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Effects of Well-Controlled HIV Infection on Complement Activation and Function

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Abstract

INTRODUCTION—Uncontrolled HIV infection is known to activate the complement system, leading to an increase in chronic inflammation. Whether or not this activation of complement persists and contributes to chronic inflammation in subjects with HIV infection that is well-controlled through use of antiretroviral therapy has not been studied.

METHODS—We conducted an observational, cross-sectional study using sera from 305 adults with well-controlled HIV infection and 30 healthy controls. Sera was tested for markers of complement activation (C3a and C5a levels), complement function (CH50 assay), and immunoglobulin levels (IgG1-IgG4) as IgG can activate complement. We evaluated the association of well-controlled HIV infection with C3a, C5a, CH50, IgG1-IgG4, and total IgG levels using both univariate and multivariate analyses, controlling for factors such as age, gender race, comorbidities (including hepatitis C co-infection), smoking status, and statin use.

RESULTS—Well-controlled HIV infection was associated with a 54% increase in complement activation as measured by C3a levels compared with healthy controls ($p < 0.0001$). Hepatitis C co-infection was associated with a further 52% increase in complement activation, as measured by C3a levels, over HIV alone ($p = 0.003$).

CONCLUSION—These results suggest that complement activation may contribute to a pro-inflammatory state even in well-controlled HIV infection. Further, HCV-co-infection may be even more pro-inflammatory, in terms of complement activation, compared with HIV infection alone.

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Keywords

Complement; HIV; Hepatitis C; C3a; C5a; CH50

INTRODUCTION

HIV infection creates a chronic inflammatory state that improves but does not resolve when HIV is well-controlled through the use of antiretroviral therapy (ART)^{1–3}. This chronic inflammation has been linked to a 2–4 fold higher risk of mortality from non-AIDS defining events such as cardiovascular disease³. It has also been linked to metabolic disorders, bone disease, kidney disease, and neurocognitive dysfunction¹.

Untreated HIV infection is known to lead to complement activation through envelope proteins on the HIV virion⁴. In addition, untreated HIV infection produces elevated levels of IgG subclasses 1 and 3, the two IgG subclasses which are strongly complement activating, in contrast with subclasses 2 and 4, which are minimal activators of complement^{5–7}. The complement system is a critical regulator of inflammation with multiple effects including opsonization, elaboration of inflammatory signaling molecules, and cell lysis. Complement activation leads to formation of the anaphylatoxins C3a and C5a, which can stimulate numerous immune cells. C3 is the central component of the complement system and is present in high concentrations in human plasma (1 g/l)⁸. C3a results from cleavage of C3, leading to the generation of opsonins, C3b/iC3b, and terminal complement cascade activation. C3a can have pro- or anti-inflammatory functions in different situations⁹. C5a is an anaphylatoxin with strong pro-inflammatory effects, including an association with cardiovascular events¹⁰. To date, the role of complement activation in well-controlled HIV infection has not been studied.

In order to better understand the potential roles of immunoglobulin and complement in the chronic inflammation seen in well-controlled HIV infection, we compared the levels of C5a, C3a, IgG subclasses, and classical pathway complement function via the CH50 assay in serum from adults with well-controlled HIV infection versus uninfected controls.

METHODS

This was an observational, cross-sectional study conducted at the primary HIV clinic (C3ID) at Eastern Virginia Medical School (EVMS) in Norfolk, VA. We used stored serum and data from 305 adults with well-controlled HIV infection who participated in one of two prior studies conducted 2012–2014, and who consented that their excess serum and data could be used in future studies. Inclusion criteria for both prior studies included documented HIV infection, age ≥ 18 years, and HIV viral load <400 copies/ml on the most recent test¹¹. We also prospectively enrolled and collected serum and questionnaire data from 30 healthy controls who were 22–63 years old, not HIV-infected, not taking immunosuppressive medications, and not acutely ill. All healthy controls underwent informed consent. The EVMS Institutional Review Board approved the study.

