Hematol Oncol Stem Cell Ther (2019) 12, 97–104

Available at www.sciencedirect.com

ScienceDirect

journal homepage: www.elsevier.com/locate/hemonc

ORIGINAL RESEARCH REPORT

The prevalence and prognostic significance of autoimmune cytopenias in a cohort of Egyptian patients with chronic lymphocytic leukemia

Basma Atef [a], Emad Azmy [a], Doaa Aladle [b], Mohamed Mabed [a,*]

[a] The Hematology Unit, Oncology Center, Mansoura University, Mansoura, Egypt
[b] The Clinical Pathology Department, Faculty of Medicine, Mansoura University, Mansoura, Egypt

Received 4 October 2017; received in revised form 12 January 2019; accepted 21 January 2019
Available online 20 February 2019

KEYWORDS
Autoimmune cytopenia;
Autoimmune hemolytic anemia;
Chronic lymphocytic leukemia;
Prognosis

Abstract
Objective/Background: The impact of autoimmune cytopenias (AICs) on the chronic lymphocytic leukemia (CLL) clinical course and its prognostic significance remain a matter of controversial debate. This could be due to exclusion of patients with cytopenia from most clinical trials for this particular complication and the lack of standard diagnostic criteria and treatment approaches. We herein evaluate the prevalence and the prognostic significance of AICs among patients with CLL.
Methods: This is an observational retrospective study. Data on 101 patients with CLL were derived from the Oncology Center, Mansoura University, Egypt, database, which contains information on demographic and clinical characteristics at diagnosis and follow-up records.
Results: The prevalence of immune cytopenias was 11.9% among patients studied. Autoimmune hemolytic anemia was the most common autoimmune form in patients with cytopenia due to pure immune etiology (C immune group) with a prevalence of 6.9%. Patients with AICs and those in the C immune subgroup presented with more unfavorable parameters. Besides, patients with AICs showed lesser response to treatment and on restaging after initial treatment, significantly more patients without AICs moved to a more favorable stage. However, no parallel significant difference in the overall survival was found between patients without AICs and those with AICs or with immune and combined or infiltrative cytopenia.

* Corresponding author at: The Hematology Unit, Oncology Center, Faculty of Medicine, Mansoura University, Mansoura, Egypt.
E-mail address: mohmabed@mans.edu.eg (M. Mabed).

https://doi.org/10.1016/j.hemonc.2019.01.004

Conclusion: We have shown a prevalence of 11.8% for AIC among our CLL patients. AIC was associated with unsatisfactory normalization of the hematological parameters even with therapy and lower number of patients with CLL downstaging in comparison with patients without AIC. These results suggest that AIC is a fingerprint of a biologically more aggressive disease even if no significant impact on overall survival was found.

Introduction

Chronic lymphocytic leukemia (CLL) is characterized by a marked clinical heterogeneity, ranging from years of stable disease to rapidly progressive disease [1]. Many staging systems and prognostic parameters are available to address the severity of disease and its outcome. Autoimmune cytopenia (AIC) has been reported since 1960s as a common complication of CLL [2]. AIC can occur in many clinical forms: autoimmune hemolytic anemia (AIHA), immune thrombocytopenia (ITP), Evan syndrome, pure red cell aplasia (PRCA), and autoimmune granulocytopenia [AIG] [3]. However, the overview of CLL-associated cytopenia as a prognostic factor has changed over the years [4]. The old staging systems, namely, the Rai and the Binet systems, considered cytopenias (irrespective to their etiology) to be associated with an advanced disease stage yielding poor prognosis [5,6].

By contrast, the new guidelines of National Cancer Institute-International Workshop on Chronic Lymphocytic Leukemia (NCI-IWCLL) have respected the etiology of the cytopenia in defining the signs of advanced disease and in allocation of the treatment indications. They considered the cytopenia due to bone marrow (BM) infiltration and the refractory immune cytopenia as indications for chemotherapy. The other correctable causes of cytopenia such as iron, vitamin B_{12}, and folate deficiency and simple AIC responding to treatment are not associated with poor prognosis [7]. AIC may be an expression of CLL aggressiveness. A strong association has been observed between AIC and various adverse clinical, laboratory, or biological prognostic features, such as older age, advanced stage, high white cell count, short lymphocyte doubling time, increased serum β_2-microglobulin (B2M) levels, CD38, and ZAP-70 positivity [8].

The aim of this study was to assess the prevalence and the prognostic impact of AICs in a sample of patients with CLL who attended the Hematology Unit, Mansoura University, Egypt.

