Cytomegalovirus antibody responses associated with increased risk of TB disease in Ugandan adults

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SUMMARY:

A dose-dependent increased risk of TB disease was seen with increasing HCMV exposure as measured by IgG in this Ugandan cohort. Increased TB risk was also associated with an inflammatory profile of IP-10 and IL1α.

ABSTRACT

Background

Recent evidence highlights human cytomegalovirus (HCMV) and immune activation as risk factors for tuberculosis (TB) disease. It is not known whether other herpes viruses are also implicated, nor if a dose-response relationship exists between TB risk and herpes co-infection.

Methods

This nested case-control study used stored serum samples from 25 TB cases up to 10 years prior to TB diagnosis and between 3 and 6 matched non-TB controls from a rural Ugandan cohort. Samples were investigated for Epstein Barr (EBV), Herpes Simplex (HSV), and HCMV-specific IgG, serum markers of inflammation, and mycobacterial antibody levels.

Results

Humoral response to HCMV, but not EBV or HSV was associated with increased risk of active TB disease up to 10 years prior to diagnosis. Individuals with medium HCMV IgG were 2.8 times more likely to have TB (p=0.055), and those with high HCMV IgG 3.4 times more likely to have TB (p=0.007). Mycobacterial antibody levels were not associated with differences in odds of TB disease. IP-10 was independently associated with increased odds of TB; OR 4.2, p=0.009.

Conclusions

These data provide evidence of a dose response between magnitude of HCMV IgG with risk of TB disease. An inflammatory environment, characterized by serum IP-10 and IL1α, are independently associated with increased risk of TB disease.

Key words: TB, Tuberculosis, HCMV, Cytomegalovirus, case-control, herpes virus

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## BACKGROUND

Epidemiological studies have identified important risk factors for TB: HIV [1], diabetes [2], IFN-γ deficiencies [3], and malnutrition [4]. Despite these findings, the vast majority who develop TB globally are HIV negative, non-diabetic and immunocompetent. The reasons why these individuals develop active TB disease, thereby propagating transmission of the pathogen, are unknown.

Human cytomegalovirus (HCMV), also known as human herpesvirus-5 (HHV-5), is a member of the *β-herpesviridae* subfamily, is widely distributed in human populations, and is transmitted through person-to-person contact. In many of the areas of the world with highest burden of TB, HCMV infection is nearly ubiquitous and evidence exists for convergent epidemiology of the two pathogens [5].

Once infected, HCMV establishes lifelong latency in a variety of cell types, including those infected by *M.tb*. Infection rarely results in serious side effects in immune competent individuals, however it can cause permanent hearing and neurological damage in neonates [6], severe non-AIDS events in HIV-infected people [7], and important clinical problems in solid organ transplant recipients [8]. Despite its ubiquitous and mostly benign status as an infectious disease, HCMV infection is highly associated with immune variation [9], T cell activation [10], immune senescence [11], and memory inflation [12]. There is currently an incomplete understanding of the biological basis for increased CMV IgG levels. Some studies suggest that accumulated HCMV burden correlates with high HCMV IgG titres, [13,14]. Seropositivity has been linked to increased overall mortality [16], and presence of virus in blood is linked to a range of chronic diseases [15]. Epidemiological evidence describes elevated HCMV-specific IgG in TB cases [17,18], and two studies report a link between increased latent and active HCMV infection in people with TB and non-tuberculous mycobacterial (NTM) disease [18,19]. Given the paucity of information of HCMV-associated TB risk, this study aims to further investigate the possible role of HCMV in TB disease using a case-control study based in a rural Ugandan longitudinal cohort. In addition, inflammatory serum markers, mycobacterial antibodies and levels of IgG against two other chronic herpes viruses; Epstein Barr (EBV) and herpes simplex 1 and 2 (HSV), were investigated to determine association with TB risk in this cohort.

## METHODS

### Sampling

The General Population Cohort (GPC) is a population-based open cohort in rural Uganda. The GPC was established in 1989 to examine trends in prevalence and incidence of HIV infection and their determinants [20]. The cohort

comprises approximately 20,000 people, half of whom are aged below 13 years. Data are collected through an annual census and blood samples are stored in a biobank at -80°C, located in Entebbe, Uganda. Testing for HIV was carried out immediately after blood collection in Uganda as previously described by Asiki et al [20]. TB is diagnosed through passive case identification of symptomatic individuals presenting for care at GPC clinics. Twenty-five individuals from this cohort who were diagnosed with sputum positive active TB disease between 1999 and 2014, and who had available serum samples, were included in this study. All available stored serum samples from these 25 TB cases from 10 years prior, up to three months after TB diagnosis were retrieved. Between one and four stored serum samples per TB case were identified and retrieved (total sample number = 51). Figure 1 shows the timing of samples obtained from TB cases aligned to time of TB diagnosis.

