Relationship between autoimmune phenomena and disease stage and therapy in B-cell chronic lymphocytic leukemia

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The aim of this multicenter GIMEMA study was to correlate autoimmune complications (AIC) in B-cell chronic lymphocytic leukemia (B-CLL) with stage and therapy. Autoimmune hemolytic anemia (129/194 cases) and autoimmune thrombocytopenia (35/194 cases) were typically present in advanced and multi-treated disease. Age over the median, stage C and first and second line therapy were identified as independent risk factors by multivariate analysis. In contrast, non-hematologic AIC (30/194 cases) and the presence of serological markers of autoimmunity were mostly observed in early B-CLL, suggesting different pathogenic mechanisms underlying hematologic and non-hematologic autoimmune phenomena in B-CLL.

Key words: B-CLL, autoimmunity, AIHA, AITP, fludarabine.

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The relationship between B-cell chronic lymphocytic leukemia (B-CLL) and the immune system is widely recognised.[1] The disease is characterized by immunodeficiency, hypogammaglobulinemia and a high prevalence of autoimmune phenomena mainly restricted to blood cell self-antigens.[2,3] Autoimmune hemolytic anemia (AIHA) and thrombocytopenia (AITP) are known complications of B-CLL, whereas there are only few reports on other autoimmune diseases and on serological markers of autoimmunity.[2-4] The occurrence of autoimmune cytopenias was commonly considered a poor prognostic factor,[5] although, more recently, this has been questioned.[6,7] Reports on other autoimmune phenomena are anecdotal.[8-10] No studies exist that correlate non-hematologic autoimmune diseases and the presence of autoantibodies with the clinical stage of B-CLL and its therapy. The aim of this multicenter GIMEMA study was to determine the presence of different autoimmune complications (AIC) in B-CLL (hematologic cytopenias, other autoimmune diseases, and serological markers of autoimmunity) and their relationship with B-CLL stage and therapy.

Design and Methods

Case series and diagnosis of AIC

The diagnosis and staging of B-CLL were performed according to the National Cancer Institute criteria and the Binet staging system.[11,12] AIC were recorded prospectively between October 2001 and May 2003 (30 cases), and retrospectively through chart reviews (164 cases) among all the B-CLL patients regularly followed (total 3150) by 18 hematology centers of the GIMEMA group. Age, gender, and B-CLL stage and therapy were recorded before the occurrence of the AIC, to avoid confounding AIHA and AITP on B-CLL stage. First line therapy consisted of chlorambucil with or without steroids; second line treatment mostly consisted of cyclophosphamide with or without an anthracycline or in fludarabine-based regimens. The diagnosis of AIHA was dependent on the presence of a positive direct antiglobulin test (DAT), that of AITP was made according to the British Committee for Standards in Haematology,[13] and the diagnosis of other AIC was made according to standard clinical and immunological criteria.[14] The same information collected for the patients with AIC (age, gender, stage and therapy) was also recorded for 434 other B-CLL patients (all the patients of the three main participating centers because data were not available for all 3,150), considered as the control B-CLL group. This study was performed after obtaining informed consent from patients and approval from the institutional Human Research Committee. The procedures followed were in accordance with the Helsinki international ethical standards on human experimentation.

Serological markers of autoimmunity

Serological studies were performed in 227 consecutive patients of the 434 B-CLL

patients without AIC forming the control group. The following antibodies were detected by standard techniques:[15] anti-nuclear (ANA), anti-smooth muscle (SMA), anti-mitochondrial (AMA), anti-parietal cells (PCA), and anti-liver-kidney-microsomal antibodies (LKM) by indirect immunofluorescence on Hep-2; anti-DNA antibodies by indirect immunofluorescence on *Crithidia luciliae*; anti-extractable nuclear antigen (ENA, in particular, SS-A, SS-B, Sm, RNP, Scl-70), anti-thyroglobulin (anti-Tg), anti-thyroid peroxidase (anti-TPO), anti-neutrophil cytoplasmic (ANCA), anti-cardiolipin IgG and IgM (ACA), and anti-β-2-glycoprotein 1 IgG and IgM antibodies (β2GP1) by ELISA; rheumatoid factor by standard nephelometry.

Statistical analysis

Differences in distribution of stage and treatment among groups were evaluated using the χ^2 test. Multivariate analyses were performed using a logistic model. Computations were performed with the SAS statistical package (8.1 Version, SAS Institute Inc., Cary, NC, USA). *p* values lower than 0.05 were considered statistically significant.

Results ad Discussion

AIC in B-CLL patients

There were 194 cases of AIC. The most frequent form of AIC was AIHA (129 cases, 66%), followed by AITP (35 cases, 18%), and 30 other autoimmune diseases (16%): bullous pemphigus (n=9), Hashimoto's thyroiditis (n=8), rheumatoid arthritis (n=4), systemic lupus erythematosus (n=1), autoimmune glomerulonephritis (n=1), autoimmune gastritis (n=1), Sjögren's syndrome (n=1), polymyositis-dermatomyositis (n=1), vasculitis (n=1), autoimmune polyneuropathy (n=1), ulcerative colitis (n=1), and Raynaud's disease (n=1). As for idiopathic AIHA, the great majority of cases of B-CLL-associated AIHA (89%) were due to *warm* autoantibodies (IgG+DAT); only 11% were cold hemagglutinin diseases due to IgM autoantibodies (anti-C'+DAT).