Patients and methods

Study design and sample size

This is an observational retrospective study that was approved by the Institutional Review Board of the Faculty of Medicine, Mansoura University, Egypt. It was conducted on 101 patients with CLL who attended the Hematology Unit of the Oncology Center, Mansoura University (OCMU), Egypt, between January 2013 and June 2016. All data were derived from the OCMU database, which contains information on demographic and clinical characteristics at diagnosis and follow-up records.

Methods

The enrolled patients were sequentially diagnosed and various data were collected and recorded. Immunophenotyping using a complete panel of markers (CD2, CD5, CD19, CD22, CD23, CD79b, sIgM, FMC7) was used to confirm diagnosis of CLL based on the scoring system (score should be $\geq$ 3/5) [9]. CD38 and ZAP-70 were tested for their prognostic value. CD38 expression by peripheral blood CLL cells was assessed by immunofluorescence with anti-CD5-fluorescein isothiocyanate, anti-CD19-phycoerythrin (PE), and anti-CD38-PE-Cy5 using appropriate isotype-matched controls. ZAP-70 protein was analyzed on cells isolated from the peripheral blood of patients with CLL by flow cytometric assessment as previously described [10]. After gating the lymphocyte population according to CD45/SSC characteristics, the $CD5^+/19^+$ cells were gated. The isotype control was used to set the cutoff for the ZAP-70-negative region. Results were expressed as a percentage of $CD5^+/19^+$ cells, with results $\geq$20% considered positive. BM aspiration and BM biopsy were performed to assess BM cellularity and percentage and pattern of BM infiltration by small lymphocytes. Direct antiglobin test (DAT) was performed at presentation and repeated when the patients with negative DAT presented with hemolytic anemia during the course of the disease or after chemotherapy.

Diagnostic criteria

CLL diagnosis was made according to NCI-IWCLL guidelines [7]. The diagnosis of AIHA was based on the presence of an unexplained hemoglobin (HB) level $\leq$ 10 g/dL or hematocrit $\leq$ 30%, positive DAT for either immunoglobulin G or C3, and the presence of $\geq$1 of the indirect markers of hemolysis (high reticulocyte count, low serum haptoglobin levels, increased serum lactate dehydrogenase [LDH], and increased indirect bilirubin levels). For patients in whom DAT was negative, the diagnosis of AIHA was made if $\geq$2 of the indirect signs of hemolysis were present. ITP was diagnosed when there is sudden and/or unexplained fall in platelet count to $\leq 100 \times 10^9$/L and the presence of $\geq$2 of the following: evidence of normal BM function (normal or increased megakaryocytes in BM), no splenomegaly, and no chemotherapy within the last month from study entry [8].

Classification of the patients

The patients were classified according to the Binet system to include five patients in Stage A, 37 patients in Stage B, and 59 patients in Stage C. Clinical and laboratory data of the enrolled patients were further recollected after initial therapy to re-evaluate their response to treatment.

Treatment strategy

The enrolled patients received different treatment approaches that were tailored for each according to the treatment indications, stage, and their comorbidities. These approaches were as follows: no treatment (watch and wait approach when there is no indication for treatment); steroids only (applied to patients with immune cytopenias associated with quiescent CLL); and chemotherapy only with fludarabine-based regimen plus rituximab or steroids plus chemotherapy, which was indicated for patients with progressive disease aged less than 65 years without significant comorbidities. The NCI-IWCLL criteria were used to define response to treatment [11].

Statistical analysis

All statistical analysis of data was performed using the Statistical Package for Social Science program (SPSS version 20; SPSS Inc., Chicago, IL, USA). Kolmogorov–Smirnov test was performed to test the normality of data distribution. Qualitative data were presented as frequency and percentage. Chi-square or Fisher exact tests were used to compare groups. Quantitative data were presented as mean, standard deviation, median, and range. For comparison between two groups, Student *t* test and Mann–Whitney test (for nonparametric data) were used. For comparison between more than two groups, analysis of variance and Kruskal–Wallis test (for nonparametric data) were used. Kaplan–Meier test was used for survival analysis and the statistical significance of differences among curves was determined by the log-rank test. All *p* values less than .05 were considered statistically significant.

