Impact of interleukin-10 polymorphisms (-1082 and –3575) on the survival of patients with lymphoid neoplasms

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ABSTRACT

Background and Objectives

Single-nucleotide polymorphisms (SNP) in interleukin-10 (IL-10) genes can influence immune responses, which may affect the outcome of patients with lymphoid neoplasms. The aim of this study was to explore the association between polymorphisms of IL-10-$_{1082A>G}$ and IL-10-$_{3575T>A}$ with the overall survival in patients with lymphoid neoplasms.

Design and Methods

We analyzed two IL-10 SNP (-1082 and –3575) in 472 consecutive cases with lymphoid neoplasms. Genotypes were tested for association with overall survival and classical prognostic factors by multivariate analysis. Haplotype analysis was carried out using the haplostats package implemented in R software. The implications for survival of patients with lymphoma were evaluated using multivariate analysis.

Results

Lymphoma patients with the IL-10-$_{3575T>A}$ genotype had a better overall survival (p=0.002), as did the subgroup with non-Hodgkin's lymphoma (NHL) (p=0.05). Patients with the IL10-$_{1082GG}$ genotype had a better median overall survival (p=0.05). When both genotypes were included in a multivariate analysis, IL-10-$_{3575AA}$ genotype was the only independent prognostic factor for survival (HR=0.20, 95%CI 0.05-0.92). Patients with the IL-10-$_{1082}$ and $_{-3575}$ G-A/G-A diplotype had a longer overall survival (p=0.003) and this combination appeared to be an independent prognostic factor for survival (HR:0.26; 95%CI 0.08-0.83).

Interpretation and Conclusions

The IL-10-$_{3575A/A}$ genotype was identified as a marker of favorable survival. Because the IL-10-$_{1082}$ and $_{-3575}$ G-A/G-A diplotype was also identified as an indicator of longer survival, we cannot exclude the potential additive role of the IL-10-$_{1082GG}$ genotype. These results need to be replicated in larger series and examined in different NHL subtypes.

Key words: IL-10 polymorphisms, lymphoid neoplasms, survival.

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Interleukin-10 (IL-10) is a cytokine normally produced by activated T cells, monocytes, B cells and thymocytes. It contributes to antigen- or mitogen-driven B-cell differentiation and acts as a growth factor. IL-10 is also an immune response modulator. Dysfunctions in the immune system are thought to be the underlying basis of lymphomagenesis.[1] Several studies have shown the possible involvement of IL-10 in the pathogenesis of lymphoid malignancies, as well as its association with prognosis.[2-4] Dysregulation of IL-10 production appears to play a role in lymphoproliferative disease and it is hypothesized that polymorphisms in the IL-10 gene promoter, which predispose an individual to high IL-10 levels, may be more frequent in patients with lymphoma than among the normal population. It has also been described that patients with high IL-10 production have a worse prognosis.[3,5,6] A number of polymorphisms of the IL-10 gene promoter have been described. These include two biallelic polymorphisms at positions –1082 (G to A substitution), and -3575 (T to A substitution). Briefly, IL-10$_{-1082A\rightarrow G}$ is localized in the proximal region of the IL-10 promoter between –4kb and –1.1 kb and IL-10$_{-3575T\rightarrow A}$ is localized in the distal region of the promoter between –1.3 kb and –4.0 kb. A number of studies have reported that IL$_{-10-1082G}$[7] and IL$_{-10-3575T}$ alleles are associated with high IL-10 production.[8] Previous studies have evaluated whether polymorphisms in IL-10-$_{3575T\rightarrow A}$ and IL-10-$_{1082A\rightarrow G}$[9] are associated with an increased risk of non-Hodgkin's lymphomas. Cunningham *et al.*[9] observed that the frequency of the low IL-10 producing AA allele (at position –1082) was significantly higher in patients with aggressive lymphomas compared to in controls. However, in a larger study group, Lech-Maranda E *et al.*[2] found that the frequency of IL-10-$_{1082G}$ allele was higher in patients with diffuse large B-cell lymphoma than in controls. Most recently, Rothman *et al.*[10] identified genotypes AA or TA at position -3575 of IL-10 to be associated with an increased risk of non-Hodgkin's lymphoma in the largest pooled analysis to date. Few studies have yet analyzed the impact of IL-10 polymorphisms on the prognosis of patients with lymphoma. Only one, by Lech-Maranda E *et al.*,[2] found that patients carrying the IL-10$_{-1082G}$ allele had higher complete remission rates, 5-year freedom from progression, and better overall survival when compared to patients carrying the IL10-$_{1082AA}$ genotype.