For controls, we selected stored serum samples collected in 2011 from people within the GPC who had no record of TB disease by the end of the dates studied here (2014). Samples were matched on known predictors of HCMV level; age, sex and HIV status at time at which sample was taken. Control samples were designated the same time before TB diagnosis as the TB case to which they were matched. Between three and six control individuals were matched per TB case sample (a maximum of one sample per control individual). Because of the ubiquity of HCMV infection within this population [17], HCMV seronegative samples were excluded (n=9, all nine were non-TB control individuals with a mean age of 37 years (26.9-50.8 years), 2/9 were HIV positive.) The 307 HCMV positive samples (256 controls and 51 TB samples, grouped into 51 case-control matched sets), were included in the analyses (Table I), such that TB cases with multiple samples could be included in more than one set. The exposure of interest was TB disease as a binary measure.

Ethical approval and consent to participate

Ethical approval for this study was obtained from London School of Hygiene & Tropical Medicine (ref 10000 and 10643), the Uganda Virus Research Institute Research and Ethics Committee (ref GC/127/15/06/512), and from the Uganda Council for Science and Technology. Written consent for the use of clinical records and biological samples for research purposes was obtained from all GPC participants following the Uganda National Council of Science and Technology guidelines.

Herpes virus-specific IgG

Measurement of HCMV IgG was conducted as previously described [17]. IgG against Epstein-Barr virus nuclear antigen 1 (EBNA-1) and HSV1 and HSV2 full antigens was measured using commercial kits (Euroimmun, Germany).

The resulting measurement (in relative units (RU)) was calculated based on a standard curve from the calibration sera and based on kit cut-offs.

Serum cytokines

Luminex multiplex cytokine platform (Merck Millipore, USA) was used to determine the concentrations of IFNα2, IFNγ, IL10, IL12p40, IL12p70, IL1Ra, IL1α, IL1b, IL6, IP-10, TNFα in serum samples. Bio-Plex manager software version 6.1 was used for bead acquisition and analysis of median fluorescence intensity (MFI). MFI was converted to pg/mL using the software.

Total IgG

Measurement of total IgG was conducted as previously described [21]. Briefly, $8 \times 10^5$ diluted test sera and IgG antibody standards (134.4–8.4 ng/ml) were incubated on mouse anti-human IgG (Abcam ab200699) coated plates. After washing and incubation with goat anti-human Fc (Abcam ab97225). Plates were read and OD measurements converted into g/l by use of the standard curve on each plate.

Mycobacterial antibodies

Measurement of Antigen 85A (Ag85A) IgG and IgM, lipoarabinomannan (LAM) IgG, purified protein derivative (PPD) IgG and ESAT6/CFP10 IgG was conducted as previously described [21]. Briefly, 1:100 diluted test sera were incubated on plates coated with recombinant Ag85A (Aeras), PPD (Lot 051815KA, Aeras, USA), LAM (NR-14848, BEI Resources), and combined CFP10 (NR-49425, BEI Resources) and ESAT6 (NR-14868, BEI Resources). After washing and incubation with either goat anti-human IgG-HRP (04-10-20, KPL) or goat anti-human IgM-HRP (Abcam ab97205), absorbance was measured to obtain optical density (OD), a surrogate marker of antibody titre.

Statistical analyses

Analyses were conducted on all 307 samples to investigate differences between individuals who progressed to diagnosis with active TB disease with those who remained TB-free. Pearson's correlation was used to investigate associations between continuous measurements of HCMV, EBV and HSV1/2 IgG levels, as well as IP-10, IL1α and herpes virus IgG levels. HCMV, EBV and HSV 1/2 seropositive samples were categorized into tertiles according to level of specific IgG response. Associations between herpes virus IgG tertiles, mycobacterial antibodies, inflammatory markers and TB disease were investigated using a conditional logistic regression model conditioned on the 51 matched case-control sets. HCMV tertile was included as a covariate. Due to different measurement ranges, all continuous exposure variable measurements were z-score transformed, hence reported odds ratios

are for a one standard deviation of the mean increase in response. We investigated the slope of serum antibody and cytokine change in TB cases over time to diagnosis using a linear random effects model.