Results

Prevalence and types of AICs among the study group

The study was conducted on 101 patients with CLL. A total of 59 patients (58.4%) presented with cytopenias (Stage C) at diagnosis. Cytopenias due to pure immune etiology (C immune) were found in 12 patients (11.9%), whereas cytopenias due to BM infiltration (C infiltrative) were found in 28 patients (27.7%). Combined immune and infiltrative etiologies (C combined) were found in 19 patients (18.8%). Among the 31 patients with cytopenias related to an immune etiology, AIHA was diagnosed in 20 (19.8%), ITP in three (3%), and Evan syndrome in eight (7.9%). AIHA, ITP, and Evan syndrome were reported in seven, three, and two patients in the C immune subgroup, respectively, whereas only AIHA and Evan syndrome were found in 13 patients (68.4%) and six patients (31.6%) in the C combined subgroup, respectively (Table 1).

Patient's demographic characteristics in both AIC and no AIC groups at the time of diagnosis

Characteristics of study patients are presented in Table 2. A total of 52 male (74.3%) and 18 female (25.7%) patients were without AIC, whereas 17 male (54.8%) and 14 female (45.2%) patients were with AIC. Patients with AIC presented with insignificantly higher TLC and ALC, but with significantly lower HB levels ($p < .001$) and platelet counts ($p = .02$). Those without AIC showed significantly lower LDH concentration and lower serum B2M level with *p* values of .001 and .04, respectively. The percentage of patients with positive CD38 and ZAP-70 expression were significantly higher in those with AIC. CD38 was tested in only 61 patients, with positive results reported in 23. ZAP-70 was tested in only 60 patients, with positive results reported in 33. In patients without AIC, 27% and 44.4% had positive CD38 and ZAP-70, respectively, whereas in those with AIC 54.2% and 70.8% presented with positive results ($p = .03$ and .04, respectively). Patients with AIC showed significantly less CLL BM infiltration compared with patients without AIC (19 patients; 61.3%; $p < .001$) with a significant lower median infiltration percentage of 65 (range, 10–95; $p = .01$). A significantly higher percentage of patients without AIC (64.3%) had diffuse infiltration pattern of the BM compared with 38.7% of patients with AIC ($p = .017$).

Laboratory data of patients with AIC versus patients without AIC and their outcome after initial therapy

A total of 83 patients were evaluated after initial treatment (25 patients with AIC and 58 patients without AIC). Patients with CLL without AIC had a significant decrease in TLC and ALC ($p \leq .001$), a significantly lower frequency of BM infiltration ($p = .002$), and a significant increase in HB concentration ($p = .001$). Patients with AIC had a significant decrease in TLC and ALC ($p = .006$ and .009, respectively). On comparing the responses of both groups, patients without AIC showed significantly lower TLC and ALC and significantly higher HB concentration when compared to those with AIC after initial treatment ($p = .006$, .005, and .01, respectively).

Response to initial treatment showed 26 patients (37.1%) without AIC having complete response (CR) and five patients (7.2%) experiencing partial response (PR). Ten patients with AIC (32.3%) showed CR and two (6.5%) showed PR. This difference was insignificant. Similarly, no significant difference was found in patients who showed stationary or progressive disease between the two groups.

All patients were restaged at the end of initial therapy. A total of 31 patients (44.3%) without AIC improved, 17 (24.3%) had stable disease, and 10 (14.3%) experienced progressive disease. Twenty-nine patients (41.4%) were in Stage A, 15 (21.4%) in Stage B, and 14 (20%) remained in Stage C. Therefore, 24 patients improved to Stage A from Stages B and C and seven patients improved to Stage B from Stage C. Among patients with AIC, 12 (35.5%) improved, nine (29%) remained stationary, and four (12.9%) experi-

Table 1 Prevalence and types of AIC among the study group.

Parameters	*N*	%
Total number	101	100
Presence of cytopenia		
No cytopenia (Stage A + B)	42 (5 + 37)	41.6
Cytopenia (Stage C)	59	58.4
Type of cytopenia		
Infiltration (C infiltrative)	28	27.7
Immune (C immune)	12	11.9
Combined (C combined)	19	18.8
Types of immune cytopenia (*N* = 31)		
AIHA	20	19.8
ITP	3	3
Evan	8	7.9
PRCA	0	0
AIG	0	0
C immune (*N* = 12)		
AIHA	7	58.4
ITP	3	25
Evan	2	16.7
C combined (*N* = 19)		
AIHA	13	68.4
ITP	0	0
Evan	6	31.6

Note. AIC = autoimmune cytopenia; AIG = autoimmune granulocytopenia; AIHA = autoimmune hemolytic anemia; ITP = immune thrombocytopenia; PRCA = pure red cell aplasia.

enced a progressive disease. On restaging, two patients (6.5%) improved to Stage A, 10 patients (32.3%) improved to Stage B, and 13 patients (41.9%) remained in Stage C. Upon comparing both groups, a significantly higher number of patients without AIC improved to Stage A ($p < .001$).

