HIV-1 Disease Progression and Survival in an Adult Population in Zimbabwe: Is There an Effect of the Mannose Binding Lectin Deficiency?

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Abstract

HIV infection remains a major global health burden since its discovery in 1983. Sub-Saharan Africa is the region hardest hit by the HIV/AIDS pandemic where 63% of the 33 million infected people live. While there is marked person-to-person variability in susceptibility, progression, and survival with HIV infection, there is a paucity of predictive diagnostics associated with these clinical endpoints. In this regard, the deficiency in plasma Mannose Binding Lectin (MBL) is a common opsonic defect reported to increase susceptibility infections, including HIV. To the best of our knowledge, we report here the first study on the putative role of MBL deficiency on HIV progression and survival in an African adult population. We hypothesized that MBL deficiency has a role to play in HIV infection by increasing HIV disease progression and decreasing survival. We assessed the role of MBL deficiency on HIV disease progression and survival in a Zimbabwean adult population enrolled in the Mupfure Schistosomiasis and HIV (MUSH) cohort. We analyzed blood samples for MBL levels, *MBL2* genotypes, HIV-1 status, viral load, and CD4⁺ T cell counts. Participants were followed for 3 years wherein the endpoints were measured at baseline, 6 weeks, and 3, 6, 12, 24, and 36 months. Disease progression was measured as the rate of decline in CD4⁺ T cell counts and the rate of increase in HIV viral load. We assessed 197 HIV positive adults where 83% (164) were women with a median age of 31 years. Prevalence of plasma MBL deficiency (less than 100 µg/L) and *MBL2* deficient genetic variants (*A/O* and *O/O* genotypes) was 21% (42 out of 197) and 39% (74 out of 190), respectively. We did not observe a significant role to explain individual variation in mortality, change of CD4⁺ T cell count, and viral load by MBL plasma deficiency or *MBL2* genetic variants from baseline to 3 years follow up period in this adult population. We suggest the need for global OMICS research and that the present findings attest to the large between-population variability in a host of factors that can predispose individuals susceptible to HIV progression and mortality. We therefore cannot recommend at this time the use of plasma MBL levels or *MBL2* genetic variants as a prognostic marker in HIV infection, disease progression, and survival in this adult population in Africa.

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Introduction

SUB-SAHARAN AFRICA IS THE REGION hardest hit by the HIV/AIDS pandemic where 63% of the 33 million infected people live (UNAIDS, 2012). HIV infection has remained a major public health challenge since its discovery in 1983 (Gallo et al., 1983). Several reports indicate that individuals worldwide differ in their susceptibility to HIV infection and it is widely agreed that genetic polymorphisms in the host genes that are important in immune regulation, can influence the risk and progression of HIV infections (Cao Qin et al., 1995; Dzwonek et al., 2006; Israels et al., 2012; Tang and Kaslow, 2003). There has been growing interest in genomics research in recent years in Africa due to the realization that diseases are influenced by both genetic and social factors (Dandara et al., 2014). Knowledge translation and multi-omics research are sorely needed within the real-life context of the African continent (Ahmed et al., 2014; Bosch et al., 2014; Dandara et al., 2014; ElRakaiby et al., 2014).

Mannose Binding Lectin (MBL), an important constituent of the innate immune system, has been widely investigated in different populations to determine association between MBL deficiency and susceptibility to several other infections (Antony et al., 2013; Garred et al., 1997; 2003; Hu et al., 2010; Zinyama-Gutsire et al., 2015) and the role of MBL deficiency in HIV disease progression (Boniotto et al., 2000; Catano et al., 2008; Garred et al., 1997; Hundt et al., 2000; Maas et al., 1998; Mangano et al., 2008; Prohaszka et al., 1997; Senaldi et al., 1995; Tan et al., 2009; Vallinoto et al., 2008) and survival (Garred et al., 1997; Maas et al., 1998).

HIV disease progression is characterized by decline in CD4⁺ T cell count and increase in HIV viral load (Lyles et al., 2000; Mellors et al., 1995; 1996; Mellors et al., 1997; Nakagawa, 2014; Phillips et al., 1991; Sabin et al., 2000) eventually leading to death in the absence of treatment (Lyles et al., 2000; Mellors et al., 1995; 1996; 1997; Nakagawa, 2014; Phillips et al., 1991).

