

## Predictive Value of CD19 Measurements for Bacterial Infections in Children Infected with Human Immunodeficiency Virus

REBECCA A. BETENSKY,<sup>1\*</sup> THERESA CALVELLI,<sup>2</sup> AND SAVITA PAHWA<sup>3</sup>

Department of Biostatistics, Harvard School of Public Health, Boston, Massachusetts 02115<sup>1</sup>; Albert Einstein College of Medicine, Bronx, New York<sup>2</sup>; and North Shore University Hospital-New York University School of Medicine, Manhasset, New York 11030<sup>3</sup>

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We investigated the predictive value of CD19 cell percentages (CD19%) for times to bacterial infections, using data from six pediatric AIDS Clinical Trials Group protocols and adjusting for other potentially prognostic variables, such as CD4%, CD8%, immunoglobulin (IgA) level, lymphocyte count, prior infections, prior zidovudine treatment, and age. In addition, we explored the combined effects of CD19% and IgG level in predicting time to infection. We found that a low CD19% is associated with a nonsignificant 1.2-fold increase in hazard of bacterial infection (95% confidence interval: 0.97, 1.49). In contrast, a high IgG level is associated with a nonsignificant 0.87-fold decrease in hazard of infection (95% confidence interval: 0.68, 1.12). CD4% was more prognostic of time to bacterial infection than CD19% or IgG level. Low CD19% and high IgG levels together lead to a significant ( $P < 0.01$ ) 0.50-fold decrease in hazard (95% confidence interval: 0.35, 0.73) relative to low CD19% and low IgG levels. Similarly, in a model involving assay result changes (from baseline to 6 months) as well as baseline values, the effect of CD19% by itself is reversed from its effect in conjunction with IgG. In this model, CD19% that are increasing and high are associated with decreases in hazard of infection ( $P < 0.01$ ), while increasing CD19% and increasing IgG levels are associated with significant (at the  $P = 0.01$  level) fourfold increases in hazard of infection relative to stable CD19% and decreasing, stable, or increasing IgG levels. Our data suggest that CD19%, in conjunction with IgG level, provides a useful prognostic tool for bacterial infections. It is highly likely that T-helper function impacts on B-cell function; thus, inclusion of CD4% in such analyses may greatly enhance the assessment of risk for bacterial infection.

In AIDS Clinical Trials Group (ACTG) pediatric protocols, measurements of participating subjects' CD19 cell percentages (CD19%) of lymphocytes and CD19 cell counts are routinely collected. This was originally motivated by a hypothesis that CD19, possibly in conjunction with measurements of immunoglobulin, is predictive of time to bacterial infection and could serve as a surrogate marker for disease progression as well as treatment response. Here, we investigate this hypothesis, using combined data from six pediatric protocols: ACTG 051, 128, 138, 144, 152, and 190. These protocols were chosen because of their large numbers of subjects and long periods of follow-up relative to other pediatric ACTG protocols.

There are no reported investigations of the predictive value of CD19 for bacterial infections in the literature. It has been observed by several authors that hypergammaglobulinemia is a common and early abnormality observed in pediatric subjects infected with human immunodeficiency virus (HIV) (for examples, see references 8, 9, 11, and 12). In addition, polyclonal hypergammaglobulinemia occurs early in the disease in infected infants. One of the proposed mechanisms for the observed hypergammaglobulinemia is that HIV and its proteins are potent B cell activators (8). Additionally, B cell superantigen-like properties have been ascribed to HIV envelope protein gp120 (2). Despite the hypergammaglobulinemia specific antibody, responses to recall antigens and to new bacterial antigens are lost as the disease progresses (3). The relationship

of immunoglobulin levels (IgG and IgA) to B-cell numbers in the periphery is unknown. For this reason, we felt it would be useful to characterize B-cell phenotypes that could serve as surrogate markers for bacterial infection and for the assessment of response to treatment and their possible interactions with immunoglobulin levels.

Rodriguez et al. (12) found phenotypic differences in CD19 subsets between HIV-infected children and a control group. Specifically, they found a significantly lower median CD19<sup>+</sup> Leu8<sup>+</sup> cell count in P2 (i.e., symptomatic) children and a significantly lower median CD19<sup>+</sup> CD23<sup>+</sup> cell count in P1 (i.e.,

TABLE 1. Normal ranges for IgG and IgA levels and CD19% by age

Age (mo)	IgG level (mg/dl)	IgA level (mg/dl)	CD19%
0–1	250–1,000	5–25	
4–6	220–650	25–70	
6–12	350–1,000	25–125	
12–24	750–1,050	25–175	
24–48	500–1,400	35–330	
48–96	650–1,500	35–400	
96–168	750–1,650	75–500	
>168	750–1,775	80–550	
2–4			25–35
4–8			24–34
8–12			21–32
12–30			18–28
>30			18–25

\* Corresponding author. Mailing address: Department of Biostatistics, Harvard School of Public Health, 655 Huntington Ave., Boston, MA 02115. Phone: (617) 432-2821. Fax: (617) 432-2832. E-mail: betensky@hsph.harvard.edu.