Relationship between AIC and B-CLL stage and therapy

The statistical analysis was conducted on the 164 retrospectively identified cases of AIC. The small group of 30 new cases was considered separately in the statistical analysis because these differed significantly for clinical stage and therapy from the retrospective group. By comparing patients with and without AIC (Table 1) we found that the former had a higher median age (p=0.03), were mostly in advanced disease stage and had already been treated with first and second line therapy (p<0.001). Multivariate analysis showed that age >69 years (median age), stage C and prior administration of first and second line therapy emerged as independent factors significantly related with the occurrence of AIC. Considering the relationship between the different types of AIC and stage/therapy (Table 2), we found that the majority of patients with AIHA were in stage C whereas cases of AITP were equally distributed according to stage and the

Table 1. Clinical data of B-CLL patients with or without autoimmune complications (control B-CLL).

	Control B-CLL (n=434)	*Autoimmune complications (n=164)*	*Multivariate analysis* OR	95%CI
Age (years)	68.3±10.3	70.3±9.6	2.78	1.75-4.43
Gender M/F (%)	239/195 (55/45)	85/79 (52/48)	0.73	0.47-1.13
Stage A (%)	291 (67)	53 (32)	1.00	Ref
Stage B (%)	100 (23)	39 (24)	1.40	0.83-2.37
Stage C (%)	43 (10)	72 (44)	2.87	1.64-5.04
No therapy (%)	272 (63)	11 (7)	1.00	Ref
First line therapy (%)	110 (25)	80 (49)	13.33	6.70-26.51
Second line therapy (%)	52 (12)	73 (44)	27.48	12.77-59.14

Age is given as mean ± SD; OR: odds ratio; CI: confidence intervals.

Table 2. Clinical data of the B-CLL patients with the different autoimmune complications. Multivariate analysis of 113 patients with AIHA.

	AIHA (n=113)	OR	CL	*AITP (n=28)*	*Other autoimmune complications (n=23)*
Age (years)	71.4±9.1	4.85	2.65-8.89	68.4±11.3	67.2±9.2
Gender M/F (%)	60/53 (53/47)	0.75	0.45-1.26	16/12 (57/43)	9/14 (39/61)
Stage A (%)	26 (23)	1.00	Ref	10 (36)	17 (74)
Stage B (%)	26 (23)	2.12	1.13-4.01	9 (32)	4 (17)
Stage C (%)	61 (54)	6.54	3.43-12.78	9 (32)	2 (9)
No therapy (%)	2 (2)	1.00	Ref	0 (0)	9 (39)
First line therapy (%)	58 (51)	47.87	11.31-202.68	13 (46)	9 (39)
Second line therapy (%)	53 (47)	99.34 479.81	20.57-(54)	15 (22)	5
II line-treated with fludarabine (%)	28 (25)	91.32	19.28-432.52	0 (0)	0 (0)

Age is given as mean ± SD; OR: odds ratio; CL: confidence limits.

other autoimmune diseases were mostly observed in stage A (p<0.001). As regards chemotherapy, both AIHA and AITP were almost exclusively observed in patients with first and second line-treated B-CLL, and the other autoimmune diseases were equally distributed. We evaluated in particular the relationship between AIHA and second line therapy with fludarabine. Fludarabine-treated patients accounted for 28/53 and 33/52 of those with AIC and control B-CLL patients, respectively. Multivariate analysis showed that the relative risk of developing AIHA was similar in patients treated with second line therapy with or without fludarabine. As found for all AIC, age above the median, disease progression (stage B and C) and therapy intensification (first and second line) emerged as independent risk factors for AIHA. To minimize the effects of disease progression on the analysis, we focused on stage A patients only. Multivariate analysis confirmed that variables more associated with AIHA were age above the median (OR 3.43, CI 1.22-9.63), first line therapy (OR 15.62, CI 5.00-48.82), and second line therapy (OR 48.64, CI 10.00-239.19).

Prospective study

This group was mainly composed of stage A (54%) and untreated (67%) patients, being more similar to the control group of B-CLL patients without AIC. For this reason and because of the small number of cases the distribution of the different AIC among stages and treatments was not significant. However, even in this small group, multivariate analysis showed that stage C emerged as an independent factor significantly related with the occurrence of AIC (OR 4.78, CI 1.61-14.17).

Follow-up of clinical AIC

Follow-up data were available for 116/194 cases of AIC. Patients with AIHA, AITP, and the other autoimmune diseases recovered in 65%, 54%, and 40% of cases, respectively. It should be noted that 14/33 (42%) cases of refractory AIHA were fatal and that death was directly due to the hemolytic crisis in 5/14.