To account for multiple comparisons, 99% confidence intervals (CIs) are reported and a p value of 0.01 is considered to represent strong evidence to reject the null hypothesis. A robust standard error was employed in conditional regression analysis to account for the fact that some TB cases contributed more than one sample. All analyses were performed using Stata version 14 (Stata Corporation, College Station, TX, USA).

## RESULTS

Of the 281 HCMV seropositive individuals included in this nested case control study, 24% were HIV positive (8/25 TB cases and 59/256 controls; Table I). Of the 307 HCMV positive samples included in analyses, 81% (250/307) were EBV positive and 98% (302/307) were HSV positive. To examine the robustness of the assay indicating HCMV, EBV and HSV1/2 seropositivity, longitudinal samples from the TB cases were investigated. Of the 17 TB cases who contributed more than one sample to analyses, seropositivity was consistent between samples taken from the same individual, with one individual seroconverting to HSV1/2 positive and two individuals seroconverting to EBV positive during the period for which samples were available. HCMV IgG and HSV1/2 IgG were positively associated (ρ 0.20, p=0.0006). HCMV IgG levels were not associated with EBV IgG (ρ -0.04, p=0.47), nor were EBV IgG levels associated with HSV1/2 IgG (ρ -0.03, p=0.65).

### Mycobacterial antibody levels were not associated with risk of progression to TB

LAM IgG and ESAT6/CFP10 IgG (measured at all time points prior to, and at point of TB diagnosis) were associated with 1.5 and 1.4 times increased odds of TB disease respectively per 1 SD increase in antibody level, however neither of these were significant at the p<0.01 threshold. Odds ratios were slightly reduced when HCMV tertile was included in the regression model (Table II).

High HCMV IgG, but not HSV or EBV, is associated with increased risk of TB

The odds associated with progression to active TB were increased 2.8 times among individuals with a medium HCMV IgG compared to low IgG (99% CI 0.908 - 8.638, p=0.055) (Table III and Figure 2). Having HCMV IgG in the upper tertile of the range was associated with a 3.4 times greater odds of having active TB disease compared to low HCMV IgG (99% CI 1.072 – 11.074, p=0.007), the directional trend to increased risk of TB with increased HCMV IgG was significant (p=0.006). The same trend of increased risk with higher IgG was not seen with either of the herpesviruses EBV or HSV1/2 (Table III).

Inflammatory markers CXCL10 (IP-10) and IL1α are associated with increased odds of TB

IP-10 is associated with 4.6 times increased odds of progression to TB disease per 1 SD increase in cytokine level (OR 4.587, 99% CI 1.064, 19.769, p=0.007). The OR does not decrease significantly when HCMV IgG levels is added into the conditional logistic regression model (OR 4.233, 99% CI 1.021, 17.547, p=0.009; Table III).

The only other cytokine with a statistically significant association with risk of TB disease is IL1α; a one SD increase in IL1α is associated with a 1.5 times greater odds of going on to develop TB disease (OR 1.521, 99% CI 1.045, 2.212, p=0.004; Table IV).

CXCL10 (IP-10) level correlated with HCMV IgG but not EBV or HSV1/2 IgG levels

Correlations between levels of IP-10 and HCMV, EBV and HSV 1/2 IgG showed that serum levels of IP-10 were positively correlated with increased HCMV IgG levels (ρ=0.40, p<0.0001) whereas no association was seen between IP-10 and EBV IgG (ρ=-0.07, p=0.22), nor between IP-10 and HSV 1/2 IgG (ρ=0.03, p=0.54) (Figure S1). IL1α levels were negatively associated with HCMV and HSV 1/2 IgG (ρ=-0.16, p=0.005; ρ=-0.17, p=0.003, respectively) and IL1α levels were not significantly associated with EBV IgG (ρ=-0.03, p=0.60) (Figure S2).