Available literature on the association between MBL deficiency and disease progression is conflicting, some report faster HIV disease progression due to MBL deficiency (Garred et al., 1997; Hundt et al., 2000; Tan et al., 2009; Vallinoto et al., 2008) but others found no association (Catano et al., 2008; Nielsen et al., 1995; Pastinen et al., 1998; Senaldi et al., 1995). Others report association between MBL deficiency and decreased survival in HIV infected people (Garred et al., 1997) but conflictingly others reported increased survival in those with variant *MBL2* genotypes and MBL deficient individuals (Maas et al., 1998).

We hypothesized that MBL deficiency had a role to play in HIV infection by increasing HIV disease progression and decreasing survival. We therefore assessed the role of plasma MBL deficiency and *MBL2* genetic variants, on HIV-1 disease progression and survival in a Zimbabwean adult population enrolled in the Mupfure Schistosomiasis and HIV cohort (MUSH) cohort.

Materials and Methods

Study design

This was a sub-study of the Mupfure Schistosomiasis and HIV (MUSH) cohort, established between 2001 and 2007. The main aim of the MUSH cohort was to investigate im-

munological interactions between HIV-1 and schistosomiasis (Kallestrup et al., 2005a; 2005b; Zinyama-Gutsire et al., 2015). Details including screening procedures, the setting, and the study population have been described in detail elsewhere (Kallestrup et al., 2005a; 2005b).

Plasma and whole blood samples collected at baseline and the six follow-up time points were used for measurement of viral load and CD4⁺ T cell count. The MUSH study was approved by the National Research Ethics Committee of the Medical Research Council of Zimbabwe (MRCZ/A/918) and this sub-study was also approved separately (MRCZ/A/1770), in addition to having been approved by the University of Witwatersrand Human Research Ethics Committee (M130348). All individuals gave written informed consent for specimen storage and future laboratory studies. The participants were all naïve to treatment for HIV-1 infection as there were no anti-HIV drugs available in public hospitals in Zimbabwe at the time of sample collection.

Laboratory procedures

Participants were screened for HIV-1 as described earlier (Kallestrup et al., 2005a; 2005b). Briefly, HIV testing was performed confidentially and pre- and post-test counselling was provided in the participants' native language (Shona) by qualified medical personnel. Initially, a rapid HIV-1/2 test kit was used on a dry blood spot in the field (Determine, Abbott Laboratories, Tokyo, Japan). This test was followed by two different rapid HIV tests Oraquick (by Orasure) and Serodia (by Fujirebio) for all who tested positive initially. CD4⁺ T cell counts and HIV RNA at the six follow-up points were used for this analysis. CD4⁺ T cell counts were measured in whole blood (FacsCalibur, Becton Dickinson) and HIV RNA was measured in plasma using Roche Amplicor (F. Hoffmann la-Roche) at the Department of Haematology of Parirenyatwa Hospital Harare, Zimbabwe.

Viral load and CD4⁺ T cell counts were available at baseline, 6 weeks, and 3, 6, 12, 24, and 36 months follow-up for the 198 HIV positive participants. HIV disease progression was defined as the magnitude of decrease in CD4⁺ T cell counts and increase in viral load (Mellors et al., 1995; 1996; 1997). Participants were classified into three CDC categories, namely A, B, and C according to CDC classification (CDC 1993; 1999). Category A were HIV positive but asymptomatic, category B showed HIV symptomatic conditions, and category C showed AIDS-indicator conditions (CDC 1993; 1999).

Plasma MBL concentration was measured in baseline plasma samples using the double enzyme immuno-assay (EIA) (Garred et al., 1999; Zinyama-Gutsire et al., 2015) using plasma stored at -20°C until analysis. The lower detection limit in the assay was 20 µg/L (Garred et al., 1997; 1999; Madsen et al., 1998; Zinyama-Gutsire et al., 2015). Plasma MBL concentration was categorized into normal (above 500 µg/L), intermediate (100 µg/L–500 µg/L) and deficient (below 100 µg/L) (Eisen et al., 2008; Egli et al., 2013; Garred et al., 1997; Kruse et al., 2002; Zinyama-Gutsire et al., 2015).