TABLE 2. Grouping of subjects by CD19%, IgG, and IgA range<sup>a</sup>

Range	Total no. (%) of subjects		
	CD19%	IgG (mg/dl)	IgA (mg/dl)
Low	592 (47.7)	27 (2.2)	58 (4.7)
Normal	384 (30.9)	147 (11.9)	830 (66.9)
High	265 (21.4)	1,067 (86.0)	353 (28.4)

<sup>a</sup> Based on age-related normal ranges for baseline analysis (1,241 subjects).

asymptomatic) and P2 children relative to the control group. They suggested that the proportion of CD19<sup>+</sup> CD23<sup>+</sup> cells could serve as a marker of progression, although the mechanism for this is not clear. They hypothesize that the observed decrease in these cells in HIV-infected children is due to stem cell fatigue or the elimination of mature B cells.

Motivated by these preliminary findings, we investigated the usefulness of CD19% as a marker for disease progression in terms of its predictive value for time to bacterial infection. A practical goal of our analysis was to determine if there is any possible justification for continuing to routinely collect CD19 measurements on all ACTG pediatric studies. If CD19% alone is predictive of time to bacterial infection or if CD19% modifies the well-accepted predictive value of CD4 cell count for time to infection, we would consider routine measurement of CD19% to be justified. Otherwise, considerable savings (approximately \$30.00 per measurement) could result from stopping the practice of routine determination of CD19%. In addition, we tested the hypotheses that combined evaluation of B-cell count and IgG level is more useful as a predictive marker for bacterial infection than evaluation of either B-cell count or IgG level alone.

#### MATERIALS AND METHODS

The data from six ACTG pediatric protocols (ACTG 051, 128, 138, 144, 152, and 190) were combined for this analysis. These protocols were chosen because of their large numbers of subjects and long periods of follow-up relative to other pediatric ACTG protocols. The subjects in these trials ranged in age from 3 months to 18 years, and all had symptomatic HIV infections. All subjects were treated with zidovudine (ZDV) or dideoxyinosine or dideoxycytosine. Bacterial infections were the end points of interest, and they were defined for the purposes of this analysis to be any mycobacterium infection, bacterial pneumonia (non-*Pneumocystis carinii* pneumonia and non-lymphocytic interstitial pneumonia), bacteremia, meningitis, osteomyelitis, septic arthritis, acute mastoiditis, abscess of an internal organ, and cellulitis. Since we did not have access to culture information, we accepted presumptive diagnoses for all infections except bacteremia and meningitis, for which we required microbiological diagnoses. While

antibody is not known to play a major role in host defense against mycobacteria, these infections were included because they fall into the larger category of bacterial infections. In addition, mycobacterial infections were so few that their inclusion has minimal, if any, impact on the results.

In all of our analyses, for enumeration of B cells we used CD19% rather than absolute CD19 count. This is because absolute counts are overwhelmed by the steep age-related decreases in total lymphocyte counts. To assess the predictive value of CD19% for time to bacterial infection, we assumed a Cox proportional hazards model (6), in which the instantaneous hazard of bacterial infection is expressed as a function of explanatory variables. We took the time to bacterial infection to be censored by death as well as by the end of follow-up. We included in the model possible prognostic variables as well as variables which might affect CD19% and IgG level: age, gender, history of bacterial infection, prior usage of ZDV, treatment with IVIG or HIVIG, CD4%, CD8%, lymphocyte count, and IgA. We adjusted for prior usage of ZDV because of the possibility of its lowering of IgG levels and for treatment with IVIG or HIVIG because of its effect of increasing the IgG concentration in serum. We did not include current therapy as a possible prognostic variable, because the protocols that we used were randomized and several of them were still blinded as to treatment assignment at the time of analysis.

Averaged predicted time-to-infection curves are presented for some of the factors of interest. These are derived by estimating the baseline time-to-infection function from the relevant Cox proportional hazards model, with the estimated time-to-infection curve for each subject calculated by using the relationship  $P\{\text{survive beyond } t \text{ given covariates } Z\} = P\{\text{survive beyond } t \text{ given } Z = 0\}^{\exp(\beta Z)}$ , and averaging these predicted curves for individuals within each level of the factor of interest.

We assessed the assumption of proportional hazards by fitting proportional hazards models stratified on a covariate of interest and comparing the resulting log cumulative baseline hazard functions for each value of the covariate (5). Parallel log cumulative baseline hazard functions indicate that the assumption of proportional hazard with respect to that covariate is plausible. Based on viewing several plots, we could not reject the proportional hazards assumption.

We addressed the hypotheses of interest in two separate analyses. Both analyses began by including potentially prognostic variables available at baseline, including gender, prior ZDV treatment, prior bacterial infection, treatment with IVIG or HIVIG, age, CD4%, CD8%, lymphocyte count, and IgG and IgA levels. We did not attempt to find the best parsimonious model, because the question of interest in this analysis is whether CD19% adds any information after adjusting for what is routinely measured. We found the best models that included these prognostic variables by using a model selection process based on likelihood ratio tests described by Collett (5). We report *P* values and 95% approximate confidence intervals for the hazard ratios of infection associated with various levels of the prognostic variables.