A combination of serum HCMV IgG and IP10 maximizes prediction of TB risk

To explore whether the risk of TB disease associated with HCMV IgG tertile was independent of that seen with IP-10 and IL1α, they were included in a conditional logistic regression including HCMV tertile. The addition of IP10 into the regression model resulted in a better fit (R2 = 0.29) compared with IL1α (R2=0.10) and so was investigated further. The inclusion of IP-10 modified the odds ratios associated with the medium tertile of HCMV IgG from 2.8

to 1.9, and the odds ratio associated with the highest HCMV IgG tertile from 3.4 to 2.4. Meanwhile the inclusion of HCMV in the model modified the odds ratio associated with IP-10 from 4.6 to 4.2 (Table III). Despite HCMV tertile explaining some of the increased risk associated with IP10 and vice versa, a likelihood ratio test provided good evidence that a model including both HCMV IgG tertile and IP-10 independently, resulted in a statistically significant improvement in model fit ($p<0.0001$). In conclusion, of the parameters studied here, the best model for prediction of risk of TB disease includes both HCMV IgG and serum IP10.

Evidence of longitudinal changes among TB cases

There was limited statistical power to detect trends over time, however, of the 17 TB positive cases who contributed more than one sample to analyses, there was evidence that levels of Ag85A IgG, IL1α and total levels of IgG changed over time leading up to diagnosis of TB disease (Figures S3,S4 and S5). Both Ag85A IgG (Figure S3) and IL1α (Figure S4) showed a trend towards decreased serum levels from 10 years prior to TB diagnosis until point of diagnosis (slope coefficients -0.04 (99% CI -0.07- -0.01, p=0.001) and -14.69 (99% CI -28.46- -0.93, p=0.006) respectively. Total IgG (Figure S4) showed a trend toward increasing over time to TB diagnosis; slope coefficient 2.10 (99% CI 0.32-3.87, p=0.002).

## DISCUSSION

Using a case-control design containing longitudinal samples taken up to 10 years before TB diagnosis, we show that magnitude of HCMV infection, as measured by IgG, is associated, in a dose-dependent manner, with risk of TB disease. The same association with TB risk was not seen with EBV or HSV despite evidence of co-prevalence of these three chronic herpes viruses [22]. Here, we see that risk of TB disease is increased 3.4 times in the those with the highest HCMV levels of IgG. There are a variety of possible mechanisms by which HCMV infection may exacerbate *M.tb* infection and lead to increased TB disease risk. HCMV encodes viral proteins which may interfere with protective immune responses including; UL111A, a homologue to the immunosuppressive cytokine IL-10 [23]; gpUS6 which blocks peptide presentation, reducing CD8 T-cell recognition [24]; and US2 which causes degradation of HLA-DR-α and DM-α, two essential proteins in the MHC class II antigen presentation pathway, thereby blocking CD4 T-cell presentation of viral antigens [25]. Many TB endemic areas have very high HCMV seropositivity rates [28,29]. If HCMV reactivation and reinfection events are driving HCMV IgG in this population,

the large number of potential HCMV reactivation and reinfection events possible given the early age at which an individual is infected with HCMV in this population (83% seropositivity by one year of age [17]), makes HCMV/TB an interesting co-infection model in which to further study mechanisms. Due to lack of cellular material in this study, cellular interactions were not investigated however the propensity of both *M.tb* and HCMV to infect the same cell type [26,27], may be an indication of a direct interaction between the two pathogens. Alternatively, myriad indirect interactions by one pathogen altering the immunologic milieu in favour of a more favourable environment for persistence of the other pathogen may be driving co-pathogenicity. In this study, both IP-10 and IL1α serum levels were associated with increased risk of TB (4.2 and 1.5 times respectively). Despite a positive correlation between IP-10 and HCMV IgG in serum, these associations were independent of HCMV IgG levels. Assuming independence, a 1 standard deviation increase in IP10 combined with a HCMV IgG level in the highest tertile is associated with a ten times increased odds of TB disease (OR 2.4 for HCMV multiplied by OR 4.2 for IP10) compared to a 1 SD increase in IP10 in the lowest HCMV tertile. Elevated levels of IP-10 have been associated with post-transplantation morbidity in HCMV discordant recipients [11]. In addition, IP-10 has been investigated as a possible diagnostic biomarker for TB disease [reviewed in 32], however its potential role in exacerbating an inflammatory response leading to a more TB-permissive environment and TB disease progression has not been previously explored.