Genomic DNA was extracted from frozen peripheral blood mononuclear cells (PBMCs) collected at baseline using the standard salting out procedure (Miller et al., 1988). The *MBL2* genotypes and promoter region alleles were detected by allele-specific oligonucleotide PCR (ASO-PCR) where specific sequences were used for each allele (Madsen et al.,

1998; Steffensen et al., 2000; full details provided earlier (Zinyama-Gutsire et al., 2015).

Statistical analysis

All statistical analysis was done using Stata 11 statistical package (STATA Corp, Timberlake Consultants, Texas, USA). Model assumptions were checked graphically using residual and Kaplan-Meier plots. Viral load was included in the model as log-transformed units. We used Mann-Whitney and Kruskal-Wallis tests to compare plasma MBL concentrations according to two CD4⁺ T-cell count groups, above or below 350 cells/ μ L (Sabin et al., 2000), the level recommended by WHO then (2007) to initiate ART and viral load above or below the median viral load of this study population of 60,000 copies per mL.

Frequencies of the *MBL2* genotypes, alleles, and promoter region genotypes and haplotypes were obtained by direct gene counting. For *MBL2* genotype analysis, normal homozygous *MBL2* was denoted as A/A and the variant *MBL2* genotypes A/B, A/C, A/D were grouped together as A/O homozygous and compound homozygotes were grouped together as O/O (Supplementary Table S1; supplementary material is available online at www.liebertpub.com/omi). Conformation to the Hardy-Weinberg equilibrium was determined using the chi square test in the SHEsis online programme (Li et al., 2009; Shi and He, 2005).

Generalized Estimating Equations (GEE) models (COHERE, 2014; Hansen and Holmskov 1998; Ma et al., 2012; Martin et al., 2014) were used to compare rates of change of the CD4⁺ T cell count and viral load measurements over the 3-year follow-up period. The role of plasma MBL deficiency and *MBL2* genetic variants on survival over the 3-year period were estimated using the Cox proportional hazard models.

Regression analysis was used to test for interaction and confounding between MBL deficiency, *MBL2* genetic variance, age, and sex. We use the Wald Chi-square statistic to choose between full and nested models. All tests were conducted at 5% level of statistical significance.

Results

Baseline demographic characteristics of the main MUSH cohort are reported elsewhere (Kallestrup et al., 2005; 2006). The majority of the HIV-infected participants were females (83%), median age was 31 (27–29) years. The plasma MBL concentration was available for 197 HIV positive individuals and *MBL2* genotyping results were available for 190 individuals. The median (interquartile range, IQR) plasma MBL concentration was 688 μ g/L (147–1904 μ g/L) among these HIV-1 positive individuals. Prevalence of plasma MBL deficiency (less than 100 μ g/L) and *MBL2* deficient variants (A/O and O/O genotypes) was 21% (42 out of 197) and 39% (74 out of 190) respectively. Plasma MBL concentration among these 197 HIV-1 infected individuals did not differ between participants in CDC categories A versus categories B/C combined, CD4 count groups below or above 350 cells/ μ L (Table 1) and viral load did not differ between the three plasma MBL groups ($p=0.867$) (Table 2).

Role of MBL deficiency in CD4 T cell count change

GEE models were used for disease progression analysis. In bivariate analysis, there was a significant change from

TABLE 1. COMPARISON OF MEDIAN PLASMA MBL CONCENTRATIONS AMONGST THE HIV-1 INFECTED INDIVIDUALS ACCORDING TO CDC HIV/AIDS DISEASE CATEGORIES AND CD4⁺ T CELL COUNT

<i>Variable</i>	<i>n (%)</i>	<i>Median (IQR)</i> <i>MBL levels μg/L</i>	<i>p-value</i>
<i>CDC category</i>			
A	132 (67)	688 (20–5792)	0.970
B and C combined	65 (33)	656 (20–3120)	
<i>CD4⁺ T cell count</i>			
Above 350 cells/μL	92 (47)	728 (20–5792)	0.468
Below 350 cells/μL	105 (53)	576 (20–3840)	

The concentration of MBL in plasma was measured in μ g/L. Differences in median plasma MBL concentration in the different infection status categories were analyzed by using nonparametric Mann-Whitney test.