The first analysis included only baseline immunologic measurements and assessed the predictive value of CD19% and its possible interaction with IgG levels. The second analysis included estimated slopes of the immunologic measures over the first 6 months of the study. This necessitated excluding subjects who went off the study or experienced a bacterial infection within the first 6 months. In this so-called landmark analysis, we tested hypotheses on the combined effects of CD19% and IgG level.

We used the tertiles of the distributions of the continuous variables to divide them into low, normal, and high levels. We also tried divisions of CD19% and IgG and IgA levels into low, normal, and high values based on a priori notions of age-related normal laboratory values. For example, if a 7-month-old child has an IgG measurement of 500 mg/dl, her IgG level would be classified as normal, if it were 200 mg/dl, her IgG level would be classified as low, and if it were 1,100

TABLE 3. Summary statistics for continuous covariates<sup>a</sup>

Variable	Baseline analysis					Landmark analysis				
	MIN	33.3%	66.7%	MAX	Median	MIN	33.3%	66.7%	MAX	Median
Age (mo)	2.9	21.0	60.0	218.4	37.1	3	21.4	60.4	218.4	38.2
CD19%	0	15	24	80	19	1	15.5	24	76	19
CD19% slope	NA	NA	NA	NA	NA	-2.5	-0.1	0.1	3.2	0.0
CD4%	0	16	26.5	66	21.5	0	17	27.5	54.4	22.3
CD4% slope	NA	NA	NA	NA	NA	-1.1	-0.04	0.2	1.9	0.04
CD8%	2	35.5	48	86	41	4.5	35.5	47	86	40.8
CD8% slope	NA	NA	NA	NA	NA	-1.6	-0.2	0.1	1.5	-0.03
Lym. count	20	284.4	1,058	12,792	455	19.6	325	1,599	12,640	569.3
Lym. slope	NA	NA	NA	NA	NA	-276	-7.1	0.2	292	-1.9
IgG level (mg/dl)	61	1,810	2,630	8,450	2,180	124	1,868	2,720	8,190	2,230
IgG slope (mg/dl)	NA	NA	NA	NA	NA	-250	-17	1.1	207.6	-8.4
IgA level (mg/dl)	1	113	255	2,720	174	7	116	257	2,720	175
IgA slope (mg/dl)	NA	NA	NA	NA	NA	-159.4	-1.0	0.7	45.1	-2.0

<sup>a</sup> The lower (33.3%) and middle (66.7%) tertiles are given. Abbreviations: MIN, minimum; MAX, maximum; Lym., lymphocyte; NA, not available.

TABLE 4. Summary statistics for categorical covariates

Covariate	Baseline analysis <sup>a</sup>		Landmark analysis <sup>b</sup>	
	Total (%)	No. of infections (%)	Total (%)	No. of infections (%)
Male	628 (50.6)	242 (50.4)	355 (51.0)	109 (52.2)
Female	613 (49.4)	238 (49.6)	341 (49.0)	100 (47.9)
Prior ZDV	392 (31.6)	166 (34.6)	132 (19.0)	50 (23.9)
Prior infection	520 (41.9)	195 (40.6)	303 (43.5)	61 (29.2)
Prior IVIG	354 (28.5)	152 (31.7)	170 (24.4)	59 (28.2)
ACTG 051	161 (13.0)	92 (19.2)	81 (11.6)	25 (12.0)
ACTG 128	268 (21.6)	111 (23.1)	197 (28.3)	56 (26.8)
ACTG 138	49 (3.9)	34 (7.1)	17 (2.4)	9 (4.3)
ACTG 144	96 (7.7)	56 (11.7)	51 (7.3)	20 (9.6)
ACTG 152	522 (42.1)	163 (34.0)	346 (49.7)	99 (47.4)
ACTG 190	145 (11.7)	24 (5.0)	4 (0.6)	0 (0.0)

<sup>a</sup> A total of 1,241 individuals and 480 infections.

<sup>b</sup> A total of 691 individuals and 209 infections.

mg/dl, her IgG level would be classified as high. Table 1 lists age-related normal ranges for CD19% and IgG and IgA levels, and Table 2 lists percentages of subjects that fall into low, normal, and high ranges of these measures by infection status. In the landmark analysis, we considered several possible divisions of the slopes of CD19%, CD4%, CD8%, IgG and IgA levels, and lymphocyte count into decreasing, stable, and increasing. One possible division is organized according to the tertiles of their distributions. According to this definition, a measure is decreasing for a particular subject if its estimated slope for that subject is less than that of at least 67% of the slopes, it is increasing if it is greater than at least 67% of the slopes, and it is stable otherwise. Another definition is based on a fixed percentage change from the baseline value. That is, a measure is decreasing for a particular subject if its estimated slope for that subject predicts a 6-month value that is less than 100*p*% of the baseline value, it is increasing if it predicts a 6-month value that is more than 100*p*% of baseline, and it is stable otherwise. We considered values of *p* of 0.1, 0.3 and 0.5.