On the day of collection, blood samples were centrifuged, and the serum aliquotted and stored at -80°C until laboratory analysis. All serum was extracted and stored by the same person using identical procedures and equipment to ensure continuity between samples. Serum was tested by six unique ELISA assays for the specific quantitative measurement of IgG 1- IgG 4 antibody levels (eBiosciences, San Diego, CA), C3a levels (eBiosciences, San Diego, CA), and C5a levels (R&D systems, Minneapolis, MN). Whole Complement Titration (CH50) was performed with the sera using Ab-sensitized sheep erythrocytes at 5×10^8 (CompTech, Tyler, TX) to test classical and terminal pathway complement function¹². Total IgG levels were calculated by adding the levels of IgG1-4.

We estimated that with a sample size of 305 subjects with well-controlled HIV infection, we would need to enroll 30 healthy controls to detect a 25% change in C5a levels between HIV-infected subjects and controls to achieve a statistical power of 80% at $\alpha = 0.05$.

Categorical variables were presented as percentages, and continuous variables were presented as means and standard deviations (SD). The primary outcomes included C3a levels, C5a levels, CH50 levels, IgG1 levels, IgG2 levels, IgG3 levels, IgG4 levels, and total IgG levels. The primary independent variable of interest was HIV infection. Covariates included in the analysis were age, gender, race, diabetes diagnosis, current smoking status, hepatitis C virus (HCV) coinfection, hepatitis B virus (HBV) coinfection, and current statin use. Among HIV-infected subjects only, covariates also included protease inhibitor use, atazanavir use, darunavir use, non-nucleoside reverse transcription inhibitor (NNRTI) use, integrase inhibitor use, tenofovir use, abacavir use, current CD4 count, history of AIDS diagnosis (defined as ever having a CD4 count < 200 cells/mm³ or ever having an AIDS-defining illness), and duration of HIV infection. In addition, for the outcomes of C3a levels, C5a levels, and CH50 levels, we analyzed the association of IgG1, IgG2, IgG3, IgG4, and total IgG levels as variables. To examine the association between the primary outcomes and variables of interest, we used linear regression for continuous variables, t-test for binary variables, and ANOVA for categorical variables with more than two choices. For multivariate analyses, we included the primary variable of interest (HIV infection) as well as variables that were significant on univariate analysis. A subanalysis was done excluding the HCV-coinfected subjects once it was determined that HCV co-infection was associated with a number of the outcomes. Two-sided statistical tests were conducted at $\alpha = 0.05$. Statistical analysis was performed using SAS version 9.3 (SAS institute, Cary, North Carolina).

RESULTS

Study Subjects

The demographics of the 305 adults with well-controlled HIV infection and the 30 healthy controls are shown in Table 1. A number of variables were significantly different between the two groups, including mean age, racial composition, smoking status, HCV infection status, and statin use.

Complement Activation as Measured by C3a Levels

HIV infection was associated with a 64% increase in C3a levels on univariate analysis ($p=0.001$), and showed a trend towards being increased on multivariate analysis ($p=0.06$). HCV co-infection appeared to strongly impact the association of C3a and HIV (Table 2, Figure 1). In the subanalysis excluding the 42 HCV co-infected subjects, HIV infection remained significantly associated with a 54% increase in C3a levels (7524 versus 4901 ng/ml, $p<0.0001$, in HIV-infected versus uninfected subjects, Figure 1).

Among the 305 subjects with well-controlled HIV infection (Table 3), HCV infection was the only covariate significantly associated with C3a levels, with HCV-infected subjects having a 52% increase in C3a levels ($p=0.03$). These results suggest that both HCV infection and well-controlled HIV infection are associated with increased complement C3 activation.

As all but one of the 30 healthy controls had C3a levels $<10,000$ ng/ml, we further investigated the 305 subjects with well-controlled HIV infection to assess whether the C3a level of 10,000 ng/ml is a natural breakpoint, and what levels $\geq 10,000$ ng/ml might correlate with. We found that C5a levels were significantly higher (83 ± 31 versus 71 ± 31 ng/ml, $p=0.01$), and IgG4 levels were significantly lower (252 ± 239 versus 382 ± 373 μ g/ml, $p=0.001$), in the 58 subjects with C3a $\geq 10,000$ versus the 247 subjects with C3a $<10,000$ ng/ml respectively. We also found that the proportions of subjects with HCV infection (24% versus 11%, $p=0.01$), protease inhibitor use (67% versus 45%, $p=0.002$), NNRTI use (41% versus 57%, $p=0.03$), integrase inhibitor use (33% versus 19%, $p=0.02$), and abacavir use (19% versus 9%, $p=0.03$) were significantly different by C3a levels $\geq 10,000$ versus $<10,000$ ng/ml respectively.