baseline in CD4 to 359 cell/count ($p<0.001$) among those with normal MBL levels. The change in CD4 among those with intermediate and deficiency MBL levels were 37 ($p=0.338$) and 15 ($p=0.717$) cell/count less than those with normal MBL levels, however the difference was not significant at 5% level (Table 3). Similarly, plasma MBL deficiency levels category 100–500 μ g/L ($p=0.338$), *MBL2* structural variants A/O/O ($p=0.633$), *MBL2* deficient genotype XA/XA, YA/YO ($p=0.851$), MBL deficient genotype XA/YO, YO/YO ($p=0.705$), and promoter region variants LY*LX combined ($p=0.602$) had no role in change in CD4⁺ T cell count change over the 3-year follow-up period (Table 3).

We found a significant difference in the rate of change in CD4⁺ T cell count by sex, males had mean (95%, CI) CD4⁺ T cell decrease of –110 cells/ μ L (–197–22, $p=0.014$) less than that of females. Multivariate analysis also showed MBL deficiency had no role in change in CD4⁺ T cell count and viral load (Table 3). There was insufficient evidence at 5% level to explain change of CD4⁺ T cell count by MBL plasma deficiency and *MBL2* genetic variants from baseline to 3-year follow-up period.

Role of MBL deficiency in change in viral load

In bivariate analysis, there was a significant change from baseline in viral load to 4.58 ($p<0.001$) among those with normal MBL levels. The changes in viral load among those

TABLE 2. COMPARISON OF VIRAL LOAD ACCORDING TO PLASMA MBL CONCENTRATIONS AMONGST THE HIV-1 INFECTED INDIVIDUALS

Variable	n	Median (IQR) viral load copies per mL	p-value
Normal plasma MBL levels	113	43250 (12700–192750)	0.867
Intermediate plasma MBL levels	41	49000 (12800–193750)	
Deficient plasma MBL levels	43	65000 (14350–157500)	
Total	197		

Viral load was measured in copies per mL. Differences in median viral load in the three plasma MBL categories shown above were analyzed using nonparametric Kruskal-Wallis test.

TABLE 3. FACTORS ASSOCIATED WITH CHANGE IN CD4⁺ T CELL COUNTS FROM BASELINE TO 3 YEAR FOLLOW-UP PERIOD

Factor	n	Parameter Estimate (cell counts)	95% CI	P value
<i>Plasma MBL level</i>				
Normal plasma MBL level > 100 µg/L (reference)	113	359	317–403	<0.001
MBL intermediate level 100–500 µg/L	43	–37	–114–39	0.338
MBL deficient level < 100 µg/L	41	–15	–99–68	0.717
<i>MBL exon 1 genotype</i>				
MBL normal genotypes A/A	116	356	312–400	0.000
MBL deficient genotypes A/O and O/O combined	74	–17	–87–53	0.633
<i>MBL haplotypes</i>				
MBL normal haplotypes (YAYA/YAXA)	110	353	309–396	0.000
1.MBL deficient genotype (XA/XA, YA/YO) (intermediate levels)	72	–6	–206–139	0.851
2.MBL deficient genotype XA/YO, YO/YO (deficient levels)	8	–33	–206–139	0.705
<i>MBL promoter region genotypes</i>				
MBL normal promoter region H/Y	14	318	196–440	0.000
MBL promoter variants LY/LX	176	33	–93–160	0.602
<i>Age at t0</i>				
Below 25 years old	21	429	327–531	0.000
Above 25 years old	177	–90	–198–16	0.097
<i>Sex</i>				
Female	16	367	331–403	0.000
Male	34	–110	–197–22	0.014

Change in CD4⁺ T cell count (cells/µL) over the 3 year follow-up period. Reference plasma MBL levels is normal levels above 500 µg/L. Reference *MBL2* genotype is normal wild type genotype A/A and YAYA/YAXA. Reference MBL promoter region genotype is H/Y. Reference age was below 25 years old. Reference sex was females.

with intermediate and deficiency MBL levels were 0.19 ($p=0.221$) and 0.02 ($p=0.892$) log₁₀ copies/mL more than those with normal MBL levels; however the difference was not significant at 5% level (Table 4). The mean (95%, CI) increase in viral load among those with plasma MBL defi-

ciency less than 100 µg/L was 0.02 log₁₀ copies/mL (–0.31–0.35, $p=0.892$) compared to those with normal plasma MBL levels (Table 4).