## RESULTS

There were 1,241 subjects included in the baseline analysis, 480 of whom experienced bacterial infections, and there were 696 subjects included in the landmark analysis, 209 of whom experienced bacterial infections. Table 3 summarizes the distributions of age and various immunologic parameters for the subjects of each analysis. Note that the distributions are similar for both analyses, suggesting that the subjects in the subset included in the landmark analysis are not different from the entire population of subjects with respect to these variables. Table 4 lists summary statistics for demographic variables of

interest for each analysis and for the subpopulations from each analysis that experienced bacterial infections. Of note, about half of the subjects were female and about 40% had a prior bacterial infection. A difference between the subpopulation of subjects that were in the landmark analysis is their frequency of prior ZDV treatment; 19% of the landmark analysis subjects used ZDV prior to the analysis, whereas 31% of the baseline analysis subjects used ZDV prior to the analysis. Table 5 summarizes the distributions of infection times and censoring times for these populations.

**Baseline analysis.** In a model with no interaction terms, a low CD19% is associated with a modest and nonsignificant increase in hazard of bacterial infection ( $P = 0.09$ ; increase of 21%) over moderate or high CD19%. The other levels of CD19% do not individually add significantly to the model. The covariates in the model that are significantly associated with an increase in hazard of infection are prior ZDV treatment ( $P < 0.01$ ; increase of 68%), high lymphocyte count ( $P < 0.01$ ; increase of 42%), and low and moderate CD4% ( $P < 0.01$ ; increases of 99 and 37%, respectively), and those that are significantly associated with a decrease in hazard of infection are prior bacterial infection ( $P = 0.02$ ; decrease of 22%), oldest age group ( $P < 0.01$ ; decrease of 43%), and moderate lymphocyte count ( $P < 0.01$ ; decrease of 36%). High IgG levels are associated with a nonsignificant decrease in hazard of bacterial infection ( $P = 0.25$ ; decrease of 13%).

Figure 1 displays average predicted distributions of time to bacterial infection based on this model for subjects with low CD19% and for subjects with moderate and high CD19%. Figure 2 displays average predicted distributions of time to bacterial infection based on this model for subjects with high, moderate, and low IgG levels. For comparison, Fig. 3 displays average predicted distributions of time to bacterial infection for subjects with high, moderate, and low CD4%. Viewed together, these figures illustrate the relative prognostic values of CD19%, IgG level, and CD4%; namely, CD4% appears to be more prognostic of time to bacterial infection than CD19% alone or IgG level alone. This is supported by simple log rank tests comparing the groups defined by these prognostic variables ( $P = 0.195$  for CD19%,  $P = 0.066$  for IgG level, and  $P < 0.0001$  for CD4%).

We also considered entering IgG and IgA levels and CD19% into the model according to the age-related ranges given in Table 1. Because of the sparse numbers within the low IgG level and low IgA level groups, we combined low and moderate IgG levels and low and moderate IgA levels into single groups. When IgG and IgA levels and CD19% are discretized in this way, none of the levels of CD19% adds significantly to the

TABLE 5. Distributions of infection times and follow-up times

Time interval (mo)	Baseline analysis			Landmark analysis		
	No. of infections	No. of off-study subjects without infection	Probability of infection-free outcome (SE)	No. of infections	No. of off-study subjects without infection	Probability of infection-free outcome (SE)
0-6	203	55	0.836 (0.010)	NA <sup>a</sup>	NA	
6-12	95	87	0.756 (0.012)	70	28	0.899 (0.011)
12-18	66	89	0.693 (0.013)	47	32	0.829 (0.014)
18-24	42	95	0.648 (0.014)	31	73	0.779 (0.016)
24-30	43	137	0.593 (0.015)	33	122	0.717 (0.018)
30-36	18	142	0.561 (0.016)	17	102	0.670 (0.020)
36-42	9	101	0.531 (0.018)	9	91	0.628 (0.023)
42-48	4	51	0.495 (0.024)	2	37	0.597 (0.031)
48-54	0	4		0	2	

<sup>a</sup> NA, not available.