The aim of this study was, therefore, to explore the association between polymorphisms of IL-10-$_{1082A\rightarrow G}$ and IL-10-$_{3575T\rightarrow A}$ with the overall survival in patients with lymphoid neoplasms.

Design and Methods

Patients

The study was based on data from the Epilymph study, a case-control study conducted in four Spanish centers: Barcelona, Tortosa, Reus and Madrid. A detailed description of the study population, composition, and interviews has been given elsewhere.[11] The cases comprised 561 consecutive non-immunosuppressed patients newly diagnosed with a lymphoid malignancy between 1998 and 2002. The diagnosis of lymphoma was confirmed by histology and in most cases by supplementary immunohistochemistry tests. A diagnosis of multiple myeloma (MM) was based on cytology and cytometry in addition to clinical parameters. A diagnosis of chronic lymphocytic leukemia (CLL) was based on cytology and cytometry.[1] Cases were categorized according to the WHO classification.[12] A board of pathologists, not involved in the original diagnoses, re-reviewed 20% of the lymphomas within each histological category as well as all cases with a diagnosis of not-otherwise-specified (NOS) lymphoma, with an agreement over 90%.

All patients provided a blood sample at diagnosis after informed consent had been obtained. The Institutional Review Boards of the participating centers approved the study.

Clinical data

The initial medical evaluation consisted of a complete history and physical examination. Blood tests as well as chest X-rays, thoracic and abdominal computed tomography and bone marrow biopsy or aspirate were performed at diagnosis. The extent of disease was categorized according to the Rai classification in CLL patients,[13] the Durie-Salmon classification for MM patients[14] and the Ann Arbor classification for patients with the remaining neoplasms.[15] Performance status was assessed using the Eastern Cooperative Oncology Group (ECOG) criteria.[16] Overall survival was determined from the date of the diagnosis of the lymphoma until the last follow-up evaluation or death.

Cases were actively followed-up from diagnosis to January 2005. The median follow-up time was 43 months (range, 17 to 65 months) and in this period, 169 (30%) deaths occurred.

Laboratory analysis

We chose two single nucleotide polymorphisms (SNP) (minor allele frequency range 0.02-0.44), each of which could be functionally important in the 1q31-32 gene: IL10–$_{1082A\rightarrow G}$ (rs1800896) and IL10–$_{3575T\rightarrow A}$ (rs1800890). DNA samples were analyzed at one laboratory using a Taqman™ platform (Applied Biosystems, Foster City, CA, USA) for genotyping. Sequence data and assay conditions for the Taqman™ assays are available on the NCI SNP500 website *http://snp500cancer.nci.nih.gov*. Further data regarding the genotype analysis done in our study patients have been described elsewhere.[10]

Statistical analysis

Each polymorphism was tested to ensure that it fitted the Hardy-Weinberg equilibrium. For the clinical or bio-

Table 1. Characteristics of the study population according to the genetic variants of IL-10$_{-3575T>A}$ and IL-10$_{-1082A>G}$.