Similarly, plasma MBL deficiency levels 100–500 µg/L ($p=0.221$), *MBL2* structural variants AO/OO ($p=0.847$),

TABLE 4. FACTORS ASSOCIATED WITH CHANGE IN VIRAL LOAD FROM BASELINE TO 3 YEARS FOLLOW-UP PERIOD

Factor	n	Parameter Estimate viral load	95% CI	P value
<i>Plasma MBL level</i>				
Normal plasma MBL level > 100 µg/L (reference)	113	4.58	4.41–4.75	0.000
MBL intermediate level 100–500 µg/L	43	0.19	–0.11–4.90	0.221
MBL deficient level < 100 µg/L	41	0.02	–0.31–0.35	0.892
<i>MBL2 exon 1 genotype</i>				
MBL normal genotypes A/A	116	4.63	4.45–4.79	0.00
MBL deficient genotypes A/O and O/O combined	74	–0.027	–0.30–0.24	0.847
<i>MBL2 haplotypes</i>				
MBL normal haplotypes (YAYA/YAXA)	110	4.62	4.44–4.79	0.000
MBL deficient genotype (XA/XA, YA/YO) (intermediate levels)	72	–0.01	–0.28–0.25	0.911
MBL deficient genotype XA/YO, YO/YO (deficient levels)	8	0.027	–0.66–0.72	0.937
<i>MBL2 promoter region genotypes</i>				
MBL normal promoter region H/Y	14	4.67	4.21–5.14	0.000
MBL promoter variants LY/LX	176	–0.067	–0.55–0.41	0.785
<i>Age at t0</i>				
Below 25 years old	21	4.49	4.10–4.88	0.000
Above 25 years old	177	0.15	–0.26–0.57	0.463
<i>Sex</i>				
Female	16	4.54	4.40–4.69	0.000
Male	34	0.471	0.13–0.81	0.006

Change in viral (load log₁₀ scale) over the 3 year follow-up period. Reference plasma MBL levels is normal levels above 500 µg/L. Reference *MBL2* genotype is normal wild type genotype A/A and YAYA/YAXA. Reference *MBL2* promoter region genotype is H/Y. Reference age was below 25 years old. Reference sex was females.

FIG. 1. Kaplan Meier plot of the 197 HIV-1 infected participants by plasma MBL concentration. Survival time was calculated from baseline data to death or upon censoring. Participants were subdivided into three groups according to plasma MBL concentration, normal levels (above 500 $\mu\text{g/L}$), reduced/intermediate (100–500 $\mu\text{g/L}$), and deficient (below 100 $\mu\text{g/L}$). One hundred and thirty four (113) participants had normal plasma MBL concentrations, 41 had reduced/intermediate levels, and 43 had deficient levels. Log Rank test 0.1129.

MBL deficient genotype *XA/XA*, *YA/YO* ($p=0.911$), MBL deficient genotype *XA/YO*, *YO/YO* ($p=0.937$), and promoter region variants *LY*LX* combined ($p=0.785$) had no role in change in viral load over the 3-year follow-up period (Table 4). However, we found a significant difference in increase in viral load between males and females, mean log (95% CI) decrease in viral in males was 0.471 log₁₀ copies/ml (0.131–0.81, $p=0.014$) ($p=0.006$) greater than in females. Multivariate analysis also showed MBL deficiency had no role in change in both CD4⁺ T cell count and viral load (results not shown).

Analysis of mortality in this study population

Participants in the main MUSH cohort were followed up for a total of 631 person-years with a median follow-up time of 3.6 years with 58 deaths, 32 due to respiratory illness, 12 unknown causes, and one suicide, as reported in more detail earlier (Erikstrup et al., 2007). The rate of death was 29% (58 out of 197) among the HIV infected compared to 1% (2 out of 181) among HIV-negative participants.