C5a Levels

A small (16%) decrease in C5a levels noticed on univariate analysis between HIV-infected versus uninfected subjects did not remain significant on multivariate analysis when age and HCV infection were included in the model (Table 2, Figure 1). In the subanalysis excluding the 42 HCV co-infected subjects, HIV status was the only variable significantly associated with C5a levels, with a similar small (14%) decrease in C5a levels in the HIV-infected subjects ($p=0.04$). Thus, as the change was very small and the association was in the opposite direction, the anaphylatoxin C5a does not appear to contribute to inflammation in well-controlled HIV infection.

Among the 305 subjects with well-controlled HIV infection (Table 3), HCV infection was the only covariate significantly associated with C5a levels, with HCV-infected subjects demonstrating a 15% lower C5a level ($p=0.03$). This small difference in C5a concentration is unlikely to be clinically relevant.

Complement Function as Measured by CH50 Levels

HIV infection was significantly associated with increased complement function as measured by CH50 levels on univariate analysis, but this did not remain significant on multivariate analysis when age, race, HCV infection, and statin use were included in the model (Table 2). Similarly, in the subanalysis excluding HCV-infected subjects, HIV infection was

significantly associated with increased CH50 levels on univariate analysis, but not on multivariate analysis when age, race, diabetes status, and statin use were included in the model.

Among the 305 subjects with well-controlled HIV infection (Table 3), black race and statin use were significantly associated with increased CH50 levels, and HCV infection was significantly associated with decreased CH50 levels. The clinical relevance of increased CH50 levels is unknown. However, decreased CH50 levels are likely due to decreased production or increased consumption of complement components. In agreement with HCV co-infection being associated with increased C3a levels, the CH50 results provide supportive evidence for increased complement activation leading to the consumption of complement components in the setting of HCV co-infection.

IgG Levels

As expected, HIV infection was significantly associated with higher IgG1, IgG3, and total IgG levels on univariate analysis, but this only remained significant for IgG3 levels on multivariate analysis (Table 2, Figure 1). This same pattern was seen when HCV co-infected subjects were excluded from the analysis.

Among the 305 subjects with well-controlled HIV infection (Table 3), history of AIDS diagnosis was significantly correlated with higher IgG1, IgG3, IgG4, and total IgG levels. Taking darunavir and black race were also significantly correlated with higher IgG1 and total IgG levels. CD4 count, statin use, and currently smoking were inversely correlated with IgG1, IgG3, and IgG4 levels respectively.

Correlation Between IgG Levels and Complement Tests

When the associations between IgG levels and C5a, C3a, or CH50 levels were examined, we found that IgG1 levels were significantly inversely related to CH50 levels on both univariate and multivariate analysis (r coefficient -0.12 , $p=0.03$ and 0.008 respectively). IgG4 was significantly related to C5a levels on univariate analysis, but the association did not remain significant on multivariate analysis (r coefficient 0.12 , $p=0.04$ and 0.06 respectively). This is consistent with IgG1 being a strong complement activator via the classical pathway and potentially leading to some depletion of complement components and hence lower CH50 levels.

DISCUSSION

We measured complement activation and function in 305 subjects with well-controlled HIV infection and 30 healthy controls, to assess whether well-controlled HIV infection is associated with complement activation, and by extension whether this might contribute to the chronic inflammatory state seen in subjects with well-controlled HIV infection. C3a data, indicative of activation of the central complement component and most important in assessing overall complement activation, was increased 1.5-fold for HIV-infected individuals. In contrast, C5a levels showed minimal difference (-16%) with HIV infection ($p=0.09$ in multivariate analysis), suggesting that this anaphylatoxin has little or no clinical impact on inflammation in well-controlled HIV infection. CH50 values are calculated as a z-

score, limiting the sensitivity of this measure unless there are very large amounts of complement component consumption or decreased component production (e.g. C6 deficiency). Other variables that were associated with complement activation and function included HCV infection, statin use, and race.