Emerging evidence suggests that HCMV may induce host production of IP-10. Initially assumed to be a silent infection, the life-long latency established after initial infection with HCMV is characterised by low level viral gene expression and induction of cytokine production from infected cells [27]. In an experimental latent HCMV model of infected monocytes, selective expression of proinflammatory cytokines, including IP-10 was seen up to 6 days post-infection [31]. While it is likely that there are other causes of elevated IP-10 levels not investigated here, these findings indicate that the risk of active TB disease associated with HCMV infection might be greater than that attributed to HCMV IgG levels alone. While further investigation is needed to understand the causal link between HCMV and IP10, the combined risk of cumulative HCMV infection (as measured by HCMV IgG), in addition to HCMV-induced IP-10 associated risk may demonstrate that HCMV is acting through multiple mechanisms to increase the risk of TB. HCMV viral load was shown to be a poor determinant of mortality in very ill HIV and TB co-infected individuals [32], indicating that HCMV IgG may be a better marker of risk of TB disease, likely being a result of cumulative reactivation as well as reinfection events throughout a lifetime.

IL1 has been identified as critical in control of TB infection [33]. IL1α and IL1β dysregulation by type I interferons (IFNα and IFNβ) has been linked to disease exacerbation via eicosanoid imbalance-induced necrotic, as opposed to apoptotic, cell death and subsequent bacterial escape and further cellular infection [34]. Although in this study we see a link between increased IL1α and increased risk of TB, rather than IL1 being associated with control of TB disease, we do have limited evidence to suggest that levels of IL1α may be decreasing as individuals progress towards diagnosed active TB disease. We also do not see a concomitant increase or decrease in IL1β or IFNα, however many samples were below the lower limit of quantitation for the assay.

In contrast to the protective effect of Ag85A as was seen among BCG-vaccinated South African infants [35], we do not see a difference in serum Ag85A IgG between TB cases and matched controls. Despite not finding evidence of a difference in antibodies against five mycobacterial antigens between TB cases and controls, we did see a trend towards decreased Ag85A IgG with time to TB diagnosis over the preceding 10 year period, which may merit further investigation. In the same population, previous work found an increase in mycobacterial antibody levels with age regardless of TB status until approximately 20 years of age, suggestive of exposure to non-tuberculous mycobacteria (NTM), and to *M.tb* itself [21]. The TB field has seen a resurgent interest in antibodies in TB, despite the failure to identify a satisfactory antibody profile which could function as a diagnostic for TB [36]. A group in China found that total immunoglobulin purified from 7/48 TB-exposed but healthy healthcare workers protected mice against virulent TB challenge whereas immunoglobulin from TB patients did not protect challenged mice [37]. Using a systems serology approach, Lu *et al* found a functional role for antibodies in TB whereby antibodies from latently infected (indicative of control) had enhanced ability to induce phagolysosomal fusion and inflammasome activation compared to people with active disease [38].

In summary, this work shows, for the first time that magnitude of humoral responses against HCMV are associated with risk of TB disease. In addition, an inflammatory environment, possibly exacerbated by HCMV infection itself, is also associated with increased risk of TB disease in this cohort. Given the ubiquity of HCMV exposure in TB endemic settings, and the excess TB disease risk associated with increased HCMV IgG responses seen here, further research should be conducted to understand if repeated HCMV reinfection and reactivation events are driving this effect and whether HCMV-specific interventions could be investigated to tackle TB.

Development of a HCMV vaccine is already underway [39], however target groups mainly consist of women of child-bearing age to protect neonates from HCMV-associated neurological disorders. The data presented here show that the need for HCMV control measures may be greater than initially considered, as controlling HCMV could contribute to the control of TB disease.

## Limitations

Although the capacity exists within the GPC to collect PBMC, cells were not available for the current study. Control samples were matched to case samples on age, sex and HIV status when the sample was taken as opposed to matching at point of diagnosis which may introduce a potential source of bias. This study is limited by the small number of TB cases. Reliance upon passive TB case detection in the GPC meant that control individuals included in this study were not investigated for TB infection. More stringent inclusion criteria for controls may have resulted in larger differences between groups. The grouping into tertiles of HCMV-specific IgG is based upon the ranges seen in this population and may not be generalizable. It will be important to measure ranges in other populations and determine if specific cut-offs of HCMV IgG are associated with increased risk of TB. Whilst we had longitudinal data for some TB cases, we only had data from one time point for non-TB controls. Ideally, we would have included longitudinal data for controls but those samples were not available.

Footnote page

Availability of data and materials

The datasets generated and/or analysed during the current study will be available upon publication on an appropriate repository.

