IgA levels at diagnosis predict for infections, time to treatment, and survival in chronic lymphocytic leukemia

Ganchimeg Ishdorj,[1,]* Erin Streu,[2,]* Pascal Lambert,[3] Harbhajan S. Dhaliwal,[4] Salaheddin M. Mahmud,[5-7] Spencer B. Gibson,[1,8,9] Versha Banerji,[1,4] Aaron J. Marshall,[9] and James B. Johnston[1,4]

[1]Research Institute in Oncology and Hematology, [2]Department of Nursing, and [3]Department of Epidemiology, CancerCare Manitoba, Winnipeg, MB, Canada; and [4]Section of Haematology/Oncology, Department of Internal Medicine, [5]Department of Community Health Sciences, [6]Vaccine and Drug Evaluation Centre, [7]College of Pharmacy, [8]Department of Biochemistry and Medical Genetics, and [9]Department of Immunology, University of Manitoba, Winnipeg, MB, Canada

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Key Points

- Abnormal levels of IgA at diagnosis predict time to treatment, survival, and need for immunoglobulin replacement in CLL.

- Abnormal levels of IgG at diagnosis predict the need for immunoglobulin replacement in CLL.

To better understand the relationship between baseline immunoglobulin measurements and subsequent clinical outcomes in chronic lymphocytic leukemia (CLL), we performed a retrospective analysis on 660 patients with CLL (72%), monoclonal B-cell lymphocytosis (MBL) (13%), and small lymphocytic lymphoma (SLL) (14%), diagnosed between 2005 and 2014 at CancerCare Manitoba. Of 511 patients who had their first immunoglobulin level determined within 3 months of diagnosis, abnormal (either increased or decreased) immunoglobulin M (IgM), IgG, and IgA values were observed in 58% of patients with CLL, 27% of patients with MBL, and 20% of patients with SLL. Immunoglobulin deviances were similar for MBL and CLL Rai stage 0 and for SLL and Rai stages I and II; for CLL, IgG and IgA abnormalities occurred with increasing frequency with advancing Rai stage. In contrast, the frequency of IgM abnormalities was similar in all patient groups. IgA abnormalities significantly correlated with high β2-microglobulin (B2M) expression, whereas abnormal IgG and IgA levels were associated with the use of *IGHV*1-69, 3-21, and 3-49 subtypes. Increases in IgG or IgM were commonly associated with the presence of a CLL-type M-band, whereas oligoclonal bands were frequently observed with increased IgA levels. Although abnormal levels of IgG and IgA at diagnosis were independent predictors for future immunoglobulin replacement, only abnormal IgA levels were associated with shorter time to first treatment and overall survival. These findings indicate that both reduced and elevated levels of IgG and IgA at diagnosis are important and independent prognostic markers for infection in CLL, with IgA being more relevant as a marker of disease progression and survival.

Introduction

Immune suppression is a fundamental feature of chronic lymphocytic leukemia (CLL) and small lymphocytic lymphoma (SLL), leading to an increased incidence of infections and second malignancies.[1-3] Interestingly, whereas immune dysfunction is apparent at diagnosis, altered serum light chain ratios, serum paraproteins, and low immunoglobulin G (IgG) levels have been observed years before the diagnosis of CLL.[4] Moreover, patients with monoclonal B-cell lymphocytosis (MBL), the precursor to CLL or SLL, also have an increased incidence of infections and second malignancies.[5,6] Hypogammaglobulinemia (a reduction in the immunoglobulins) is a commonly seen immune defect and a major cause of infections in patients with CLL.[7] In addition, a reduction in more than 1 immunoglobulin or a decrease in a specific IgG subclass may be associated with the development of infections.[8,9]

The full-text version of this article contains a data supplement.

The use of immunoglobulin replacement therapy, either intravenous or subcutaneous, reduces the incidence and severity of infections in patients with CLL.[7]

Several studies have evaluated whether baseline immune function reflects the underlying biology of CLL and is of prognostic value.[10-17] Decreased IgG levels have been most widely reported and are also the most variable, occurring in 9.9% to 76% of patients, whereas reductions in IgA occur in 12% to 68% of patients and reductions in IgM occur in 4% to 56% of patients.[10-19] The clinical significance of decreased immunoglobulins at diagnosis is uncertain, because some authors have reported that low IgG or IgA levels may be independent predictors for time to first treatment (TTFT),[14,17] findings that have not been observed by others.[10,12,13,16,19] In addition, an older study by Rozman et al[11] demonstrated that reduced levels of IgA were an independent prognostic marker for overall survival (OS), although this study was performed in an era before fludarabine and chemoimmunotherapy. Moreover, low IgM has been reported to be an independent predictor of OS, but others have found it to have no prognostic significance.[11,18] Decreased IgG at the time of CLL diagnosis has been shown to be associated with later development of severe infections, but this significance was lost in multivariable analysis.[20] The variability of these findings is likely a result of small sample sizes and variations in the patient populations. Furthermore, little is known about the incidence and significance of immunoglobulin abnormalities in patients with SLL and MBL.