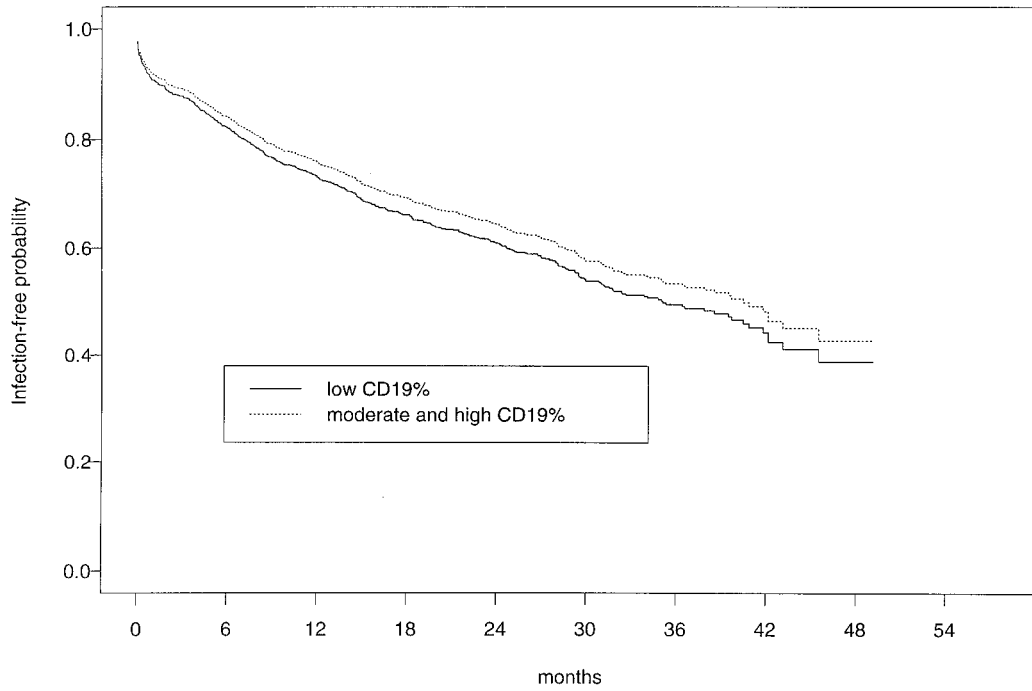


FIG. 1. Predictive time to bacterial infection based on baseline Cox model by CD19%.

model. This is true as well when age is discretized according to the divisions for IgG and IgA levels in Table 1, again with some groups collapsed because of low numbers. In addition, we removed age from the model to see if an age effect was obscuring an effect of CD19% in the age-related divisions of CD19%. However, CD19% did not add significantly to the model.

Next we considered interactions between level of IgG and CD19% (based on the tertiles of their distribution). Only the interaction between the highest level of IgG and the lowest of CD19% added significantly to the model ( $P < 0.01$ ). Thus, low CD19%, which independently was not significant in its effect, appears to decrease the hazard of infection when combined with high IgG level. Table 6 lists the hazard ratios and 95%

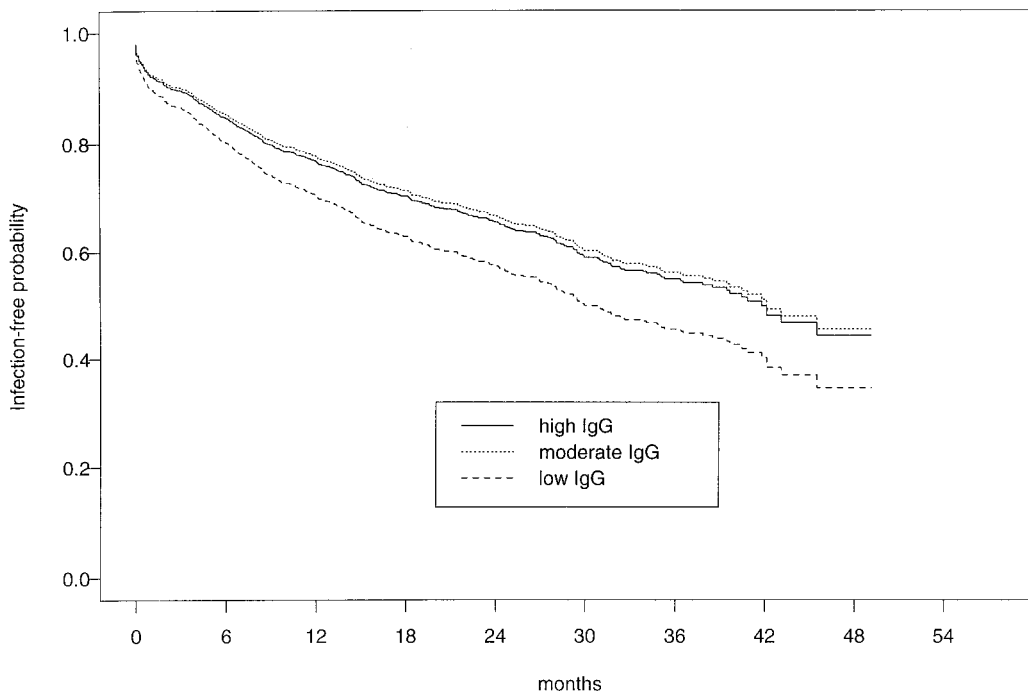


FIG. 2. Predictive time to bacterial infection based on baseline Cox model by IgG level.