Conflict of Interest

The authors declare that they do not have a commercial or other association which might pose a conflict of interest.

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Author's contributions

LS conducted laboratory assays, analysed data and wrote the manuscript. RN, HF and SN designed the study and supervised the work. RF and SN designed the statistical analyses. JR and SM contributed to analysis and interpretation of results. All authors read, edited and approved the final manuscript.

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Figures and tables

Table I – Number of TB case samples and matched non-TB controls. Age, sex and HIV status for the individuals included in this study (for TB case individuals, the mean age is age at sampling closest to diagnosis).

| | Non-TB Controls | TB Cases | Total |
|---|---|---|---|
| Number of individuals | 256 | 25 | 281 |
| Mean age (range) yrs | 34.2 (2.75-56.5) | 36.1 (13.1-56.5) | 34.3 (2.75-56.5) |
| Number female (%) | 157 (61%) | 15 (60%) | 172 (61%) |
| Number HIV positive (%) | 59 (23%) | 8 (32%) | 67 (24%) |
| Mean number of samples per individual (range) | 1 (1) | 1.45 (1-4) | |
| Total number of samples | 256 | 51 | 307 |

Table II: Conditional logistic regression of mycobacterial antibodies with odds of TB disease.

| | Mean OD (SD) | Unadjusted | | | HCMV adjusted | | |
|---|---|---|---|---|---|---|---|
| | | Odds Ratio (OR) | 99% CI | P value | Odds Ratio (OR) | 99% CI | P value |
| Ag85A IgG | 0.83 (0.4) | 0.962 | 0.645, 1.436 | 0.805 | 0.919 | 0.624, 1.353 | 0.573 |
| PPD IgG | 1.14 (0.36) | 1.035 | 0.687, 1.559 | 0.831 | 1.059 | 0.704, 1.592 | 0.717 |
| LAM IgG | 2.00 (0.58) | 1.515 | 0.948, 2.424 | 0.023 | 1.51 | 0.925, 2.467 | 0.030 |
| Ag85A IgM | 1.27 (0.82) | 1.026 | 0.666, 1.579 | 0.880 | 0.912 | 0.590, 1.409 | 0.585 |
| CFP10 ESAT6 IgG | 0.79 (0.56) | 1.434 | 0.974, 2.110 | 0.016 | 1.347 | 0.916, 1.982 | 0.047 |

OD; optical density SD; standard deviation, Ag85A; antigen 85A, PPD; purified protein derivative, LAM; lipoarabinomannan, CFP10/ESAT6; 10kDa culture filtrate protein, 6 kDa early secretory antigenic target

Table III – Odds of TB disease by chronic herpes virus IgG level. Medium and high tertiles are compared to the lowest tertile of IgG level for each virus in a conditional logistic regression model. Odds ratios and 99% confidence intervals are given. The P value is from a likelihood ratio test (LRT) for trend.

| Herpes virus IgG level (range; n) | Odds Ratio (99% CI) | $P_{trend}$ value |
|---|---|---|
| HCMV | | |
| Low (0.52-1.03 OD n=102) | 1.0 | 0.0063 |
| Medium (1.04-1.34 OD n=102) | 2.801 (0.908 - 8.638) | |
| High (1.35-2.84 OD n=103) | 3.446 (1.072 – 11.074) | |
| HSV1/2 | | |
| Low (25-132 RU; n=100) | 1.0 | 0.1718 |
| Medium (133-163 RU; n=101) | 0.811 (0.280-2.348) | |
| High (164-245 RU; n=101) | 0.527 (0.149-1.862) | |
| EBV | | |
| Low (22-61 RU; n= 83) | 1.0 | 0.4778 |
| Medium (62-106 RU; n=83) | 1.429 (0.451 – 4.528) | |
| High (107-237 RU; n=84) | 0.717 (0.208 – 2.471) | |

OD; optical density, RU; relative units

Table IV: Conditional logistic regression of cytokines, and total IgG with odds of TB disease.