Survival analysis in this study population

Analysis was done only among the 197 HIV-1 positive participants to determine the effect of plasma MBL concentration and *MBL2* polymorphism in the *MBL2* coding and

promoter region, on survival. There was no difference in rate of death amongst the HIV-positive participants according to plasma MBL concentration when participants were divided into three plasma MBL concentration groups normal ($n=113$), intermediate ($n=41$) and deficient levels ($n=43$) (Fig. 1, Kaplan Meier plot, Log Rank test 0.1129) and according to MBL levels quartiles (Supplementary Fig. S1, Kaplan Meier plot, Log Rank test 0.2916). There was also no difference in rate of death, measured as time to death, between those with normal *MBL2* genotype compared to those with the combined *MBL2* variants (Fig. 2, Kaplan Meier plot, Log Rank test 0.263).

The survival of those with the homozygous *XA/XA* promoter type, which is a low expression promoter, was compared to those with the *A/A*, *A/O*, and *O/O* genotypes. There was no difference in rate of death when *XA/XA* was compared to individuals with normal and variant *MBL2* genotypes, (Supplementary Fig. S2, Kaplan Meier plot Log Rank test 0.4175). It is known that individuals can have one or two or none *MBL2* X promoter. Analysis was done in the homozygous wild-type *A/A* genotype group to compare the dose-dependent effect of the number of X promoters on survival among the HIV-positive participants. There was no difference in rate of death between those with 1, 2, or none X promoter (Fig. 3, Kaplan Meier plot, Log Rank test 0.6125).

FIG. 2. Kaplan Meier plot of HIV-1 infected participants comparison by *MBL2* genotype. Survival time was calculated from baseline data to death or upon censoring. One hundred and sixteen participants (116) had normal *A/A* *MBL2* genotype, 66 had *A/O* variant *MBL2* genotype, and 8 had *O/O* genotype. Log rank test 0.263.

FIG. 3. Kaplan Meier plot showing the comparative dose-dependent effect of number of XA haplotypes on survival in the normal A/A *MBL2* genotype group only, within the HIV positive stratum. Log rank test 0.6125. Zero X $n=65$, One X $n=41$, Two X $n=8$.

The *MBL2* haplotypes were further combined and subdivided into three groups, namely haplotypes that give normal plasma *MBL2* levels (YA/YA, YA/XA), intermediate levels (XA/XA, YA/YO), and deficient levels (XA/YO, YO/YO), showed no differences in rate of death (Fig. 4, Kaplan Meier plot, Log Rank test 0.3176).

Cox regression models, adjusted for gender and age, were fitted to determine if MBL deficiency predicted mortality amongst the HIV positive. For this analysis, participants were subdivided into two groups for each variable (Table 5). MBL concentration ($p=0.72$), *MBL2* genotype ($p=0.69$), and *MBL2* promoter haplotypes ($p=0.58$) were all not predictors of mortality in this study amongst those who were HIV positive (Table 5). The other predictors of mortality for these participants were reported earlier (Erikstrup et al., 2007; 2008) such as low CD4⁺ T-cell count, viral load level, hemoglobin, and CDC category.

In addition, we found no correlation between plasma MBL concentration and the CD4⁺ T-cell counts (Spearman's $\rho=0.0275$, $p=0.702$). Similarly, there was no correlation between plasma MBL concentration and plasma HIV-1 RNA (Spearman's $\rho=0.01$, $p=0.822$). Also CD4⁺ T cell count (Table 6, χ^2 test $p=0.962$, Fig. 5) and plasma viral load (χ^2 test $p=0.675$, Fig. 5) did not differ according to *MBL2* genotype.

Discussion

To our knowledge, this is the first study in adults to investigate role of MBL deficiency on disease progression and survival in an adult African population. Our results showed that plasma MBL deficiency, low producer *MBL2* variant genotypes, and *MBL2* promoter region variants played no role in change in CD4⁺ T cell, change in viral load, disease progression, and survival in this ART-naïve adult population. Plasma MBL concentration was not a predictor of HIV status, CD4⁺ T cell count, and viral load.