A number of studies have demonstrated an association between untreated HIV infection and complement activation, along with a decrease in available uncleaved complement factors and thus complement function^{13–17}. Untreated HIV infection is believed to activate the classical pathway both through HIV-specific antibodies binding the HIV virions, and by the HIV surface protein gp41 binding C1q¹⁸. In addition, the HIV surface protein gp120 can bind mannose-binding lectin and activate the lectin pathway¹⁸. However, to our knowledge, only one prior study on complement activation in HIV-infected subjects included any subjects on ART¹⁹. This study took place in Gabon, and included 40 HIV-infected subjects on and 46 subjects off ART, as well as uninfected controls. They found that HIV infection was associated with complement activation both in asymptomatic and septic subjects, but that complement activation correlated with HIV viral load¹⁹. Our study included much larger numbers of HIV-infected subjects on ART, all with HIV viral loads <400 copies/ml, and suggests that even in the setting of well-controlled HIV infection, there is significant activation of the central complement component C3. This suggests that even well-controlled HIV infection is associated with complement activation, which may be contributing to a pro-inflammatory state.

Our data show significant elevation of C3a levels without a corresponding increase in C5a levels in the HIV-infected cohort. However, HIV-infected subjects with C3a levels >10,000 ng/ml had significantly elevated C5a levels compared to HIV-infected subjects with C3a levels <10,000 ng/ml. Despite the cascade nature of complement, it has been shown that C3a and C5a levels do not correlate for a variety of diseases including intestinal ischemia reperfusion²⁰, sepsis²¹ and cystic fibrosis²². C3a and C5a have described receptors including, C3aR, C5aR1 and C5aR2 that modulate different effects, although there can be some crosstalk where C3a-desArg may also bind to C5aR2 and mediate downstream pro-inflammatory effects without generation of C5a²³. It has been shown that C5a may increase the susceptibility of monocyte derived macrophages to HIV infection²⁴. Our data suggest this may be attenuated in well-controlled HIV infection. We speculate that in well-controlled HIV, C3b may be rapidly converted to its inactivated isoform iC3b, preventing further propagation of the terminal complement cascade and its downstream effects²⁵.

In addition to HIV infection by itself being associated with C3 activation, HCV co-infection was associated with a further increase in C3 activation, as evidenced by a 1.5-fold increase in C3a which was significant on both univariate and multivariate analysis. This was supported by decreased CH50 levels in HCV coinfection, suggesting complement component consumption as a result of increased complement activation. To our knowledge, this is the first report of HCV co-infection with HIV being associated with increased complement activation. A previous report showed lower serum C3 and C4 levels in HCV-infected subjects compared with uninfected controls²⁶, potentially reflecting complement activation and consumption or decreased component synthesis in the liver. Additionally, it has been shown that elevated C3a in HCV-infected patients is associated with the

development of hepatocellular carcinoma²⁷. Thus, the association of HCV co-infection in subjects with well-controlled HIV infection with elevated complement activation appears to suggest a pro-inflammatory state.

Well-controlled HIV infection was associated with increased levels of IgG1, IgG3, and total IgG, but this only remained significant for IgG3 on multivariate analysis. Our findings are consistent with a number of studies that have demonstrated that uncontrolled HIV infection is associated with increased IgG1, IgG3, and total IgG levels^{5,6,28}. IgG1 and total IgG levels have been shown to markedly decrease in HIV-infected subjects after ART initiation²⁸. As our subjects were all on ART with well-controlled HIV, it makes sense that the increased levels of IgG1, IgG3, and total IgG were not as marked as in studies of subjects with uncontrolled HIV. Our finding that a diagnosis of AIDS was significantly associated with higher levels of three of the four IgG subtypes is consistent with a prior study showing that advanced HIV infection is associated with elevated levels of IgG2 and IgG4 in addition to IgG1 and IgG3⁵, and suggests that these elevated levels may persist even after the HIV is well-controlled.

We found that being on a lipid-lowering drug of the statin class was associated with significantly increased CH50 levels and decreased levels of IgG3. Statin drugs are known to have anti-inflammatory properties, and have been shown to decrease complement activation in a mouse model²⁹. One could hypothesize that the increased complement function could be a result of less complement activation and thus more available uncleaved complement factors. However, our study did not see an association between statin use and C5a or C3a levels, our measures of complement activation.