Serum paraproteins have been observed in <10% of patients at diagnosis, and usually they are IgM.[10,12,13,21] Previous studies have shown that these paraproteins have no prognostic significance, but a recent study demonstrated that the presence of a CLL-derived IgM paraprotein is associated with more aggressive disease and shorter OS.[21]

Materials and methods

Patients

CancerCare Manitoba (CCMB) is the primary referral center for patients with cancer who are seen in the province of Manitoba, Canada, with a catchment population of 1.2 million. In this retrospective review, all newly diagnosed patients referred to the CLL clinic at CCMB from 1 January 2005 to 31 December 2014 were evaluated. Patient follow-up continued until 30 September 2017. Patients were diagnosed on the basis of flow cytometry results and were classified as having MBL, CLL, or SLL, according to 2008 International Workshop on Chronic Lymphocytic Leukemia criteria.[22] The majority of MBL patients were high-count patients, with a median lymphocyte count of 5.5×10^9/L (range, 1.2 to 9.4×10^9/L). Clinical characteristics and prognostic markers were obtained from our clinical database, and all baseline values reflect laboratory values at the time of diagnosis or within 3 months of diagnosis. Relevant clinical characteristics included age, sex, Rai stage, and Cumulative Illness Rating Scale (CIRS) score.[23] Prognostic markers included β-2 microglobulin (B2M), CD38, and ZAP-70 expression (measured by flow cytometry, with positivity defined as greater than 20% of cells staining positively), and *IGHV* mutational status/gene family use.[24,25] Normal immunoglobulin levels in our clinical laboratory are as follows: IgG, 6.9 to 16.2 g/L; IgA, 0.7 to 3.8 g/L; and IgM, 0.6 to 2.6 g/L. The normal B2M range was 1.1 to 2.4 mg/L or 708 to 1315 μg/L. Fluorescence in situ

hybridization analysis was not available at diagnosis in these patients.

Study approval was obtained from the Research Ethics Board at the University of Manitoba. Study end points were TTFT, time to immunoglobulin replacement (TTIR), and OS. Immunoglobulin replacement was considered for patients with CLL who demonstrated hypogammaglobulinemia (documented IgG <4 g/L or a reduction in a specific IgG subtype) requiring at least 2 courses of oral antibiotics in the previous 12-month period or the occurrence of 1 severe infection requiring hospital admission or intravenous antibiotics. IgG subclass was not routinely measured but was assessed in the few patients with IgG >4 g/L who had an excessive number of infections.[8,9]

Statistical analysis

Immunoglobulin distributions were plotted using density plots and Spearman correlations to measure association between immunoglobulin types. Cox regression was used to predict OS, and competing risk regression models (with death as a competing risk) were used to predict TTFT and TTIR. Schoenfeld plots were used to test the proportional hazard assumption, linearity was tested with fractional polynomials, and influence plots were used to detect influential outliers. Immunoglobulin variables were analyzed as continuous variables and included a polynomial function if the assumption of linearity was not satisfied. Dichotomizing continuous predictors is often a poor approach in which there is a loss in statistical power and a loss of variation between groups (eg, individuals on opposite sides of a cut point are seen as being very different); it does not demonstrate the possible nonlinear relationship (eg, those found in this study).[26-28] When not being analyzed as outcomes, TTFT and TTIR were included in models as time-varying predictors. Likelihood ratio testing was used for model building.

Because there were missing data, results were also produced with imputed values, which can potentially correct coefficients and increase power in the analyses. We attempted to correct the impact of missing data through multiple imputation. Imputations were created by using a substantive model-compatible modification of fully conditional specifications,[29] which has demonstrated better performance in imputing values when the outcome is nonlinear (eg, survival analysis) or when a model includes interactions or nonlinear effects compared with the traditional fully conditional specification. Fifty imputations were run with 20 iterations. Convergence based on the number of iterations was also assessed. Time-varying covariates were entered by including an event indicator and the Nelson-Aalen estimator.[30] One exception was the time-varying predictor of IgG replacement therapy in the competing risk regression model that predicted treatment, which did not reach convergence. Results from both unimputed and imputed analyses are presented to demonstrate the impact of imputations on the results, because unspecified imputations typically provide results biased toward a null effect.[31] Significant immunoglobulin relationships found in the analysis with imputed data were plotted with covariates held at their mean; plots of coefficients were produced, as well as Kaplan-Meier curves and cumulative incidence plots but with predicted values at the 10th, 50th, and 90th percentiles of immunoglobulin values. STATA 14.2 was used for the analyses and included the mfp (fractional polynomials),

Table 1. Clinical features of patients newly diagnosed between 2005 and 2014

Variable	CLL (n = 479; 72%)	SLL (n = 95; 14%)	MBL (n = 86; 13%)	All patients (N = 660)
Ratio of males to females	299:179 (1.6:1)	57:38 (1.5:1)	53:34 (1.6:1)	409:251 (1.6:1)
Median age (range), y	68 (37-99)	68 (34-91)	70 (45-93)	68 (34-99)
CLL Rai stage				
0	263 (55)			
I/II	167 (35)			
III/IV	48 (48)			
Lymphocyte doubling time, mo				
<6	38 (8)	3 (3)	1 (1)	42 (6)
6-12	67 (14)	10 (11)	3 (3)	80 (12)
>12	361 (76)	82 (86)	80 (92)	523 (79)
Ratio of mutated *IGHV* to unmutated *IGHV*	189/150 (1.3:1)	25/24 (1:1)	42/11 (3.8:1)	256/185 (1.4:1)
Ratio of ZAP-70 positive/ZAP-70 negative	122/275 (1:2.3)	20/40 (1:2)	14/54 (1:3.9)	156/369 (1:2.4)
Ratio of CD38 positive/CD38 negative	118/299 (1:2.5)	37/34 (1.1:1)	10/63 (1:6.3)	165/396 (1:2.5)
Ratio of B2M high/B2M normal	152/90 (1.7:1)	37/29 (1.3:1)	14/30 (1:2.1)	203/149 (1.4:1)
Chemotherapy				
For CLL	232 (48)	55 (58)	7 (8)	294 (44)
For solid tumors	6 (1)	2 (1)	1 (1)	9 (1)
IgG replacement	41 (9)	4 (4)	4 (5)	49 (7)
Deaths	144 (30)	25 (26)	11 (13)	180 (27)
Mean CIRS	5	5	4	5

All data are n (%) unless otherwise indicated. All values are rounded up to the nearest whole number.

smcfcs (multiple imputation), stcox (Cox regression), and stcrreg (competing risk) procedures.

