures and FEV_1 z-score. Participant age, sex, height-for-age z-score, previous TB treatment, HIV viral load and cART regimen were included as covariates in all models. A sensitivity analysis where enrolment into the CLD group was defined by FEV_1 z-score <-1.64 was performed.

Ethics

Consent from individuals within the BREATHE study was sought from the guardian and age-appropriate assent from the participant (for those aged <18 years). Ethical approval for this sub-study was granted by the Medical Research Council of Zimbabwe.

Results

A total of 241 cases and 55 controls were included in this study. A higher proportion of cases were female, reported previous treatment for TB, were stunted and wasted then the control group (Supplementary table 1). Cases had a lower mean CD4 T-cell count than the controls. There was no evidence of significant difference in HIV viral load or duration of cART between groups (Supplementary Table 1). Across both groups the median (IQR) time on cART was 6.44 (4.15-8.42). The mean (standard deviation (SD)) CMV-specific IgG level was higher in the group with CLD than the group without (48.4 ± 12.1 vs 39.7 ± 13.0, p=<0.001). 100% of participants were CMV seropositive. No control participants had detectable CMV DNA in plasma compared to 29/241 cases (12%).

Top and mid tertiles of CMV-specific IgG titre were significantly associated with increased odds of CLD compared to the bottom tertile (top-tertile OR = 3.33; 95% CI= 1.37-8.85; p=0.010 and mid-tertile OR= 2.17; 95% CI =1.60-4.55; p=0.036)) (Table 1). CMV-Specific IgG as a continuous measure was also associated with increased odds of CLD (OR=1.05; 95% CI= 1.02-1.08; p=0.003). CMV DNA in plasma was significantly associated with reduced FEV_1 z-score in cases (Coefficient ± SE = -0.30 ± 0.14; p=0.028). Neither tertile nor CMV specific-IgG as a continuous measure were not associated with FEV_1 z-score in the case group. In all analyses, duration of ART had no significant effect model results. CMV-specific IgG titre negatively correlated with CD4 T-cell count in both groups. Spearman rank correlation coefficients are presented in Supplementary Figure 1. Only the association between CMV specific IgG and FEV_1 z-score is modified in the sensitivity analysis (Supplementary table 2).

Discussion

As a common cause of LRTI in HIV-infected infants, we hypothesized that CMV may be associated with CLD in the PHIV population (9). Our results show that top tertile CMV-specific IgG titre is associated with obstructive lung disease, supporting previous associations between CMV presence in plasma and reduced lung function (7). These findings contribute to our understanding of the association of CMV with HIV-1-associated airway disease in Sub-Saharan Africa.

Infant coinfection of CMV and HIV leads to rapid disease progression and often pneumonitis, which alongside systemic inflammation may drive chronic lung disease (9). CMV-specific IgG titre can be used as a putative marker of lifelong CMV exposure and increases with impaired control of the virus. CMV Specific IgG is associated with elevated immune activation markers and with the CD45RA+ CD27- T-cell memory phenotype, indicative of multiple rounds of restimulation (10,11). High antibody levels could represent increased exposure to CMV antigens before cART, or persistent B-cell in activation individuals with CLD. CMV viremia in the plasma of study participants is likely to indicate viral reactivation. The complete absence of CMV DNA in the control group is consistent with increased lifelong exposure to CMV within the case group.

This study is limited by its cross-sectional and associative design. Levels of CMV-specific IgG were generally high in the cohort, reflecting the overall burden of CMV infection in sub-Saharan Africa. Prevalence of CMV viremia and CMV viral load in the plasma of participants was lower than recent reports, likely explained by more stable cART use in BREATHE compared to previous studies (7). As a result, the number of individuals with detectable CMV in the plasma was small. Further work is required to address whether CMV is a marker of impaired cellular immunity and/or the driver of the pathology described (12). Lower respiratory tract samples would strengthen these findings.

In conclusion, we provide further evidence that CMV co-infection in HIV-infected individuals is associated with CLD. We extend previous findings, reporting associations between CMV specific IgG titre and CLD. HIV associated comorbidities, such as CLD, represent a growing burden of disease in PHIV adolescents on stable cART. These results underline the need for future studies to assess causality, and suggest that available CMV-specific antiviral drugs such as valganciclovir may be beneficial within this population of PHIV individuals at the time of acute infection or reactivation. Trials of such drugs would further help to determine the causality of CMV-associated pathogenesis.

NOTES

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Legend for table:

Table 1: Factors associated with CLD in the case-control study (logistic regression) and factors associated with FEV-1 in children with CLD (linear regression).

*Linear regressions are performed in the CLD group only. **All multivariable analysis includes age, sex, height for age z-score, previous TB treatment, cART regime and HIV viral load as confounding variables. Tertile comparisons are compared to lowest tertile within the group compared. CMV DNA presence in plasma could not be included in logistic regression models due to no cases in the control group. P= <0.05 are highlighted in bold. CI= Confidence Interval, SE= Standard Error.

Variable	CLD logistic regression in whole cohort (n=296)		FEV_1 z-score linear regression in CLD cases* (n=241)	
	Odds Ratio (CI, P) univariable	Adjusted Odds Ratio (CI, P) **	Coefficient ± SE, P univariable	Coefficient ± SE, P multivariable**
Middle tertile CMV specific IgG	**2.34 (1.19-4.72. p=0.015)**	**2.17 (1.06-4.55, p=0.036)**	0.09 ± 0.12, p= 0.445	-0.05 ± 0.11, p=0.655
Top tertile CMV specific IgG	**5.19 (2.34-12.76, p=0 <.001)**	**3.33 (1.37-8.85, p=0.010)**	**-0.24 ± 0.11 p=0.032**	-0.09 ± 0.12, p= 0.431
CMV specific IgG (IU)	**1.06 (1.03-1.09, p<0.001)**	**1.05 (1.02-1.08, p=0.003)**	**-0.01 ± <0.01, p=0.032**	<-0.01 ± <-0.01, p= 0.461
CMV DNA Presence in Plasma	N/A	N/A	**-0.28 ± 0.14, p=0.047**	**-0.30 ± 0.14, p=0.028**