We found race was associated with increased CH50 levels, and increased levels of IgG1 and total IgG. There is very little data on the effect of race on complement activation and function. We found one study from 1975 in 163 infants and children that noted no difference by race of levels of CH50, C3, C4, or C5³⁰. However, consistent with our findings, black race has been linked to increased IgG levels in prior studies³¹.

Our finding that currently smoking was associated with decreased levels of IgG4 is consistent with prior literature that showed an association of smoking with decreased IgG levels³¹. Our finding that subjects taking darunavir versus other antiretroviral agents had higher levels of IgG1 and total IgG was surprising. To our knowledge, there is no prior data in the literature on the effect of darunavir on IgG levels.

Our study has several limitations. Demographics of our HIV-infected subjects and uninfected subjects were significantly different, which reflects real life, but makes teasing out the contribution of HIV infection more difficult. However, to account for this, we conducted multivariate analysis to control for demographic differences that influenced our outcomes, and we did a subanalysis excluding the HCV co-infected subjects. In addition, it would have been ideal to also include subjects with uncontrolled HIV-infection in the study, but we were unable to do this because of budget constraints.

Despite these limitations, we are the first to evaluate complement activation in the setting of well-controlled HIV infection. Well-controlled HIV infection was associated with increased

complement activation as measured by C3a levels in our study. HCV co-infection was associated with a further increase in complement activation, suggesting a pro-inflammatory state for patients with both HCV and HIV infection, even when their HIV infection is well-controlled. The clinical importance of this finding deserves further investigation.

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Figure 1.

Mean Levels of C3a, C5a, IgG1, and IgG3 by HIV and HCV status; HIV=human immunodeficiency virus; HCV=hepatitis C virus. Lines represent mean and bars represent standard deviation. Of note, all HIV-infected subjects had viral loads <400 copies/ml.

Table 1

Demographics of the Study Subjects

Variables	Legend	HIV +	HIV –	p Value
Study Population	(freq, %)	305 (91)	30 (9)	-
Age in years	(mean ± SD)	45.2 ± 11.1	34.8 ± 10.9	<0.0001
Gender	(freq, %)			0.07
Male		212 (70)	16 (53)	
Female		93 (30)	14 (47)	
Race	(freq, %)			<0.0001
White		95 (31)	26 (86)	
Black		208 (68)	2 (7)	
Other		2 (0.7)	2 (7)	
Diabetic	(freq, %)			0.06
No		273 (90)	30 (100)	
Yes		32 (10)	0	
Current smoker	(freq, %)			<0.0001
No		176 (58)	29 (97)	
Yes		129 (42)	1 (3)	
Hepatitis C	(freq, %)			0.03
No		263 (86)	30 (100)	
Yes		42 (14)	0	
Hepatitis B	(freq, %)			0.2
No		289 (95)	30 (100)	
Yes		16 (5)	0	
Statin Use	(freq, %)			0.03
No		233 (76)	28 (93)	
Yes		72 (24)	2 (7)	
Current CD4 Count in cells/mm ³	(mean ± SD)	638 ± 326	NA	
Duration of HIV Infection	(mean ± SD)	11 ± 8	NA	
History of AIDs Diagnosis	(freq, %)		NA	
No		128 (42)		
Yes		176 (58)		

Variables	Legend	HIV +	HIV –	p Value
On Protease Inhibitor	(<i>freq</i> , %)		NA	
No		153 (50)		
Yes		149 (49)		
On Integrase Inhibitor	(<i>freq</i> , %)		NA	
No		237 (78)		
Yes		65 (22)		
On NNRTI	(<i>freq</i> , %)		NA	
No		138 (45)		
Yes		164 (55)		

HIV = Human immunodeficiency Virus; *Freq* = frequency; SD = standard deviation; NNRTI=Non-Nucleoside Reverse Transcriptase Inhibitors

Table 2

Association of Well-Controlled HIV Infection with Complement Activation and Function and IgG Levels