Results

Clinical features and description of cohort

We retrospectively analyzed 660 CLL, SLL, and MBL patients newly diagnosed between 2005 and 2014 who had been referred to the CLL clinic at CCMB (Table 1). Seventy-two percent of the patients had CLL, 14% had SLL, and 13% had MBL. The median age of patients was 68 years at diagnosis for CLL and SLL but 70 years for MBL. The CLL patients were slightly younger than patients seen in the general population, but were similar in age to patients at other CLL clinics.[32] The median follow-up was 66 months (range, 0.6-137 months). MBL patients were slightly older and had more favorable prognostic indicators (CD38⁻, ZAP-70⁻, and *IGHV* mutated) than did CLL and SLL patients. Approximately half the patients with CLL (48%) and SLL (58%) required treatment of their CLL during follow-up, whereas 8% of MBL patients required treatment for disease progression. Only 1% of all patients required chemotherapy for a solid tumor. Twenty-seven percent of patients died during follow-up and 7.4% of patients required immunoglobulin replacement (46 patients for infectious complications, 2 for immune thrombocytopenia secondary to CLL, and 1 for an associated dermatitis).

In all, 511 patients (77%) had baseline immunoglobulins measured at diagnosis (or within the first 3 months) and 23% of patients had reduced IgG, 16% reduced IgA, and 54% reduced IgM values (Table 2). There was a progressive increase in the incidence of reduced IgG or IgA with advancing Rai stage, but this was not

observed for IgM. IgM levels were reduced by 43% to 63% in patients with MBL, CLL, or SLL. In contrast, the decreases in IgG and IgA were similar for MBL and Rai 0 CLL, whereas patients with SLL had a reduction in IgA and IgG similar to that in patients with Rai 0 to II CLL. To visualize the immunoglobulin distribution within

Table 2. Immunoglobulin levels in MBL, CLL, and SLL patients at diagnosis

|	CLL, n (%)			SLL, n (%)	MBL, n (%)	All, N (%)
Immunoglobulin	Rai 0	Rai I/II	Rai III/IV			
IgG						
Low	36 (18)	36 (29)	13 (39)	17 (21)	13 (19)	115 (23)
Normal	162 (80)	87 (67)	14 (42)	58 (71)	51 (78)	372 (73)
High	4 (2)	6 (4)	6 (18)	6 (8)	2 (3)	24 (5)
Total patients	202	129	33	81	66	511
IgA						
Low	19 (9)	29 (23)	12 (37)	16 (20)	6 (9)	82 (16)
Normal	174 (87)	95 (73)	17 (53)	60 (74)	60 (91)	406 (80)
High	8 (4)	5 (4)	3 (9)	5 (6)	0 (0)	21 (4)
Total patients	201	129	32	81	66	509
IgM						
Low	112 (55)	82 (63)	20 (61)	35 (43)	29 (45)	278 (54)
Normal	85 (42)	42 (33)	9 (27)	43 (53)	33 (50)	212 (42)
High	5 (3)	5 (4)	4 (12)	3 (4)	3 (5)	20 (4)
Total patients	202	129	33	81	65	510
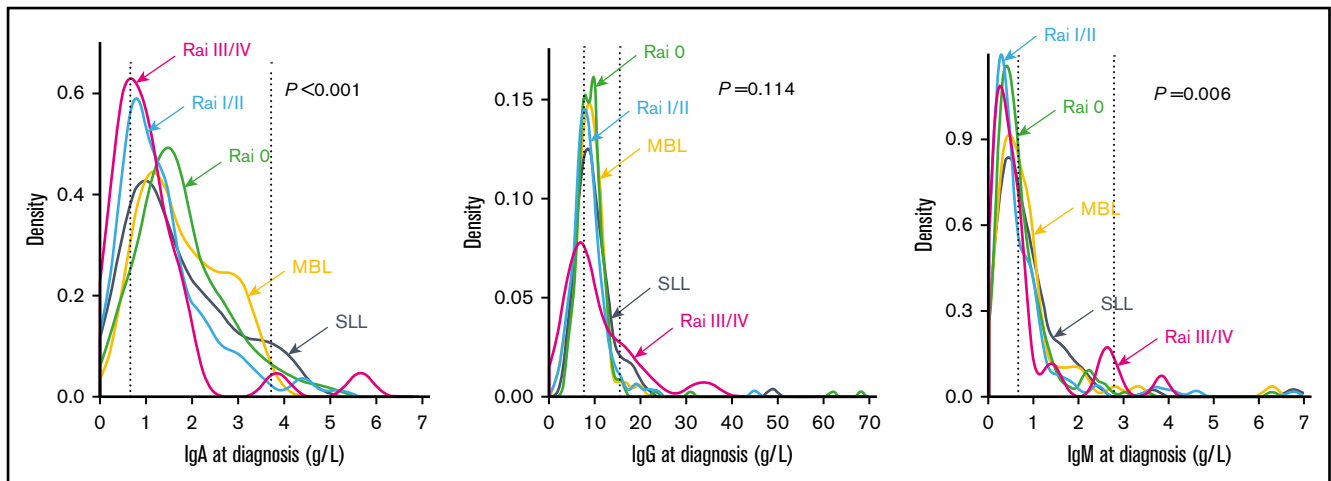
Figure 1. Distribution of IgG, IgA, and IgM baseline levels in the cohort, with hatched vertical lines showing normal ranges. IgM levels were most frequently reduced (P = .006), followed by IgA (P = .001) whereas the reductions in IgG were least significant (0.114). Most patients had levels in the low-normal range. About 5% of patients had increased levels.

each disease group, the continuous variation in immunoglobulin levels was examined by density plots (Figure 1). The majority of patients had IgM values clustering below the normal range. In contrast, the majority of the IgG and IgA values were within the low-normal range. IgA and IgG levels tended to congregate together (ρ, 0.46), with most cases congregating at the lower levels of normal or below the normal range (Figure 2).