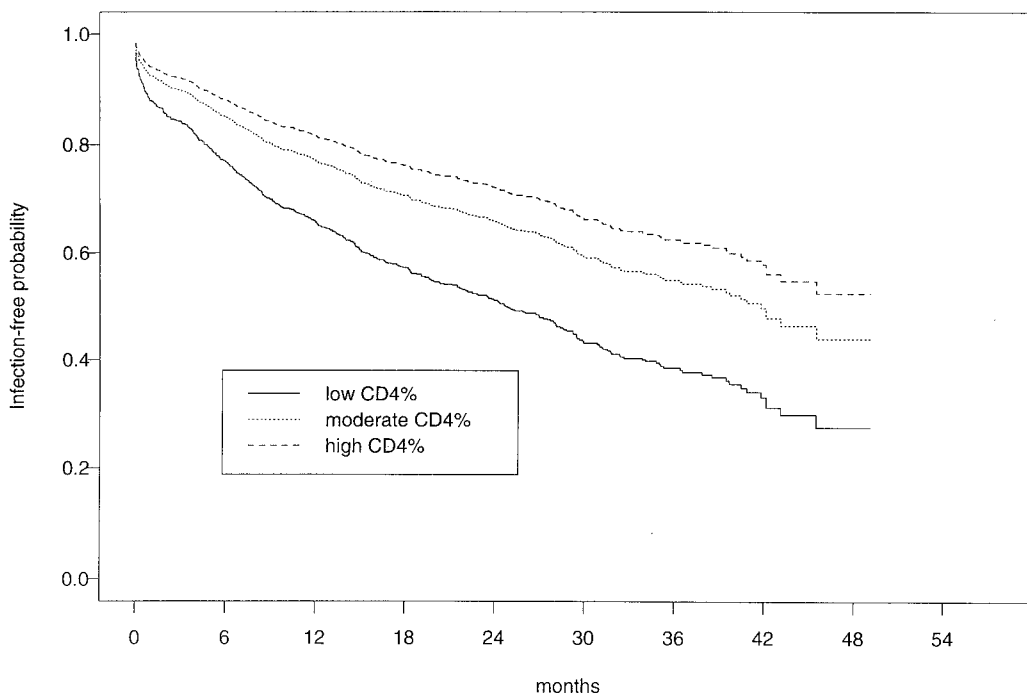


FIG. 3. Predictive time to bacterial infection based on baseline Cox model by CD4 level.

confidence intervals for high IgG levels and low CD19% relative to all other combinations. Subjects with high IgG and low CD19% have a significantly decreased hazard of infection relative to subjects with low IgG and low CD19% ( $P < 0.01$ ; decrease of 50%) and a marginally significant decreased hazard of infection relative to subjects with moderate IgG and low CD19% ( $P = 0.06$ ; decrease of 30%).

**Landmark analysis.** In the landmark analysis, we attempted to assess the predictive value of CD19% and its rate of change over time. First, we investigated whether the baseline CD19% value as well as its slope over the first 6 months of the study (discretized according to the tertiles of its distribution) jointly added significantly to a model containing all variables from the baseline analysis in addition to the estimated slopes of the immunologic measures. Moderate and high CD19% are each associated with significant decreases in the hazard for infection

relative to low CD19% ( $P = 0.01$  and  $P = 0.02$ , respectively; decrease of about 40% each), as are stable and increasing CD19% ( $P < 0.01$ ; decrease of about 45% each). By itself, IgG level does not add significantly to the multivariate model. Similar to the baseline model, the covariates that are significantly associated with an increase in hazard of infection are prior ZDV treatment ( $P = 0.02$ ; increase of 53%), high lymphocyte count ( $P < 0.03$ ; increase of 28%), and low CD4% ( $P < 0.01$ ; increase of 140%), and those that are significantly associated with a decrease in hazard of infection are prior bacterial infection ( $P < 0.01$ ; decrease of 61%), oldest age group ( $P = 0.06$ ; decrease of 37%), and moderate lymphocyte count ( $P = 0.05$ ; decrease of 32%).

Table 7 lists the hazard ratios, confidence intervals, and  $P$  values for CD19% that were high and increasing relative to all other combinations. It is seen in the table that individuals with high and increasing CD19% have a hazard of infection significantly lower than that of (i) individuals with decreasing CD19% and (ii) individuals with low CD19%. Table 8 lists the hazard ratios, confidence intervals, and  $P$  values for IgG levels

TABLE 6. Hazard of bacterial infection for subjects with high IgG levels and low CD19%<sup>a</sup>

IgG level-CD19% combination	<i>P</i>	Hazard ratio	Lower limit	Upper limit
Low, low	<0.01	0.50 <sup>b</sup>	0.35	0.73
Low, moderate	0.71	0.93	0.63	1.38
Low, high	0.34	0.83	0.56	1.22
Moderate, low	0.06	0.70	0.49	1.01
Moderate, moderate	0.24	1.30	0.84	2.01
Moderate, high	0.46	1.16	0.78	1.72
High, moderate	0.25	0.81	0.56	1.16
High, high	0.09	0.72	0.49	1.06

<sup>a</sup> Relative to all other IgG level-CD19% combinations in baseline analysis. (In this and subsequent tables, descriptors are given in the same order as the parameters are given atop the first column.) The limits given are 95% confidence intervals.

<sup>b</sup> For example, an individual with a high IgG level and a low CD19% has half the hazard for a bacterial infection of an individual with a low IgG level and a low CD19%.

TABLE 7. Hazard of bacterial infection for subjects with high baseline CD19% and increasing CD19%<sup>a</sup>

CD19%-CD19% slope combination	<i>P</i>	Hazard ratio	Lower limit	Upper limit
Low, decreasing	<0.01	0.30	0.15	0.59
Moderate, decreasing	0.02	0.50	0.28	0.91
High, decreasing	<0.01	0.50	0.33	0.74
Low, stable	0.03	0.52	0.29	0.93
Moderate, stable	0.59	0.86	0.50	1.48
High, stable	0.41	0.85	0.58	1.24
Low, increasing	0.02	0.61	0.40	0.93
Moderate, increasing	0.96	1.01	0.69	1.49

<sup>a</sup> Relative to all other CD19%-CD19% slope combinations. See Table 6 for details.