Plasma MBL deficiency and *MBL2* genetic variants had no role in HIV disease progression in our study population defined as decrease in CD4⁺ T cell count and increase in viral load, in accord with other findings (Catano et al., 2008; Nielsen et al., 1995; Senaldi et al., 1995) but in contrast to other reports of a significant association (Garred et al., 1997; Hundt et al., 2000; Tan et al., 2009; Vallinoto et al., 2008). The studies that showed similar results to ours were done in the USA among those with European origins and African Americans (Catano et al., 2008), UK (Senaldi et al., 1995), and Denmark (Nielsen et al., 1995). Studies that showed contrasting results to ours were done in Denmark (Garred et al., 1997), China (Tan et al., 2009), Germany (Hundt et al., 2000), and Brazil (Vallinoto et al., 2008).

FIG. 4. Kaplan Meier plot of HIV-1 infected participants comparison by *MBL2* genotype. The *MBL2* haplotypes were further combined and subdivided into three groups, namely haplotypes that give normal plasma MBL levels (YA/YA, YA/XA), intermediate levels (XA/XA, YA/YO), and deficient levels (XA/YO, YO/YO). Survival time was calculated from baseline data to death or upon censoring. One hundred and ten participants (110) had (YA/YA, YA/XA) *MBL2* genotype, 72 had variant (XA/XA, YA/YO) *MBL2* genotype, and 8 had (XA/YO, YO/YO) genotype). Log rank test 0.3176.

TABLE 5. UNIVARIATE COX REGRESSION ANALYSIS FOR PREDICTORS OF MORTALITY AMONG THE HIV-POSITIVE PARTICIPANTS

Variables	n	HR	95% CI	p-value
<i>Plasma MBL levels</i>				
MBL normal	113	—		
MBL deficient level 100–500 µg/L	41	0.91	0.48–1.70	0.764
MBL deficient level < 100 µg/L	43	0.50	0.22–1.12	0.090
<i>MBL2 exon 1 genotype</i>				
MBL genotype A/A	116	—		
MBL genotype A/O + O/O (combined)	74	0.69	0.39–1.23	0.209
MBL promoter region variants				
MBL haplotypes H/Y	14	—		
MBL haplotypes LY/LX (combined)	176	0.58	0.18–1.86	0.362
<i>MBL2 haplotypes</i>				
YAYA/YAXA (normal MBL levels)	110	—		
XA/XA, YA/YO (intermediate levels)	72	0.69	0.38–1.24	0.215
XA/YO, YO/YO (deficient levels)	8	1.14	0.35–3.75	0.829

Participants were subdivided into groups for each variable listed above. Results are shown as hazard ratios and confidence intervals after controlling for both age and sex. Reference categories were normal plasma MBL levels and wild type *MBL2* genotype.

The possible reasons why our results are different to others who found an association between MBL deficiency and disease progression might be due to differences in characteristics of the different populations studied or might be due to differences in sample sizes used. Our sample size was 198 HIV-positive male and female participants compared to analysis in 96 HIV-positive homosexual men (Garred et al., 1997), 128 HIV positive adult males and females (Vallinoto et al., 2008), 1075 adult males and females (Tan et al., 2009), and only 6 long-term HIV positive non-progressors (Hundt et al., 2000).

Available literature on association between MBL deficiency and HIV disease progression is conflicting, with some reporting faster HIV disease progression among those with MBL deficiency (Garred et al., 1997; Hundt et al., 2000; Tan et al., 2009; Vallinoto et al., 2008) but others have reported no association (Catano et al., 2008; Nielsen et al., 1995; Senaldi et al., 1995). Presence of an association between MBL deficiency and decreased survival in HIV infected people has been reported (Garred et al., 1997), but conflictingly others reported increased survival in those with variant *MBL2* genotypes and MBL deficient individuals (Maas et al., 1998), where MBL deficiency was reported to confer protection.

In contrast to our findings of no association between HIV disease progression and MBL deficiency, other studies showed a higher median plasma MBL concentration in patients who progressed to AIDS compared to those who did not, indicating a protective role of MBL deficiency (Mangano et al., 2008). In addition, in our study population plasma MBL

concentration was not a predictor of HIV-1 status, CD4⁺ T cell count, or viral load, similar to other reports (Catano et al., 2008), but in contrast to other findings (Vallinoto et al., 2008).