Elevated levels of immunoglobulin occurred in ~5% of patients, affecting all 3 classes of immunoglobulin (Table 2). In CLL, high immunoglobulin levels (IgG, IgA, and IgM) occurred more frequently in patients with advanced Rai stage. In MBL, high IgG and IgM occurred at rates similar to those in patients with Rai stage 0 CLL, and no patients had elevated IgA. Increased immunoglobulin levels occurred in 4% to 8% of SLL patients (Table 2). To determine the potential cause for the increased levels of Ig, serum electrophoresis was assessed in each patient who had known immunoglobulin levels (Table 3). For IgG and IgM, about half the patients had a paraprotein, which usually had light chain restriction similar to that of the CLL cells (CLL-like). In contrast, for IgA, half the patients had oligoclonal bands, and a single paraprotein was less common. Thirty-six patients with elevated immunoglobulins required treatment: 34 patients received chemotherapy for progressive CLL, 1 for Richter transformation, and 1 for concomitant multiple myeloma.

Abnormal immunoglobulin levels and prognostic markers

A significant correlation was observed between abnormal (reduced or increased) levels of all immunoglobulins (IgA, IgG, IgM) and the diagnosis of MBL, SLL, or CLL (Table 4). Abnormal IgA levels showed a highly significant positive correlation with elevated B2M levels (P = .005) and CD38 positivity (P < .001), both of which are markers of aggressive disease (Table 4). Abnormal IgM levels correlated with increased CD38 positivity (P = .034) and mutated *IGHV* (P = .005). In contrast, abnormal IgG levels did not correlate with any of these prognostic markers apart from advancing Rai stage. Unfortunately, fluorescence in situ hybridization analysis

was not carried out at diagnosis, so correlations with genomic abnormalities could not be determined.

Because it has recently been demonstrated that serum IgM and IgG paraproteins in CLL are associated with increased use of *IGHV*3-74 and *IGHV*4-39, respectively, we evaluated *IGHV* gene family use in patients with abnormal immunoglobulins.[21] Abnormal levels of IgG and IgA were associated with overrepresentation of *IGHV*1-69, *IGHV*3-21, and *IGHV*3-49, whereas this pattern was not seen for IgM (Figure 3). Reduced levels of IgG have previously been associated with *IGHV*3-21 and 3-23.[21] In contrast, patients with abnormal IgA seemed less likely to use *IGHV*3-7 and *IGHV*4-34 than patients with abnormal IgG. Statistical analysis was not feasible because of the small sample size, but these results suggest that abnormal levels of IgA or IgG may be associated with specific
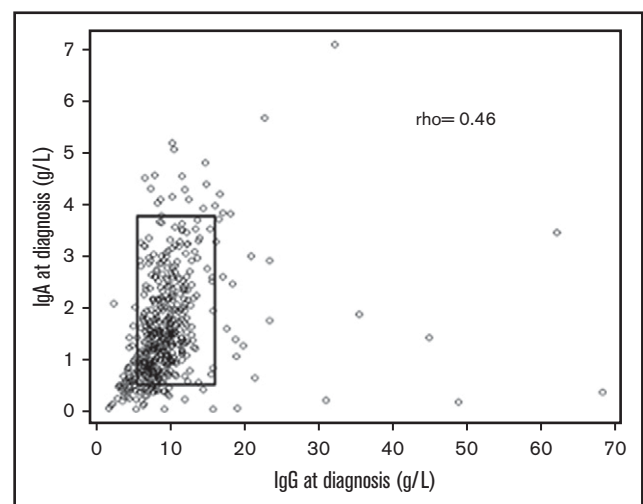

Figure 2. Relationship between baseline IgG and IgA levels and by Spearman's ρ calculation (r = 0.46). The boxed area shows the normal ranges, and a small percentage of patients had abnormal levels of 1 immunoglobulin and normal levels of the other.

Table 3. Causes of increased immunoglobulins footnote:

	Increased IgM, n (%)	Increased IgA, n (%)	Increased IgG, n (%)
Total, n /N (%)	20/510 (4)	21/509 (4)	24/511 (5)
M-bands			
CLL-type	9 (45)	2 (9.5)	7 (29)
Non-CLL type	3 (15)	1 (4.5)	3 (12.5)
Oligoclonal bands	3 (15)	8 (38)	6 (25)
Polyclonal	2 (10)	5 (24)	3 (12.5)
Unknown	3 (15)	5 (24)	5 (21)

IGHV gene subtypes, which in turn may be associated with more aggressive and advanced disease.