Demographic characteristics and laboratory data of Stage C patients during diagnosis

The subgroups of Stage C comprised: C infiltrative (28 cases), C immune (12 cases), and C combined (19 cases) (Table 3). The immune subgroup showed less TLC, ALC, HB level, and platelets count than the infiltrative subgroup. However, it showed higher LDH, serum B2M, and a higher percentage of cases expressing positive CD38 than the infiltrative subgroup. The combined subgroup showed significantly higher TLC, ALC, LDH, and serum B2M ($p = .04$, .05, .001, .019, respectively). Furthermore, the percentage of patients expressing positive CD38 and ZAP-70 were higher in the combined group (although not significant). Hypercellular BM was more frequent in the infiltrative and combined subgroups when compared with the immune subgroup ($p = .013$). The diffuse pattern of infiltration was significantly higher in the C infiltrative than in the combined subgroup ($p < .001$).

Laboratory findings of stage C patients and their course after initial therapy

After initial treatment, the C infiltrative subgroup showed a significant decrease in TLC and ALC ($p = .003$ and .004, respectively) as well as a significant increase in the HB level (Table 4). The combined subgroup showed a significant decrease in TLC and ALC ($p = .03$ and .04, respectively), whereas the immune subgroup did not show any significant improvement. On comparing these results among the three subgroups, no significant differences were found. Response rates after initial treatment were not significantly different among the three subgroups.

On restaging, the infiltrative group included 14 patients who improved (50%; 7 patients to Stage A and 7 patients to Stage B), the immune group had three (25%; to Stage B), and the combined group had nine (47.3%; 2 patients to Stage A and 7 patients to Stage B). The infiltrative subgroup showed the highest percentage of improved patients (although not significant).

Overall survival and progression-free survival of patients with AIC versus without AIC

The mean overall survival (OS) of all patients was 45.4 months with 95% confidence interval (CI) of 39.8—50.9. The mean OS of patients without AIC was 48.49 months with 95% CI of 41.5—55.5, whereas for patients with AIC this was 37.1 months with 95% CI of 28.9—45.2. However, no significant difference in OS was found between studied groups ($p = .097$; Fig. 1). Similarly, no significant progression-free survival (PFS) difference was seen between the two groups with mean PFS of 36.9 months (95% CI 40.1—50.5) for patients without AICs versus 34.7 months (95% CI 27.9—43.2) for patients with AICs ($p = .12$).

OS of the stage C patients

The mean OS was 40.7 months with 95% CI of 32.5—49 for the infiltrative subgroups, 34.3 months with 95% CI of 21.1—47.5 for the immune subgroup, and 38 months with 95% CI of 28.5—48.9 for the combined subgroups. No significant OS differences were found between the different subgroups ($p = .709$) (Fig. 2).

Discussion

CLL is a disease characterized by marked clinical heterogeneity with a wide range of possible complications that are related mainly to cellular and immune dysfunction. This immune dysfunction is manifested mainly as AICs [12]. AIHA occurs in 10—25% of cases. ITP, Evan syndrome, and PRCA are less common [13]. Several other autoimmune disorders have been observed to accompany CLL [14,15]. To our knowledge, there are no population-based studies about the prevalence of autoimmune complications in CLL in our country. Among our CLL sample, the prevalence of immune cytopenia was 11.9%. AIHA was the most common autoimmune form encountered in the C immune group with a

Table 2 Patients characteristics and the laboratory data of patients without AIC versus with AIC at time of diagnosis.