TABLE 8. Hazard of bacterial infection for subjects with high baseline IgG levels and increasing IgG levels<sup>a</sup>

IgG level-IgG slope combination	<i>P</i>	Hazard ratio	Lower limit	Upper limit
Low, decreasing	0.71	0.88	0.45	1.72
Moderate, decreasing	0.57	1.18	0.67	2.07
High, decreasing	0.63	1.10	0.74	1.65
Low, stable	0.99	0.99	0.57	1.75
Moderate, stable	0.25	1.33	0.82	2.17
High, stable	0.23	1.25	0.87	1.79
Low, increasing	0.27	0.78	0.53	1.20
Moderate, increasing	0.72	1.07	0.75	1.52

<sup>a</sup> Relative to all other IgG level-IgG slope combinations. See Table 6 for details.

that were high and increasing relative to all other combinations. As in the baseline analysis, IgG level by itself does not modify the hazard for bacterial infection. Figure 4 displays average predicted distributions of time to bacterial infection for subjects with all combinations of baseline CD4% and CD4% slope. As expected, moderate and high CD4 with stable or increasing values had the best probability for an infection-free outcome.

We then tested specific interactions between CD19%, IgG level, and age, discretizing CD19% and IgG level according to fixed percentage changes from baseline. As in the baseline analysis, we found that the effect of CD19% is modified by IgG. Specifically, we found that when increasing CD19% and increasing IgG level were associated, it increased the hazard of bacterial infection when the slopes of the immunologic measures were discretized according to whether they predict changes of 30% or more at 6 months ( $P = 0.01$ ). Table 9 lists the hazard ratios and confidence intervals for all combinations

of CD19% slope and IgG level slope. For example, increasing CD19% and increasing IgG level are associated with an increase in hazard of infection of (i) 365% relative to stable CD19% and decreasing IgG level ( $P < 0.01$ ), (ii) 259% relative to stable CD19% and stable IgG level ( $P = 0.01$ ), and (iii) 288% relative to stable CD19% and increasing IgG level ( $P < 0.01$ ). It is associated with an increase in hazard of infection of 172% relative to increasing CD19% and decreasing IgG level ( $P = 0.01$ ) and an increase of 151% relative to increasing CD19% and stable IgG level ( $P = 0.04$ ).

## DISCUSSION

CD19 is a marker for B cells that is routinely applied to phenotypic lymphocyte analysis in pediatric ACTG trials, but the utility of this marker remains undefined. In the present study we have investigated the predictive value of CD19% for bacterial infections in children with HIV infection based on data from six ACTG protocols. In our baseline analysis, we have found that a low CD19% was associated with a marginal and nonsignificant increase in hazard of bacterial infection relative to high CD19% after adjusting for age, gender, prior bacterial infection, prior treatment with ZDV, prior treatment with IVIG or HIVIG, lymphocyte count, CD4%, CD8%, and IgG and IgA levels. In contrast, a low baseline CD19% together with a high baseline IgG level decreases the hazard of infection by a factor of 0.50 ( $P < 0.01$ ; 95% confidence interval: 0.35, 0.73), relative to low CD19% and low IgG level. In our landmark analysis, we found that high and increasing CD19% is significantly associated with a decrease in hazard of infection relative to decreasing CD19%. Again, consideration of IgG level modifies this conclusion; among subjects with increasing CD19%, an increasing IgG level increases the hazard of infection relative to subjects with stable CD19% and decreasing, stable, or increasing IgG level by a factor of about