Predictive value of abnormal immunoglobulins for TTFT, OS, and immunoglobulin replacement

Results from both imputed and unimputed data were compared in these analyses to demonstrate the impact of missing data and imputations (supplemental Tables 1 and 2). The ratios produced from the OS analyses were similar between unimputed and imputed data, suggesting that the imputations could have been properly specified. Ratios produced for the TTFT and TTIR analyses were closer to the null effect for the immunoglobulin variables in the imputed data than in the unimputed data. This could indicate that the imputations were partially misspecified. However, the differences between unimputed and imputed data for immunoglobulin variables were much greater when using alternative imputation approaches (passive imputation and "just another variable").[33]

By using multivariable analysis and the imputed model, we showed that several baseline variables (supplemental Table 1) correlated with shorter TTFT. Variables associated with a shorter TTFT were low IgA levels (subdistribution hazard ratio [SHR], 0.34/1.09; 95% CI, 0.18-0.64/1.04-1.14; P = .001), unmutated *IGHV* (SHR, 3.01; 95% CI, 2.19-4.15; P < .001), and high B2M level (SHR, 1.57; 95% CI, 1.17-2.10; P = .002) (see supplemental Table 1 for details). Additional significant variables, compared with MBL, included SLL (SHR, 7.32; 95% CI, 3.58-14.99; P ≤ .001), Rai stages III to IV CLL (SHR, 12.24; 95% CI, 5.50-27.25; P ≤ .001), and Rai stages I to II CLL (SHR, 8.25; 95% CI, 4.17-16.30; P ≤ .001). Interestingly, a profound U-shaped relationship was observed between baseline IgA and TTFT (Figure 4A, left graph), suggesting that patients with low and high or very high IgA levels at diagnosis have more aggressive or advanced disease, which necessitates earlier treatment. Patients with low IgA values (10th percentile) had substantially more need for treatment than patients with values at the 50th and 90th percentiles who had similar risk (Figure 4A, right graph). Thus, independent prognostic markers for TTFT included either very high IgA (>4 g/L) or low IgA (<1 g/L) (Figure 4A, left graph), unmutated *IGHV*, high B2M level, and advanced disease.

By using multivariable imputed models, prognostic factors at diagnosis that significantly influenced survival included abnormal IgA level (SHR, 0.81/1.01; 95% CI, 0.61-1.08/1.00-1.03; P = .026), sex (SHR, 0.52; 95% CI, 0.38-0.73; P < .001), advancing age (SHR, 1.72; 95% CI, 1.52-1.95; P < .001), high B2M level (SHR, 3.01; 95% CI, 1.84-4.92; P < .001), advancing Rai stage (P = .045), and treatment for CLL (SHR, 1.83; 95% CI, 1.29-2.59; P = .001) (supplemental Table 2). Interestingly, the plot based on imputed data showed a U-shaped nonlinear relationship between

Table 4. Differences between baseline immunoglobulin levels and prognostic markers

Variables	IgA, n (%)			IgG			IgM, n (%)		
	Abnormal (n = 103)	Normal (n = 406)	P	Abnormal (n = 139)	Normal (n = 372)	P	Abnormal (n = 298)	Normal (n = 212)	P
Ratio of males to females	1.5:1	1.8:1	.457	1.5:1	1.8:1	.562	1.8:1	1.6:1	.585
Median age (range), y	70 (40-94)	68 (34-99)	.081	68 (40-90)	68 (34-99)	.58	68 (34-99)	68 (44-93)	.646
CLL Rai stage			<.001			<.001			.008
0	27 (26)	174 (43)		40 (29)	162 (44)		117 (39)	85 (40)	
I/II	34 (33)	95 (23)		42 (30)	87 (23)		87 (29)	42 (20)	
III/IV	15 (15)	17 (4)		19 (14)	14 (4)		24 (8)	9 (4)	
MBL	6 (6)	60 (15)		15 (11)	51 (14)		32 (11)	33 (16)	
SLL	21 (20)	60 (15)		23 (17)	58 (16)		38 (13)	43 (20)	
LDT, mo			.071			.269			.899
>12	77 (75)	331 (82)		106 (76)	303 (81)		237 (80)	33 (16)	
6-12	12 (12)	44 (11)		17 (12)	40 (11)		32 (11)	44 (21)	
<6	12 (12)	22 (5)		13 (9)	21 (6)		21 (7)	22 (10)	
ZAP-70 positive/ZAP-70 negative	31/49 (30/48)	92/245 (23/60)	.06	35/78 (25/56)	88/218 (24/59)	.748	74/177 (25/59)	49/118 (23/56)	1
CD38 positive/CD38 negative	39/47 (38/46)	84/260 (21/64)	<.001	41/76 (30/55)	82/233 (22/63)	.085	83/174 (28/58)	39/135 (18/64)	.034
B2M			.005			.169			.723
High	55 (53)	141 (35)		65 (47)	131 (35)		115 (39)	81 (38)	
Normal	21 (20)	124 (31)		37 (27)	108 (29)		89 (30)	56 (26)	
IGHV mutated/unmutated	38/36 (37/35)	168/112 (41/28)	.227	56/47 (40/34)	150/103 (40/28)	.463	138/78 (46/26)	67/72 (32/34)	.005

All data are n (%) of cases where data available, unless otherwise indicated. Abnormal immunoglobulins include patients with both high and low levels.