Parameters	All patients, *N* (%)	Without AIC, *N* (%)	With AIC, *N* (%)	*p*
	101 (100)	70 (69.3)	31 (30.7)	
Sex				
Male	69 (68.3)	52 (74.3)	17 (54.8)	.053
Female	32 (31.7)	18 (25.7)	14 (45.2)	.053
Age (y)				
Mean ± SD	60.9 ± 9.9	59.8 ± 9.9	61 ± 9.9	.580
CBC, median (range)				
TLC ($\times 10^9$/L)	75 (13.7—475)	56.5 (11.4—450)	65 (10—475)	.215
ALC ($\times 10^9$/L)	55 (6.3—425)	48.5 (6.5—425)	58 (6.3—300)	.331
HB (g/dL)	10 (4.4—16.4)	11 (4.4—16.4)	8.5 (4.9—14.5)	<.001
Platelets ($\times 10^9$/L)	129 (5—583)	138 (26—583)	111 (5—283)	.023
Chemistry, median (range)				
LDH (U/L)	420 (217—1954)	395 (217—1680)	630 (327—1954)	.001
β_2-Microglobulin (μg/dL)	415 (50—2745)	355 (50—2745)	810 (120—2717)	.04
Biological prognostic markers				
Positive CD38 (23/61)		10/37 (27)	13/24 (54.2)	.033
Positive ZAP-70 (33/60)		16/36 (44.4)	17/24 (70.8)	.044
BM cellularity				
Normocellular	15 (14.9)	9 (12.9)	6 (19.4)	.545
Hypercellular	86 (85.1)	61 (87.1)	25 (80.6)	
BM infiltration				
Free		3 (4.3)	12 (38.7)	<.001
Infiltrated		67 (95.7)	19 (61.3)	
Pattern of infiltration				
Focal	13 (12.9)	11 (15.7)	2 (6.5)	>.99
Diffuse	57 (56.4)	45 (64.3)	12 (38.7)	.017
Interstitial	16 (15.8)	11 (15.7)	5 (16.1)	>.99
Infiltration %, median (range)	75 (10—95)	75 (15—95)	65 (10—95)	.016

Note. ALC = absolute lymphocyte count; BM = bone marrow; CBC = complete blood count; HB = hemoglobin; LDH = lactate dehydrogenase; SD = standard deviation; TLC = total leukocyte count.

prevalence of 6.9%. Most studies concluded a prevalence range of 5—10% [15—17]. This wide range of prevalence was attributed to the lack of strict diagnostic criteria that hinder performing an accurate diagnosis. While AIHA is relatively easy to diagnose, CLL-associated ITP, PRCA, and AIG require more attention, as concomitant lymphocyte infiltration may cause an error in diagnosis. For this reason, ITP, PRCA, and AIG in patients with CLL are probably underestimated [17].

The impact of AIC on the CLL clinical course and its prognostic significance remain a matter of controversial debate. This could be due to exclusion of cytopenic patients from most clinical trials for this particular complication and the lack of standard diagnostic criteria and treatment approaches [18].

To assess the possible impact of AIC on the CLL outcomes, we compared patients without immune cytopenias with patients having cytopenias due to an immune etiology. Patients with AIC presented with more unfavorable parameters such as higher TLC and ALC (although not statistically significant) and significantly lower HB concentration and platelets counts. Moreover, significantly higher LDH, serum B2M, and percentage of cases with positive CD38 and ZAP-70 expression were observed in this group when compared with those without AIC. Similarly, Moreno et al. [8] and Shvidel et al. [16] concluded that AIC was associated with high blood lymphocyte count and unfavorable biomarkers, including increased LDH and B2M serum levels and high expression of CD38 or ZAP-70 in their studies.

For more clarification of the actual impact of pure immune cytopenia on the CLL presentation and course, we compared C infiltrative patients, C immune, and C combined patients. The C immune subgroup showed apparent higher LDH, B2M, and higher percentage of cases expressing positive CD38 than the infiltrative group, which is consistent with Duek et al. [13] who found a strong association between immune cytopenia and high levels of B2M and high CD38. In addition, Moreno et al. [8] reported a correlation of AIC with the high levels of CD38, ZAP-70, and B2M. Not surprisingly, the combined group was found to have the highest values with regard to TLC, ALC, LDH, B2M, and percentage of cases expressing positive CD38 and ZAP-70.

Table 3 Demographic and the laboratory data of stage C Patients at time of diagnosis.