Important to note is that even though plasma MBL levels can be categorized into normal, intermediate, and deficient levels according to *MBL2* genotype, evidence in literature shows that there is a wide variation in plasma MBL levels by up to 1000-fold regardless of genetic make-up (Madsen et al., 1995; 1998; Turner, 1996; Ytting et al., 2011). Our earlier results also showed this wide variation in plasma MBL levels despite genetic make-up (Zinyama-Gutsire et al., 2015).

Our results showed a significant decrease in CD4⁺ T cell count and viral load as also reported. The increases in CD4⁺ T cell count and decrease in viral load have been reported in our other publications (Kallestrup et al., 2005) where we reported that there was a significant increase in CD4⁺ T cell count and decrease in viral load after treatment of schistosomiasis. An improvement in the immune system was noted in this study population, even without HAART (Kallestrup et al., 2005).

Survival analysis was done within the HIV-1 infected stratum. Our results showed that MBL deficiency and *MBL2* genetic variants were not associated with increased rate of death, in contrast to findings of faster rate of death among HIV-infected participants with *MBL2* genetic variants (Garred et al., 1997) and among those with XA/XA *MBL2* promoter variant genotype (Catano et al., 2008). The absence of an association in our cohort might be due to analysis in a smaller cohort (379 with only 197 HIV-positive individuals) compared to another report from a significantly larger cohort

TABLE 6. COMPARISON OF MEDIAN CD4⁺ T CELL COUNT AND VIRAL LOAD AMONG THE HIV-1 INFECTED ACCORDING TO *MBL2* GENOTYPE

<i>MBL2</i> genotype	n	Median (IQR) CD4 ⁺ T cell count	p value	Median (IQR) viral load	p value
A/A	116	319 (11–1222)		65000 (20–1250 000)	
A/B	66	325 (52–1893)	0.962	40125 (74–1000 000)	0.675
O/O	8	301 (87–550)		86500 (4115–342 500)	

Differences in median CD4 T cell count and viral load according to *MBL2* genotype were analyzed by the Kruskal-Wallis test, *n* = 190.

FIG. 5. (A) CD4⁺ T cell count (cells/ μ L) according to genotype. (B) Viral load (copies per mL) according to *MBL2* genotype A/A: normal *MBL2* genotype, A/O: heterozygous and O/O: *MBL2* variant homozygous ($n = 190$).

of 3315 with 1102 HIV-positive participants (Catano et al., 2008).

Cox proportional hazards models showed plasma MBL deficiency, *MBL2* exon 1 region variants, and promoter region variants were not predictors of mortality in our study population. We found no differences in *MBL2* levels between CDC HIV categories, CD4⁺ T cell count, and viral load in our study, similar to findings by others (Senaldi et al., 1995), in contrast to other findings (Senaldi et al., 1995).

The role of MBL deficiency due to polymorphism in the *MBL2* gene has also been investigated in other infections, and is associated with development of pulmonary TB in younger patients (Hijikata et al., 2014), no role in hepatitis B (Osthoft et al., 2014; Xu, 2013), high MBL levels protective in schistosomiasis (Antony et al., 2013) and malaria (Garred et al., 2003; Luty et al., 1998).

The main limitation of our study was inclusion of few men in the main MUSH study (Kallestrup et al., 2005; 2006; Zinyama-Gutsire et al., 2015). There is a gold panning area in the neighboring district, most men work away from their rural homes, and were not available during our study recruitment phase. This has the potential to bias our findings, but every effort was done to enroll all eligible men into the main MUSH study. The results of our study cannot support recommendations to use *MBL2* polymorphism as a prognostic tool for

AIDS progression using CD4⁺ T cell counts and viral load as has been recommended by others (Vallinoto et al., 2008).

Conclusions

Available literature shows that many diseases are influenced by both genetic and social factors results that are relevant to African populations bearing a huge burden of viral, parasitic, and bacterial infections (Dandara et al., 2014). However, our results showed that plasma MBL deficiency, *MBL2* coding region variants, and *MBL2* promoter region variants had no role in change in both CD4⁺ T cell counts and viral load from baseline to 3-year follow up. We therefore cannot recommend use of plasma MBL levels and *MBL2* genetic variants as a prognostic marker in HIV infection, disease progression, and survival in this population.

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Author Disclosure Statement

No competing financial interests exist.

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