Figure 3. Normal and abnormal expression of IgG, IgA, and IgM within the different *IGHV* family subfamilies. Relative frequency is the percentage of patients within the patient population.
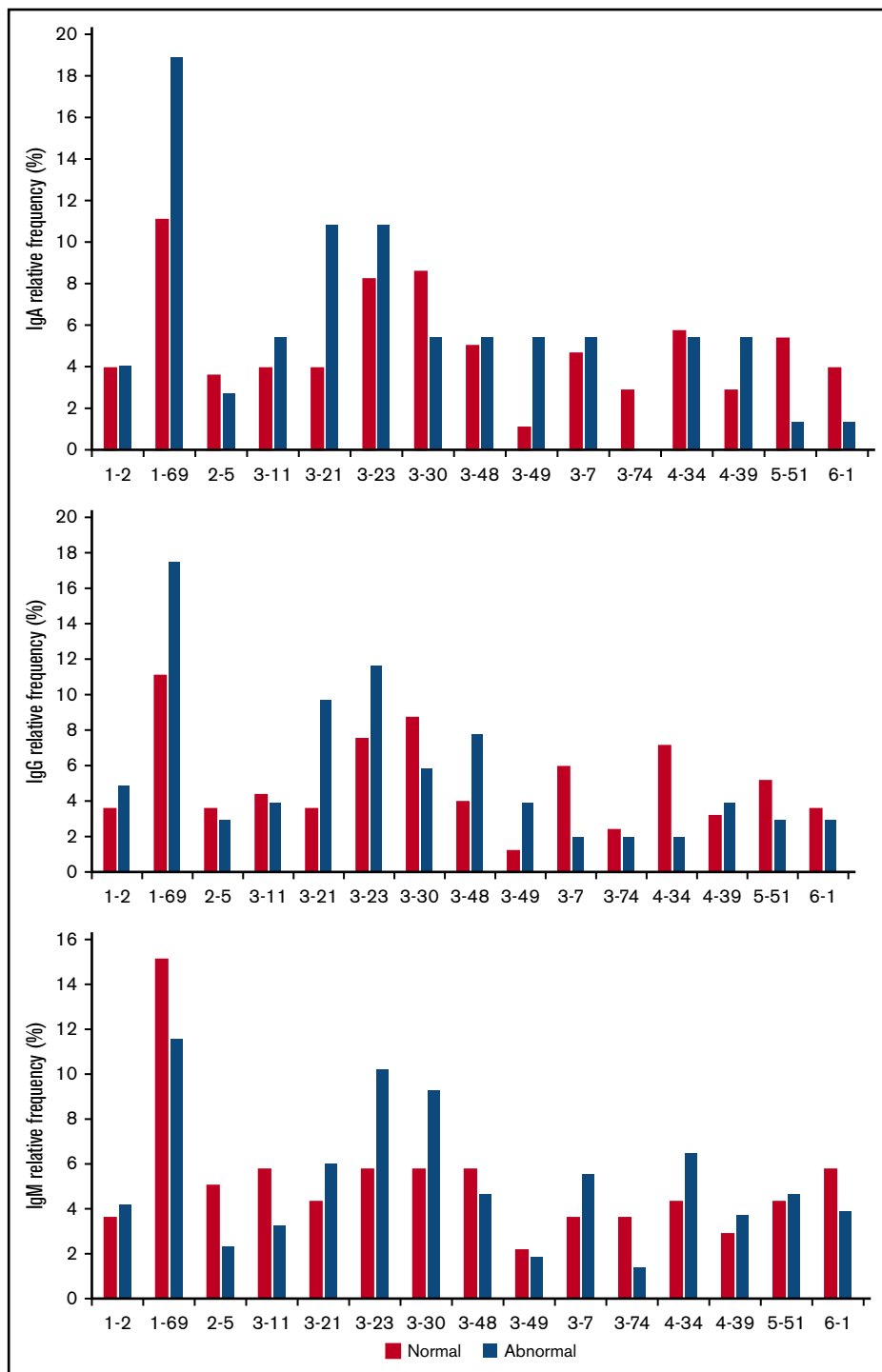
the continuum of IgA level at diagnosis and OS, indicating that decreased survival was associated with both low and very high levels of the immunoglobulin (Figure 4B, left graph). However, the Kaplan-Meier plot showing OS demonstrated that survival rates differed slightly between patients with IgA values at the 10th, 50th, and 90th percentiles (Figure 4B, right graph). Thus, an abnormal IgA level, high B2M level, male sex, older age, advanced disease, and need for CLL or SLL treatment were independently associated with decreased survival.