Parameter	Stage C infiltrative ($N = 28$), n (%)	Stage C immune ($N = 12$), n (%)	Stage C combined ($N = 19$), n (%)	p
Sex				
Male	19 (67.9)	8 (66.7)	10 (52.6)	.580
Female	9 (32.1)	4 (33.3)	9 (47.4)	.580
Age (y), mean ± SD	62.2 ± 9.6	65.2 ± 8.1	58.4 ± 10.2	.148
CBC, median (range)				
TLC ($\times 10^9$/L)	60 (9.7—445)	42 (10—160)	110 (15—475)	.04
ALC ($\times 10^9$/L)	53 (5.8—227)	35 (6.3—145)	83 (11—300)	.05
HB (g/dL)	9 (4.4—14.5)	7 (5—11)	8.5 (4.9—14.5)	.05
Platelets ($\times$109/L):	90 (26—583)	73 (5—170)	116 (6—283)	.15
Chemistry, median (range)				
LDH (U/L)	402.5 (217—1680)	605 (380—1906)	630 (327—1954)	.001
β_2-Microglobulin (μg/dL)	312.5 (150—1765)	310 (120—710)	1900 (180—2717)	.019
Biologic prognostic markers, N (%)				
Positive CD38 (19/43)	6/19 (31.6)	2/6 (33.3)	11/18 (61.1)	.19
Positive ZAP-70 (33/60)	10/18 (55.6)	3/6 (50)	14/18 (77.8)	.36
BM cellularity				
Normocellular	2 (7.1)	5 (41.7)	1 (5.3)	.113
Hypercellular	26 (92.9)	7 (58.3)	18 (94.7)	.013
BM infiltration				
Free	0 (0)	12 (100)	0 (0)	<.001
Infiltrated	28 (100)	0 (0)	19 (100)	
Pattern of infiltration				
Focal	3 (10.7)	0 (0)	2 (5.3)	.234
Diffuse	21 (75)	0 (0)	12 (63.2)	<.001
Interstitial	4 (14.3)	0 (0)	5 (26.3)	.130

Note. ALC = absolute lymphocyte count; BM = bone marrow; CBC = complete blood count; HB = hemoglobin; LDH = lactate dehydrogenase; SD = standard deviation; TLC = total leukocyte count.

These findings indicate the severity of combined group presentation, which could be explained by the effect of both the infiltrative and immune elements.

After initial therapy, patients without immune cytopenia showed more improvement with significant response with regard to the BM infiltration, leukocytosis, lymphocytosis, and HB level. By contrast, patients with AIC showed less response (no significant improvement except the decrease in TLC and ALC). On restaging after initial treatment, 44.3% of the patients without AIC were improved compared with 38.7% of the patients with AIC, with significantly more patients without AIC improving to Stage A. The patients in the C immune subgroup showed no significant clinical or laboratory improvement, whereas those in the C infiltrative subgroup showed the best response with regard to the decrease in the TLC and ALC levels and the increase in the HB level. On restaging the patients after initial treatment, the immune subgroup showed the least percentage of improved patients when compared with the other two subgroups.

Despite the apparent more advanced presentation and course in the immune group, no parallel significant difference in the OS was found between patients without AIC and patients with AIC or between patients with immune and combined or infiltrative cytopenia. AIHA has been associated with active disease, but without a negative impact on survival [19]. Visco et al. [20] reported an association between ITP and U-CLL with a possible inferior outcome. Interestingly, the same group has demonstrated a similar association between an unmutated *IgHV* gene and AIHA without a negative impact on survival [20]. In addition, other studies reported that AIHA had a negative impact on OS [16,21]. Besides, AIHA had an independent negative effect on the survival rate (dropped from 58% to 37% with the occurrence of AIHA) [21]. Shvidel et al. [16] concluded that AIHA was associated with more progression and a worse prognosis. Furthermore, both studies found that patients with positive DAT even without hemolysis had a worse survival compared with those with a negative DAT.

Contradictory to these results, Zent et al. [22] and Kyasa et al. [23] reported that patients with AIC have a significantly better prognosis than those with cytopenia due to BM infiltration. Moreover, the development of AIC at any time of the disease course did not significantly influence the prognosis [8].

Our study has some limitations. First, *IgHV* testing was performed for only few patients despite the recognized importance of genetic testing in determining the CLL prognosis and management. Hence, these data were insufficient

Table 4 Laboratory findings of stage C patients and their course and outcome after initial therapy.