		IL-10$_{-3575}$ n=463 (%)			*IL-10$_{-1082}$ n=464 (%)*		
	n (%)	*TT*	*TA*	*AA*	*AA*	*AG*	*GG*
Gender							
Male	264 (55.9)	137 (59.3)	102 (51.8)	21 (60)	97 (58.8)	129 (55.8)	35 (51.5)
Female	208 (44.1)	94 (40.7)	95 (48.2)	14 (40)	68 (41.2)	102 (44.2)	33 (48.5)
p-value			0.262	0.583			
Age							
< 65 yrs	234 (49.6)	109 (47.2)	104 (52.3)	18 (51.4)	80 (48.5)	115 (49.8)	34 (50)
≥ 65 yrs	238 (50.4)	122 (52.8)	94 (47.7)	17 (48.6)	85 (51.5)	116 (50.2)	34 (50)
p-value			0.562	0.962			
Center							
BCN	361 (76.5)	185 (80.1)	139 (70.6)	29 (82.9)	131 (79.4)	168 (72.7)	56 (81.0)
Madrid	63 (13.4)	28 (12.1)	29 (14.7)	6 (17.1)	20 (12.1)	33 (14.3)	8 (11.8)
Tarragona	48 (10.2)	18 (7.8)	29 (14.7)	0 (0%)	14 (8.5)	30 (13.0)	4 (5.9)
p-value			0.024	0.309			
Lymphoma sub-entities							
Chronic lymphocytic leukemia	110 (23.3)	61 (26.4)	41 (20.8)	7 (20.0)	39 (23.6)	58 (25.1)	12 (17.7)
Lymphoplasmacytic lymphoma	19 (4.0)	6 (2.6)	9 (4.6)	4 (11.4)	5 (3.0)	8 (3.5)	4 (5.9)
Splenic marginal zone lymphoma	20 (4.2)	10 (4.3)	9 (4.6)	1 (2.9)	6 (3.6)	10 (4.3)	3 (4.4)
Multiple myeloma	69 (14.6)	38 (16.5)	26 (13.2)	5 (14.3)	24 (14.6)	34 (14.7)	10 (14.7)
Marginal zone B-cell lymphoma	20 (4.2)	5 (2.2)	10 (5.1)	4 (11.4)	4 (2.4)	10 (4.3)	6 (8.8)
Follicular lymphoma	37 (7.8)	17 (7.4)	17 (8.6)	1 (2.9)	15 (9.1)	16 (6.9)	5 (7.4)
Diffuse large B-cell lymphoma	77 (16.3)	33 (14.3)	38 (19.3)	4 (11.4)	26 (15.8)	37 (16.0)	12 (17.7)
Other B-cell lymphoma*	34 (7.2)	20 (8.7)	10 (5.1)	3 (8.6)	16 (9.7)	12 (5.2)	6 (8.8)
Hodgkin's lymphoma	52 (11.0)	25 (10.8)	22 (11.2)	4 (11.4)	18 (10.9)	29 (12.6)	5 (7.4)
Mycosis Fungoides/Sezary's lymphoma	15 (3.2)	10 (4.3)	4 (2.0)	1 (2.9)	8 (4.9)	4 (1.7)	3 (4.4)
Other T-cell lymphomas**	19 (4.0)	6 (2.6)	11 (5.6)	1 (2.9)	4 (2.4)	13 (5.6)	2 (2.9)
	0.182	0.595					
IL-10$_{-1082}$***							
AA	165 (35.6)	162 (70.1)	2 (1.0)	–			
AG	231 (49.8)	61 (26.4)	165 (85.1)	1 (3.3)			
GG	68 (14.7)	8 (3.5)	27 (13.9)	29 (96.7)			
p-value				<0.0001			

Other B-cell lymphomas include: mantle cell lymphomas (n=9), precursor B-lymphoblastic lymphoma/leukemia (n=9), hairy cell leukemia (n=2), high grade B-cell Burkitt-like lymphoma (n=3), Burkitt's lymphoma (n=1), low grade lymphoma B not otherwise specified (NOS) (n=5), high grade lymphoma B NOS (n=1), B lymphoma NOS (n=4). Other T-cell lymphomas include: anaplastic large cell lymphoma CD30+ (n=7), large granular lymphocytic leukemia (n=2), angioimmunoblastic T-cell lymphomas (n=3), angiocentric lymphoma (n=3), anaplastic large cell lymphoma Hodgkin-like (n=1), peripheral T-cell lymphomas (n=2), T- lymphoma NOS (n=1) p refers to the χ^2 test.