Abnormal low levels of IgG ($P = .047$) or IgA ($P = .031$) at diagnosis were marginally significant for predicting the need for immunoglobulin replacement (supplemental Table 3). However, the number of patients with elevated IgG and IgA at diagnosis was low. Future need for IgG replacement also significantly correlated with increasing CIRS score ($P < .001$), advancing age ($P = .001$), and need for CLL treatment ($P = .002$) (supplemental Table 3). The cumulative incidence plot for IgA (Figure 4C, left graph) demonstrates the rates of immunoglobulin replacement for the 10th, 50th,
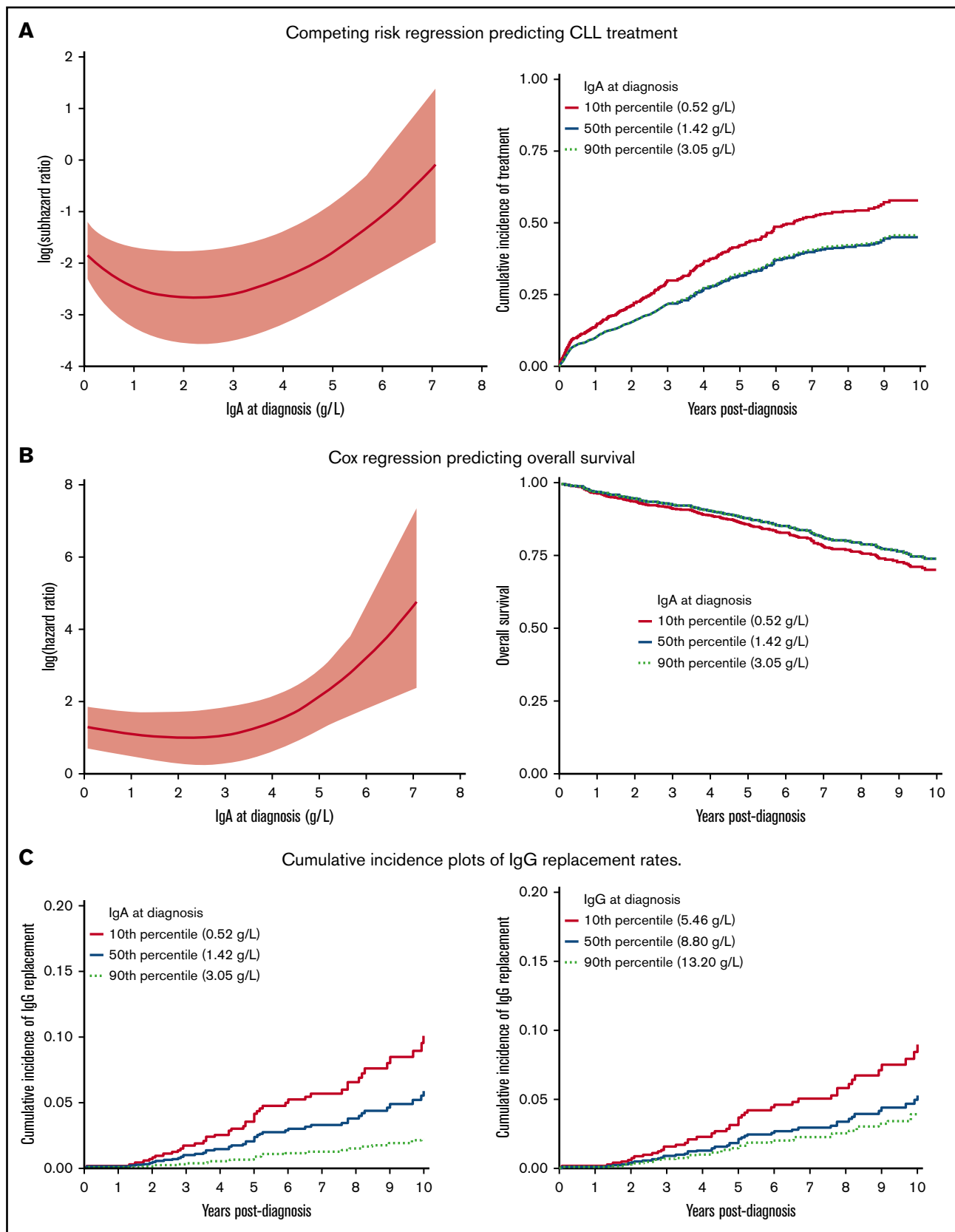
Figure 4. Influence of immunoglobulin levels on clinical outcome. The relationship between altered levels of IgA and TTFT (A), OS (B), and requirement for immunoglobulin replacement (C) for severe infections.

blood advances

and 90th percentile values showing the close relationship between baseline IgA and subsequent need for immunoglobulin replacement. In contrast, the cumulative incidence plot for IgG (Figure 4C, right graph) shows that patients at the 10th percentile value of IgG had an increased rate of immunoglobulin replacement compared with those who had either 50th or 90th percentile values, which were similar. Thus, there is a closer relationship between absolute level of IgA at diagnosis and need for replacement therapy.

Discussion

Findings in this study indicate the importance of measuring immunoglobulin levels in CLL and its variants at diagnosis. Previous studies have focused on the clinical and prognostic relevance of low immunoglobulin levels and have been limited by sample size and composition, typically excluding patients with MBL and SLL. Our retrospective review included these patient populations and examined the significance of both low and high immunoglobulin levels at the time of diagnosis. Highly significant differences were observed between abnormal IgG or IgA (either increased or decreased) in MBL, SLL, and in the different Rai stages of CLL at diagnosis, whereas the difference was less marked with IgM.

We have confirmed that the immunoglobulins are decreased in a significant number of patients with CLL, SLL, and MBL at diagnosis, with half the patients reporting low IgM (54%), 23% low IgG, and IgA (the least likely to be reduced) at 16%. Interestingly, significant reductions in immunoglobulins (IgG, 19%; IgA, 9%; IgM, 45%) were even seen in the 66 MBL patients. Glancy et al[34] previously noted low IgG in one-third of patients with MBL but did not report on IgA or IgM levels. In this study, the degree of suppression correlated with tumor burden; low IgG and IgA levels were more likely to be present in patients with CLL than in patients with MBL, and there was a greater decline in those patients with advanced Rai stage. The decreases in IgG and IgA levels in SLL were similar to those in Rai stage 0 to II CLL. In contrast, IgM reduction occurred at equal frequency in all patients. The reason for this is unclear, but it is unlikely to be related to aging, because IgM levels remain stable throughout life, whereas IgG and IgA levels actually increase with age.[35,36]

Elevated immunoglobulin levels were observed in about 5% of patients, with the incidence depending on tumor burden, being most frequent in patients with advanced Rai stage CLL. Serum protein electrophoresis reported a monoclonal band (M-band) in half the patients with elevated IgG and IgM, with most bands being CLL-like. However, although IgA paraproteins were uncommon, oligoclonal bands were frequently seen with increased IgA.[21,37] By using a highly sensitive immunoblotting technique, Beaume et al[37] showed that multiple monoclonal proteins can be detected in 80% of patients with early-stage CLL, with a mean of 3.3 bands in affected patients. These oligoclonal bands are also present in the elderly and in individuals with immune abnormalities, suggesting that oligoclonal banding in CLL is related to the presence of multiple B-cell clones as a result of T-cell dysfunction. Thus, the presence of hypogammaglobulinemia and increased immunoglobulin levels with oligoclonal bands likely reflect underlying immune deficiency.