| | Mean pg/mL (SD) | Unadjusted | | | HCMV adjusted | | |
|---|---|---|---|---|---|---|---|
| | | Odds Ratio (OR) | 99% CI | P value | Odds Ratio (OR) | 99% CI | P value |
| IFNα2 | 16.55 (40.03) | 1.412 | 0.894, 2.230 | 0.052 | 1.403 | 0.898, 2.192 | 0.050 |
| IFNγ | 3.94 (11.22) | 1.238 | 0.902, 1.700 | 0.083 | 1.206 | 0.881, 1.651 | 0.124 |
| IL10 | 20.24 (160.02) | 1.763 | 0.699, 4.449 | 0.115 | 1.893 | 0.695, 5.154 | 0.101 |
| IL12p40 | 5.34 (30.2) | 1.033 | 0.739, 1.445 | 0.802 | 1.002 | 0.708, 1.418 | 0.988 |
| IL12p70 | 0.93 (3.39) | 0.956 | 0.671, 1.362 | 0.744 | 0.982 | 0.699, 1.380 | 0.892 |
| IL1Ra | 11.93 (67.97) | 1.168 | 0.802, 1.700 | 0.287 | 1.219 | 0.908, 1.637 | 0.083 |
| IL1α | 51.67 (122.07) | 1.415 | 0.976, 2.051 | 0.016 | 1.521 | 1.045, 2.212 | 0.004 |
| IL1b | 10.63 (51.41) | 0.955 | 0.754, 1.209 | 0.614 | 1.022 | 0.820, 1.274 | 0.797 |
| IL6 | 117.3 (536.12) | 1.101 | 0.795, 1.525 | 0.446 | 1.147 | 0.857, 1.536 | 0.225 |
| IP-10 | 279.7 (577.57) | 4.587 | 1.064, 19.769 | 0.007 | 4.233 | 1.021, 17.547 | 0.009 |
| TNFα | 23.02 (85.85) | 0.977 | 0.772, 1.236 | 0.801 | 1.023 | 0.788, 1.330 | 0.820 |
| Total IgG | 68.78 (16.21) | 0.640 | 0.388, 1.055 | 0.021 | 0.590 | 0.342, 1.018 | 0.013 |

SD; standard deviation

Figure 1. Twenty five individuals diagnosed with active TB disease had between 1 and 4 samples taken prior to, and at point of TB diagnosis (total case samples n=51). Black markers represent a sample.

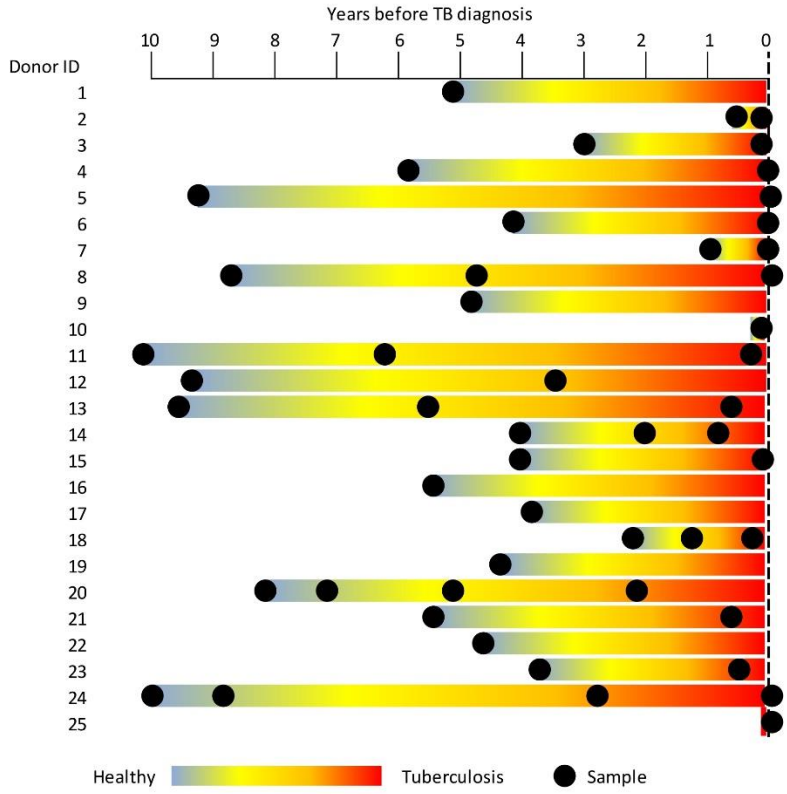

Figure 2 – Odds of TB disease by HCMV IgG. Results shown are from a conditional logistic regression model. HCMV low is used as the reference category. HCMV low n=102, HCMV medium n=102 and HCMV high n=103. Horizontal lines represent 99% CI.