	HIV + (mean $\pm$ SD)	HIV – (mean $\pm$ SD)	Unadjusted <i>p</i> value	Adjusted <i>p</i> value
C5a (ng/mL)	73 $\pm$ 31	87 $\pm$ 33	0.02	0.09
C3a (ng/mL)	8059 $\pm$ 7632	4901 $\pm$ 2240	<0.0001	0.06
CH50 (U/mL)	46 $\pm$ 8	42 $\pm$ 6	0.0006	0.2
IgG1 (μ g/mL)	8811 $\pm$ 5942	6760 $\pm$ 3268	0.004	0.5
IgG2 (μ g/mL)	1960 $\pm$ 1278	2361 $\pm$ 1330	0.10	
IgG3 (μ g/mL)	1955 $\pm$ 984	1433 $\pm$ 660	0.0003	0.04
IgG4 (μ g/mL)	357 $\pm$ 356	481 $\pm$ 296	0.07	0.2
Total IgG (μ g/mL)	13082 $\pm$ 6891	11035 $\pm$ 3906	0.02	0.6

Covariates were included in the multivariate model if they were significant on univariate analysis. Multivariate models included age and HCV infection for C5a; HCV infection for C3a; age, race, HCV infection, and statin use for CH50; race for IgG1, nothing for IgG2 (no covariates were significant on univariate analysis); age and HCV infection for IgG3; smoking status for IgG4; and race for IgG total.

Table 3

Other Variables Associated with Complement Activation and Function and IgG Levels among Subjects with Well-Controlled HIV Infection

Variable	Mean $\pm$ SD	Unadjusted <i>p</i> value	Adjusted <i>p</i> value
C5a (ng/mL)			
Hepatitis C		$\dot{0}.03$	$\dot{0}.03$
No	75 $\pm$ 30		
Yes	64 $\pm$ 31		
C3a (ng/mL)			
Hepatitis C		$\dot{0}.03$	$\dot{0}.03$
No	7524 $\pm$ 6819		
Yes	11407 $\pm$ 11022		
CH50 (U/mL)			
Race		0.03	0.005
Black	47 $\pm$ 8		
White	44 $\pm$ 8		
Other	45 $\pm$ 6		
Hepatitis C		0.01	0.0009
No	47 $\pm$ 7		
Yes	42 $\pm$ 12		
Statin Use		<0.0001	0.0003
No	45 $\pm$ 8		
Yes	49 $\pm$ 7		
IgG1 (μ g/mL)			
Race		0.005	0.0007
Black	9566 $\pm$ 6212		
White	7178 $\pm$ 5012		
Other	7790 $\pm$ 3063		
Current CD4 count (continuous variable)		0.0009	0.01
On Darunavir		0.02	0.01
No	8220 $\pm$ 4733		
Yes	12164 $\pm$ 10214		
History of AIDs Diagnosis		<0.0001	0.003
No	7222 $\pm$ 3688		
Yes	9959 $\pm$ 6929		
IgG2 (μ g/mL)			
IgG3 (μ g/mL)			

Variable	Mean $\pm$ SD	Unadjusted <i>p</i> value	Adjusted <i>p</i> value
Age (continuous variable)		0.02	0.008
Statin Use		0.04	0.002
No	2020 $\pm$ 980		
Yes	1744 $\pm$ 975		
History of AIDs Diagnosis		0.01	0.02
No	1793 $\pm$ 868		
Yes	2071 $\pm$ 1047		
IgG4 (μ g/mL)			
Current Smoker		0.03	0.04
No	393 $\pm$ 391		
Yes	309 $\pm$ 295		
History of AIDs Diagnosis		0.03	0.04
No	307 $\pm$ 278		
Yes	394 $\pm$ 399		
Total IgG (μ g/mL)			
Race		0.02	0.004
Black	13852 $\pm$ 7031		
White	11437 $\pm$ 6361		
Other	11175 $\pm$ 3381		
On Darunavir		0.03	0.01
No	12476 $\pm$ 5822		
Yes	16435 $\pm$ 10938		
Current CD4 count (continuous variable)		0.003	0.04
History of AIDs Diagnosis		<0.0001	0.004
No	11322 $\pm$ 4651		
Yes	14356 $\pm$ 7908		

[†] only variable significant on univariate analysis.

Current CD4 count in cells/mm³ inversely correlated with IgG1 levels, and age in years directly correlated with IgG3 levels. Of note, smoking status, atazanavir use, and hepatitis C infection were significantly associated on univariate analysis with CH50, IgG1, and IgG3 levels respectively, but the significance did not remain on multivariate analysis.