logical features, *p* values were calculated using the χ^2 test. Survival probabilities were estimated using the Kaplan-Meier method, and differences between individual curves were evaluated by the log-rank test. Analyses were adjusted for age, sex, center of recruitment, lactate dehydrogenase (LDH) levels, ECOG performance status, and disease stage. Hazard ratios (HR) and 95% confidence intervals (CI) were estimated. Likelihood ratio tests were used to assess the prognostic value of the genotypes and haplotypes. Genotypes for each SNP were analyzed as a three-group categorical variable (co-dominant model), and were also grouped according to the dominant and recessive models. Haplotype frequencies were estimated using the statistical package haplo.stats (*http://mayoresearch.mayo.edu/mayo/research/schaid_lab/software.cfm*), in R software (version 2.4.1).[17] This program reconstructs haplotypes based on an expectation-maximization algorithm.[18] Posterior probabilities for each compatible haplotype were used as weights in the Cox models to account for uncertainty in the identification of phase-unknown haplotypes.

Results

Of the 561 consecutive, non-immunosuppressed cases included in the Epilymph Spain study, IL-10$_{-3575T\rightarrow A}$ and IL-10$_{-1082A\rightarrow G}$ were evaluated in 463 (83%) and 464 (83%) subjects, respectively, of the 472 subjects for whom survival information was available. A case-control analysis of IL-$_{10-3575T>A}$ and IL-$_{10-1082A\rightarrow G}$, which included the cases presented here, was recently published by Rothman *et al.*[10]

The distribution of IL10-$_{3575T\rightarrow A}$ and IL10-$_{1082A\rightarrow G}$ according to age, gender, participating center, and lymphoma category is shown in Table 1. No differences in the genetic distribution were seen according to age groups or gender. Subjects from Tarragona were more likely to have a TA IL-10-$_{3575T\rightarrow A}$. Homozygous subjects for the minor allele of IL10-$_{3575AA}$ were more likely to be also homozygous for the minor allele of IL10-$_{1082GG}$ (97%, $p<0.0001$). Age <65 years, ECOG status 0-2, localized disease, normal LDH level, normal β_2-microglobulin level, and absence of B-symptoms were associated with a better