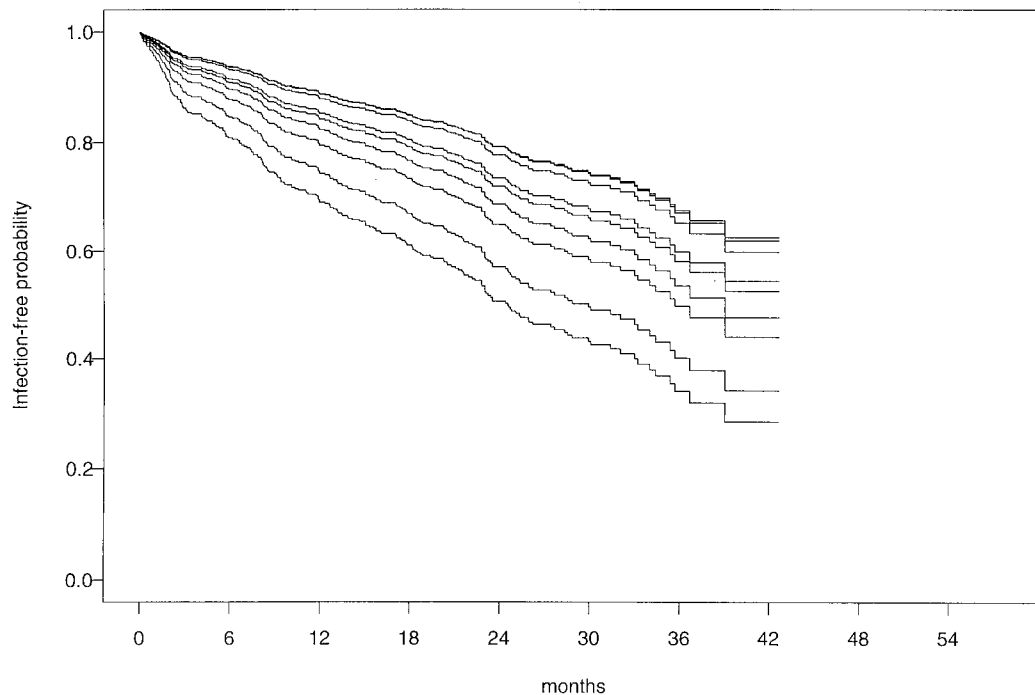


FIG. 4. Predictive time to bacterial infection based on landmark Cox model by CD4% 6-month slope and CD4% baseline level. From top to bottom, the CD4% descriptions are as follows: increasing, moderate; increasing, high; stable, high; decreasing, high; stable, moderate; decreasing, moderate; increasing, low; stable, low; and decreasing, low.

TABLE 9. Hazard of bacterial infection for subjects with increasing CD19% and increasing IgG levels<sup>a</sup>

CD19% slope-IgG slope combination	P	Hazard ratio	Lower limit	Upper limit
Decreasing, decreasing	0.43	1.34	0.65	2.79
Decreasing, stable	0.52	1.24	0.64	2.41
Decreasing, increasing	0.76	1.12	0.54	2.34
Stable, decreasing	<0.01	4.65	2.11	10.23
Stable, stable	0.01	3.59	1.41	9.12
Stable, increasing	<0.01	3.88	1.67	9.01
Increasing, decreasing	0.01	2.72	1.27	5.79
Increasing, stable	0.04	2.51	1.06	5.97

<sup>a</sup> Relative to all other CD19% slope-IgG slope combinations. Slopes are categorized according to whether they predict a 30% change from baseline at 6 months.

4. We did not find any associations between the age-related divisions of CD19% and IgG level shown in Table 1 and increased hazard of bacterial infection.

Therapy with trimethoprim-sulfamethoxazole may have a dramatic effect on risk for bacterial infection. However, this effect is likely to be negligible in this study because at the time that these studies were conducted only a small subset of children were being treated with trimethoprim-sulfamethoxazole. In addition, it would not have any effect on B-cell counts or IgG levels. Thus, not adjusting for it is likely to have no impact on our results.

The importance of the CD19 marker is that it allows analysis of B lymphocytes, which are responsible for humoral immune responses. Hypergammaglobulinemia develops early in HIV infection, possibly as a result of HIV-induced chronic immune activation. An integral component of the hypergammaglobulinemia is the production of anti-HIV antibodies to a variety of epitopes, mainly directed to envelope and Gag proteins (4). Production of antibodies to non-HIV antigens following routine immunization, however, is frequently impaired, indicating a functional humoral immune deficiency despite hypergammaglobulinemia (3). The impaired antibody responses are considered central to the increased incidence of bacterial infections in children with HIV infection and formed the basis of a placebo-controlled trial with IVIG (7). The results of that study corroborated the humoral immune deficiency in that the IVIG arm had a statistically significantly reduced risk of bacterial infections as compared to the placebo arm.

The key finding in the present study is that the predictive value of CD19% cells on incidence of bacterial infection is influenced by IgG levels, which of themselves appear to have little impact on the incidence of bacterial infections. Thus, although increasing CD19% from a relatively high baseline level had significantly better predictive value than low and decreasing CD19%, this benefit was lost in the face of increasing IgG levels. Likewise, at baseline a low CD19% had a low hazard for bacterial infection in association with high IgG levels, but with low IgG levels, it was associated with increased risk of infection. The adverse effect of a low IgG level at baseline could be attributable to poor antibody levels, which are reflected in the total IgG pool. During the course of infection, the beneficial relationship to decreasing IgG levels could be explained on the basis of reduced stimulation with HIV antigens. Spontaneous HIV antibody production by B cells of HIV-positive patients (1, 10, 14), which contributes to the total IgG pool, has been shown to decrease following effective anti-retroviral therapy or in advanced disease states, when the immune system collapses (13). It should be noted, however, that

by itself, the IgG levels were not predictive of risk for bacterial infections.

Pediatric ACTG protocols often do not measure IgG levels. The findings in this study suggest that, whereas evaluation of CD19% itself may be informative of the risk of bacterial infection, the combined analysis with IgG data modifies the predictive value and may increase the risk assessment for bacterial infections. For analysis of the functional capability of B cells, however, assays of specific antibody production rather than total IgG may prove more useful. In all the analyses performed herein, the predictive value of CD4% for risk of bacterial infection was superior to that of CD19%, even when combined with IgG data. The reason for this observation resides most probably in the requirement for T-helper function for optimal B-cell responses. We conclude that if the goal is to identify predictive markers for bacterial infection, assays for B cells should be evaluated in conjunction with analyses of CD4%. Possibly, specific antibody responses to naive or recall antigens in this context, together with CD4 values, could greatly enhance the predictability for risk of bacterial infections obtained by analyses of CD19 B cells alone.

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