Parameter	Stage C infiltrative			Stage C Immune			Stage C combined			*p*
	At diagnosis (*N* = 28)	After therapy (*N* = 21)	*p*	At diagnosis (*N* = 12)	After therapy (*N* = 8)	*p*	At diagnosis (*N* = 19)	After therapy (*N* = 17)	*p*	
TLC ($\times 10^9$/L), median (range)	60 (9.7—445)	18 (6—163)	.003	42 (10—160)	13.25 (4—99)	.09	110 (15—475)	55.5 (5—190)	.03	.06
ALC ($\times 10^9$/L), median (range)	53 (5.6—227)	16 (3.5—154)	.004	35 (6.3—145)	7.9 (2—88)	.09	83 (11—300)	44.5 (2—145)	.04	.06
HB (g/dL), median (range)	9 (4.4—14.5)	10.5 (4.6—15)	.005	7 (5—11)	12.5 (4.5—13.6)	.09	8.5 (4.9—14.5)	10 (6.4—14)	.38	.44
Platelets ($\times 10^9$/L), median (range)	90 (26—583)	104 (45—405)	.945	73 (5—170)	127.5 (14—264)	>.99	116 (6—283)	120 (11—290)	.61	.98
BM infiltration, *n* (%)	28 (100)	18 (64)	.25	3 (25)	4 (33.3)	.5	18 (94.7)	15 (78.9)	>.99	.06
Response to initial treatment, microglobulin, *n* (%)										
CR	10 (35.7)			2 (16.7)			7 (36.8)			.201
PR	4 (14.3)			1 (8.3)			2 (10.5)			.103
SD	5 (17.9)			5 (41.7)			4 (21.1)			.189
PD	2(7.1)			0 (0)			4 (21.1)			.121
Restaging after initial treatment, *n* (%)										
A	7 (25)			0 (0)			2 (10.5)			.312
B	7 (25)			3 (41.7)			7 (36.8)			.861
C	7 (25)			5 (25)			8 (42.1)			.471
Lost	7 (25)			4 (33.3)			2 (10.5)			.562

Note. BM = bone marrow; CR = complete response; HB = hemoglobin; PR = partial response; SD = stable disease; PD = progressive disease.

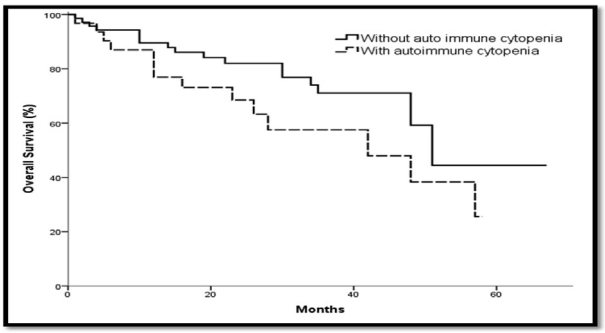

Fig. 1 The overall survival of patients with AIC versus without AIC.

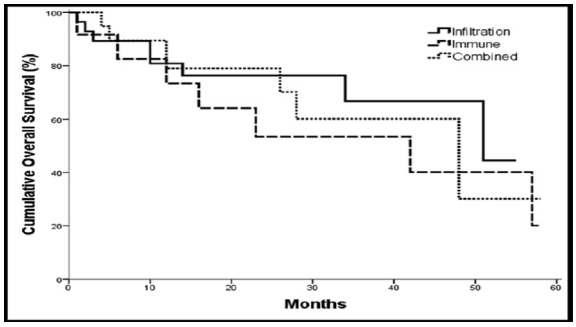

Fig. 2 The overall survival of stage C patients.

to perform meaningful analysis. The other limitation is the low number of events in patients with immune cytopenias.

In conclusion, we have shown a prevalence of 11.8% for AIC among our CLL patients. AIC was associated with unsatisfactory normalization of the hematological parameters even with therapy and lower number of patients with CLL downstaging in comparison to patients without AIC. These results suggest that AIC is a fingerprint of a biologically more aggressive disease even though no significant impact on OS was found.

Conflicts of interest

The authors have no conflict of interest.