Prevalence of serological markers of autoimmunity

We found that 93/227 (41%) of B-CLL patients, mostly in stage A, had at least one positive test for a marker of autoimmunity, distributed as follows: 36 ANA, 25 ACA, 23 RF, 23 anti-TPO/anti-TG, 20 SMA, and 10 miscellaneous (AMA, anti-DNA, anti-PCA). Considering all positive autoimmune phenomena grouped as positive serological autoantibodies only, hematologic diseases (AIHA and AITP), and non-hematologic diseases (Figure 1), there was a statistically significant distribution with respect to the clinical stage (p<0.001). In fact, serological and clinical non-hematologic complications were mostly observed in stage A patients, whereas more than 70% of hematologic complications were found in patients with advanced disease (B+C stage).

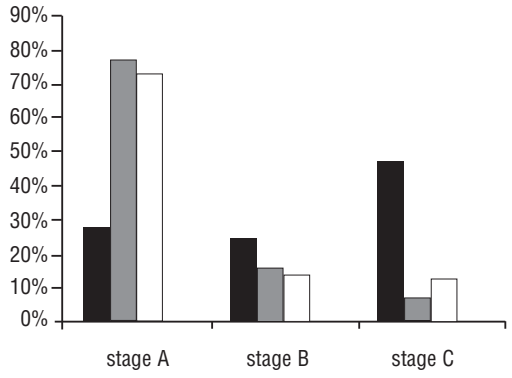

Figure 1. Distribution of autoimmune phenomena grouped into hematologic complications (AIHA and AITP, ■), non-hematologic diseases (■), and serological autoantibodies only (□), χ^2 test: p<0.001.

The results of this study showed that AIC occur in roughly 5% of patients with B-CLL. The more frequent complication was AIHA, as already reported,[2-4] followed by AITP and other autoimmune diseases, the latter two with a similar prevalence. AIC were associated with advanced and multi-treated disease, as demonstrated by multivariate analysis, which identified age over the median, stage C and first and second line therapy as independent risk factors. This finding is in line with the recently reported close correlation between ZAP-70 expression and incidence of autoimmune cytopenias in B-CLL.[16] Considering only AIHA, the more frequent and potentially fatal complication, multivariate analysis gave comparable results, adding stage B to stage C, and confirming first and second line therapy as independent risk factors. Also considering stage A patients only, to avoid the confounding effect of anemia of advanced and multi-treated disease, variables more associated with the presence of AIHA were age over the median, and first line and second line therapy. Interestingly, multivariate analysis showed that patients treated with or without fludarabine have a comparable risk of AIHA, at variance with the reported association between AIHA and fludarabine treatment,[17,18] but in agreement with recent observations by Catowsky *et al.*[19]

The novel finding of our study is that the distribution of the different AIC differed with respect to stage and therapy, in that AIHA and AITP were typically present in advanced and multi-treated disease, whereas the non-hematologic autoimmune diseases and serological markers of autoimmunity were mostly observed in early B-CLL. Hematologists are usually familiar with AIHA and AITP, but less so with non-hematologic AIC. The latter complications, even if usually less severe than hematologic ones, should be carefully searched for, particularly in patients with early B-CLL. Although it cannot be excluded that steroids and cytotoxic drugs may

successfully treat non-hematologic AIC so that they are less prominent in more heavily treated patients, this different distribution of complications is open to other interpretations. Hall *et al.*[20] demonstrated that malignant B-CLL cells are able to present purified Rh protein to autoreactive T-helper cells, driving an autoimmune response against erythrocytes. Autoimmunity against blood cells might, therefore, reflect a late event in the natural history of B-CLL, related to the accumulation of malignant B-CLL antigen-presenting cells. In contrast, non-hematologic AIC, as well as serological autoimmunity, might have a different pathogenic mechanism, involving deficient control by normally-competent T cells, already present in early disease stage.

Appendix

Other participating centres and physicians in the GIMEMA study were: Marco Candela, Giovanni Danieli, Università Politecnica delle Marche, Ancona; Giovanni Pizzolo, Università degli Studi, Verona; Giuseppe Leone, Università Cattolica del Sacro Cuore, Roma; Albano Del Favero, Università degli Studi, Perugia; Francesco Zaja, Renato Fanin, Università degli Studi, Udine; Federico Chiurazzi, Bruno Rotoli, Università Federico II, Napoli; Maurizio Longinotti, Università degli Studi, Sassari; Sara Galimberti, Mario Petrini, Università degli Studi, Pisa; Luigi Gugliotta, Arcispedale Santa Maria Nuova, Reggio Emilia; Nunzio Filardi, Francesco Ricciuti, Ospedale S. Carlo, Potenza; Tambone Reyes, Ospedale di Cefalù; Daniele Dini, Giuseppe Torelli, Università degli Studi, Modena; Rossella Paolini, Ospedale di Rovigo.

WB designed the study and prepared the manuscript; SC contributed by collecting the patients' data collection and with laboratory work; RMA, FRM, AA, RC, LL, GG, EMP helped to collect the patients' data; PP conducted the statistical analysis; AC, VL, FM, and AZ contributed to discussion of the manuscript.

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