Recent studies have demonstrated that low levels of IgG, IgA, or IgM may predict for TTFT or OS by univariable analysis, but the correlations were usually lost with multivariable analysis.[18,19] Parikh et al[14] did note, however, that reduced IgG at diagnosis predicted for TTFT by multivariable analysis, but not OS. An important observation in our study was the association of either low or high IgA levels at diagnosis with shortened TTFT and OS, with abnormal levels being an independent prognostic factor in multivariable analysis. These findings support previous observations that reduced levels of IgA at diagnosis are an independent predictor of TTFT in CLL but also demonstrate the additional prognostic relevance of increased levels of IgA.[11,17] As with IgG and IgM, abnormal levels of IgA correlated with disease burden, but IgA was unique in that abnormal levels also significantly correlated with increased B2M levels and CD38 positivity. B2M is noncovalently linked to the α-chain of the class I major histocompatibility complex on all nucleated cells, including CLL cells, increasing with tumor burden, renal dysfunction, and elevated plasma inflammatory cytokines (interleukin-6 [IL-6], IL-8, and tumor necrosis factor).[38] In contrast, CD38 is involved in cell proliferation and survival.[39] In addition, although abnormal IgM levels highly correlated with mutated IGHV, this was not observed with IgG or IgA. However, in contradistinction to IgM, altered levels of IgG and IgA were associated with IGHV1-69 and IGHV3-21, subgroups that are associated with aggressive disease. These results suggest that alterations in the immunoglobulins are likely a function of the CLL cell of origin and tumor burden. The patient numbers in the other IGHV subgroups were too small to make a meaningful comparison between IgG and IgA. IgA differs from the other immunoglobulins in that recombination occurs in Peyer's patches in the distal ileum, other gut lymphoid tissue, and nasopharyngeal lymphoid tissue, with subsequent passage of the memory B cells to mucosal tissue where they may become secreting plasma cells.[40] Thus, alterations in plasma IgA levels may reflect involvement of these sites by CLL.

In our study, 7.4% of patients required immunoglobulin replacement, almost always for frequent or severe infections, and independent prognostic factors for replacement therapy were advancing age, high CIRS score, chemotherapy, or abnormal levels of IgA or IgG at diagnosis. Moreover, when stratified according to the level of immunoglobulin, patients with the lowest levels of IgG or IgA had the greatest likelihood of requiring replacement. Similarly, Anderson et al[41] and Crassini et al[42] have demonstrated that low levels of IgA at diagnosis is a better predictor of serious infections than low levels of IgG or IgM. Infections in CLL usually involve the respiratory and urinary tracts, areas that are protected by locally secreted dimeric IgA and IgG from the systemic circulation.[40,43] Despite the fact that IgA is produced and secreted by the mucosa, plasma IgA levels do predict for the need for immunoglobulin replacement, which suggests that they correlate with secreted levels. This is an important finding because although patients may not be experiencing infectious complications at the time of diagnosis, those patients with low levels of IgG and IgA should be counseled more thoroughly regarding their future risk of infections and the importance of early assessment and intervention. As previously reported,[11,14] immunoglobulin levels also tend to decrease during the course of CLL, and patients have a high incidence of infections early in their disease, even before treatment. These infections are a significant problem, and they predict for reduced survival.[41,42] The fact that the Bruton tyrosine kinase

inhibitor ibrutinib can increase plasma IgA levels in CLL suggests that early used of this agent in patients presenting with low or decreasing levels of IgA may prevent these early infections.[44,45]

The mechanisms responsible for decreased immunoglobulins in CLL at diagnosis is still unclear, but the CLL cells themselves or factors secreted by the CLL cells may suppress normal B and plasma cells.[2] CLL cells may have a direct effect on plasma cells through the surface expression of Fas ligand, CD27, and PD-L1.[2] In addition, transforming growth factor-β is secreted by CLL and marrow stromal cells in CLL and is a potent inhibitor of normal B and T cells.[46,47] Finally, CLL cells have features similar to those of regulatory B cells, producing IL-10 which has immunosuppressive properties.[48,49] Interestingly, IL-10 production has been shown to be greater in *IGHV*-mutated cells than in unmutated cells as a result of reduced DNA methylation.[49] Plasma levels of IL-10 are similar in *IGHV*-mutated and unmutated disease, possibly reflecting the greater tumor burden with unmutated disease.[49] However, although chemoimmunotherapy could further reduce immunoglobulin levels, presumably through effects on normal B cells, agents acting through the microenvironment may actually improve these levels.[44,50]

In summary, this study demonstrates that alterations in IgG and IgA at diagnosis reflect both CLL biology and tumor burden. Although abnormalities in both immunoglobulins predict for subsequent infections, IgA is unique because alterations reflect aggressive disease with shortened TTFT and OS.

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Authorship

Contribution: J.B.J., G.I., and E.S. contributed to study design, literature review, data interpretation, and preparation of the manuscript; P.L. performed statistical analyses; and S.B.G., V.B., H.S.D., S.M.M., and A.J.M. provided conceptual advice and editing.

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ORCID profiles: A.J.M., 0000-0002-1175-5498; J.B.J., 0000-0001-6614-083X.

Correspondence: James B. Johnston, Section of Hematology/Oncology, Department of Internal Medicine, University of Manitoba, CancerCare Manitoba, 675 McDermot Ave, Winnipeg, MB R3E 0V9, Canada; e-mail: james.johnston@cancercare.mb.ca.

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