References

[1] Chiorazzi N, Rai KR, Ferrarini M. Chronic lymphocytic leukemia. N Engl J Med 2005;352:804—15.
[2] Hodgson K, Ferrer G, Montserrat E, Moreno C. Chronic lymphocytic leukemia and autoimmunity: a systematic review. Haematologica 2011;96:752—61.
[3] Visco C, Barcellini W, Maura F, Neri A, Cortelezzi A, Rodeghiero F. Autoimmune cytopenias in chronic lymphocytic leukemia. Am J Hematol 2014;89:1055—62.
[4] Visco C, Cortelezzi A, Moretta F, Falisi E, Maura F, Finotto S, et al. Autoimmune cytopenias in chronic lymphocytic leukemia at disease presentation in the modern treatment era: is stage C always stage C? Leuk Lymphoma 2014;55:1261—5.
[5] Rai KR, Sawitsky A, Cronkite EP, Chanana AD, Levy RN, Pasternack BS. Clinical staging of chronic lymphocytic leukemia. Blood 1975;46:219—34.
[6] Binet JL, Leporrier M, Dighiero G, Charron D, D'Athis P, Vaugier G, et al. A clinical staging system for chronic lymphocytic leukemia: prognostic significance. Cancer 1977;40:855—64.
[7] Hallek M, Cheson BD, Catovsky D, Caligaris-Cappio F, Dighiero G, Döhner H, et al. Guidelines for the diagnosis and treatment of chronic lymphocytic leukemia: a report from the International Workshop on Chronic Lymphocytic Leukemia updating the National Cancer Institute-Working Group 1996 guidelines. Blood 2008;111:5446—56.
[8] Moreno C, Hodgson K, Ferrer G, Elena M, Filella X, Pereira A, et al. Autoimmune cytopenia in chronic lymphocytic leukemia: prevalence, clinical associations, and prognostic significance. Blood 2010;116:4771—6.
[9] Matutes E, Polliack A. Morphological and immunophenotypic features of chronic lymphocytic leukemia. Rev Clin Exp Hematol 2000;4:22—47.
[10] Shankey TV, Forman M, Scibelli P, Cobb J, Smith CM, Mills R, et al. An optimized whole blood method for flow cytometric measurement of ZAP-70 protein expression in chronic lymphocytic leukemia. Cytometry B Clin Cytom 2006;70:259—69.
[11] Hallek M, Cheson BD, Catovsky D, Caligaris-Cappio F, Dighiero G, Döhner H, et al. Guidelines for diagnosis, indications for treatment, response assessment and supportive management of chronic lymphocytic leukemia. Blood 2018;131:2745—60.
[12] Gribben JG. How I treat CLL up front. Blood 2010;115:187—97.
[13] Duek A, Shvidel L, Braester A, Berrebi A. Clinical and immunologic aspects of B chronic lymphocytic leukemia associated with autoimmune disorders. Isr Med Assoc J 2006;8:828—31.
[14] Pritsch O, Maloum K, Dighiero G. Basic biology of autoimmune phenomena in chronic lymphocytic leukemia. Semin Oncol 1998;25:34—41.
[15] Hamblin TJ. Autoimmune complications of chronic lymphocytic leukemia. Semin Oncol 2006;33:230—9.
[16] Shvidel L, Tadmor T, Braester A, Bairey O, Rahimi-Levene N, Herishanu Y, et al. Pathogenesis, prevalence, and prognostic significance of cytopenias in chronic lymphocytic leukemia (CLL): a retrospective comparative study of 213 patients from a national CLL database of 1,518 cases. Ann Hematol 2013;92:661—7.
[17] D'Arena G, Guariglia R, La Rocca F, Trino S, Condelli V, De Martino L, et al. Autoimmune cytopenias in chronic lymphocytic leukemia. Clin Dev Immunol 2013;8:730131.
[18] Quinquenel A, Al Nawakil C, Baran-Marszak F, Eclache V, Letestu R, Khalloufi M, et al. Old DAT and new data: positive direct antiglobulin test identifies a subgroup with poor outcome among chronic lymphocytic leukemia stage A patients. Am J Hematol 2015;90:E5—8.
[19] Mauro FR, Foa R, Cerretti R, Giannarelli D, Coluzzi S, Mandelli F, et al. Autoimmune hemolytic anemia in chronic lymphocytic leukemia: clinical, therapeutic, and prognostic features. Blood 2000;95:2786—92.
[20] Visco C, Novella E, Peotta E, Paolini R, Giaretta I, Rodeghiero F. Autoimmune hemolytic anemia in patients with chronic lymphocytic leukemia is associated with IgVH status. Haematologica 2010;95:1230—2.
[21] Dearden C, Wade R, Else M, Richards S, Milligan D, Hamblin T, et al. The prognostic significance of a positive direct antiglobulin test in chronic lymphocytic leukemia: a beneficial effect of the combination of fludarabine and cyclophosphamide on the incidence of hemolytic anemia. Blood 2008;111:1820—6.
[22] Zent CS, Ding W, Schwager SM, Reinalda MS, Hoyer JD, Jelinek DF, et al. The prognostic significance of cytopenia in chronic lymphocytic leukaemia/small lymphocytic lymphoma. Br J Haematol 2008;141:615—21.
[23] Kyasa MJ, Parrish RS, Schichman SA, Zent CS. Autoimmune cytopenia does not predict poor prognosis in chronic lymphocytic leukemia/small lymphocytic lymphoma. Am J Hematol 2003;74:1—8.