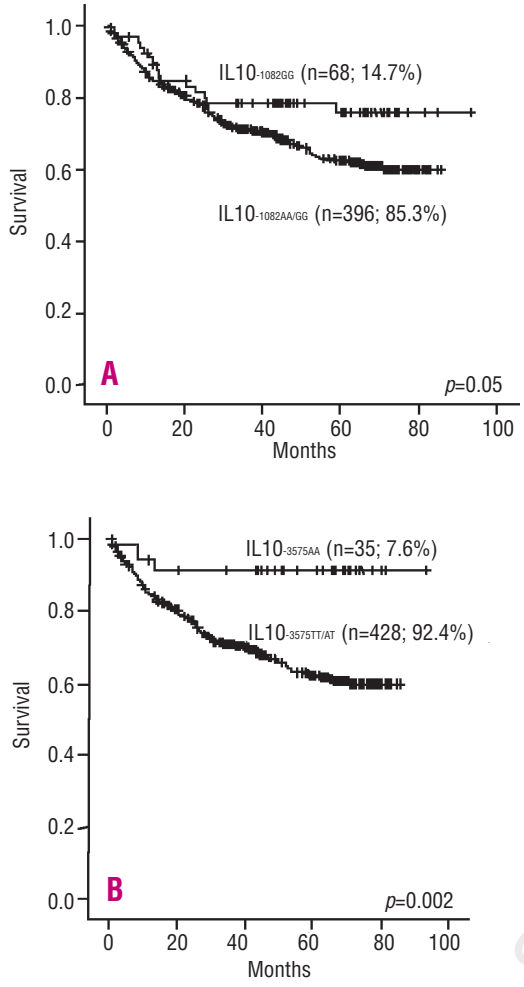

Figure 1 (left). Kaplan-Meier overall survival estimate according to IL10-1082 and IL10-3575 polymorphisms. Kaplan-Meier analysis for the recessive model was performed including all lymphoid neoplasms; (A) Analysis for IL10-$_{1082A>G}$ polymorphism (B) Analysis for IL10-$_{3575T>A}$ polymorphism; *p* refers to log-rank test.

overall survival (*data not shown*), as were IL-10-$_{3575AA}$ and IL-10-$_{1082GG}$ genotypes (Figure 1), although the association between IL-10-$_{1082A\rightarrow G}$ and survival was of borderline statistical significance. The association between overall survival and IL-10-$_{3575AA}$ genotype was also observed in the CLL, myeloma and non-Hodgkin's lymphoma groups and it was almost statistically significant in the last two (Figure 2). For the other lymphoma subtypes numbers were too small for evaluation. A significant association was observed between IL-10-$_{3575\ T\rightarrow A}$ genotypes and ECOG status: patients with the IL-10-$_{3575AA}$ genotype were more likely to have ECOG status 0-2 than patients with IL-10-$_{3575TT}$ and TA genotypes (82.9%; 61.9%; 69.3%; p= 0.034). However, differences in survival among subjects in each of the IL-10-$_{3575T\rightarrow A}$ genotype groups could not be explained by ECOG status (*data not shown*) and associations with prognosis were independent of patients' and clinical characteristics, as described below. Furthermore, IL-10-$_{1082A\rightarrow G}$ was not associated with established prognostic factors.

Association with prognosis

Table 2 shows the univariate HR and 95% CI adjusted for age, sex, center of recruitment, ECOG status, LDH levels and stage. The strongest association was observed

Table 2. Univariate and multivariate Cox models for IL10-$_{1082A>G}$ and IL10-$_{3575T>A}$ polymorphisms adjusted by prognostic factors for survival.

	Cases, n (%)	*Univariate deaths, n (%)*	*Hazard Ratio*	*Multivariate 95% CI*	*Hazard Ratio**	*95% CI*
ALL Lymphoma						
IL-10-$_{1082A>G}$ genotypes n=411						
Recessive Model						
AA+AG	348 (84.7)	235 (67.5)	1			
GG	63 (15.3)	49 (77.8)	0.73	(0.41-1.27)	1.25	(0.68-2.27)
IL-10-$_{3575T>A}$ genotypes n=411						
Recessive Model						
TT+TA	378 (92.0)	124 (32.8)	1		1	
AA	33 (8.0)	3 (9.1)	0.33	(0.10-1.06)	0.20	(0.05-0.92)
Non-Hodgkin's lymphomas						
IL-10-$_{1082A>G}$ genotypes n=206						
Recessive Model						
AA+AG	175 (82.6)	65 (37.1)	1		1	
GG	37 (17.5)	10 (27.0)	0.72	(0.37-1.42)	1.07	(0.50-2.27)
IL-10-$_{3575T>A}$ genotypes n=206						
Recessive Model						
TT+TA	195 (92.0)	72 (36.9)	1		1	
AA	17 (8.0)	3 (17.7)	0.65	(0.19-2.15)	0.41	(0.08-2.04)

*Non Hodgkin's lymphoma exclude chronic lymphoytic leukemia, multiple myeloma and Hodgkin's lymphomas. Cox models were adjusted for age, sex, center of recruitment, LDH, ECOG status and disease stage. *Model that includes both polymorphisms in addition to adjustment for age, sex, center of recruitment, LDH levels, ECOG status, and diesease stage.*

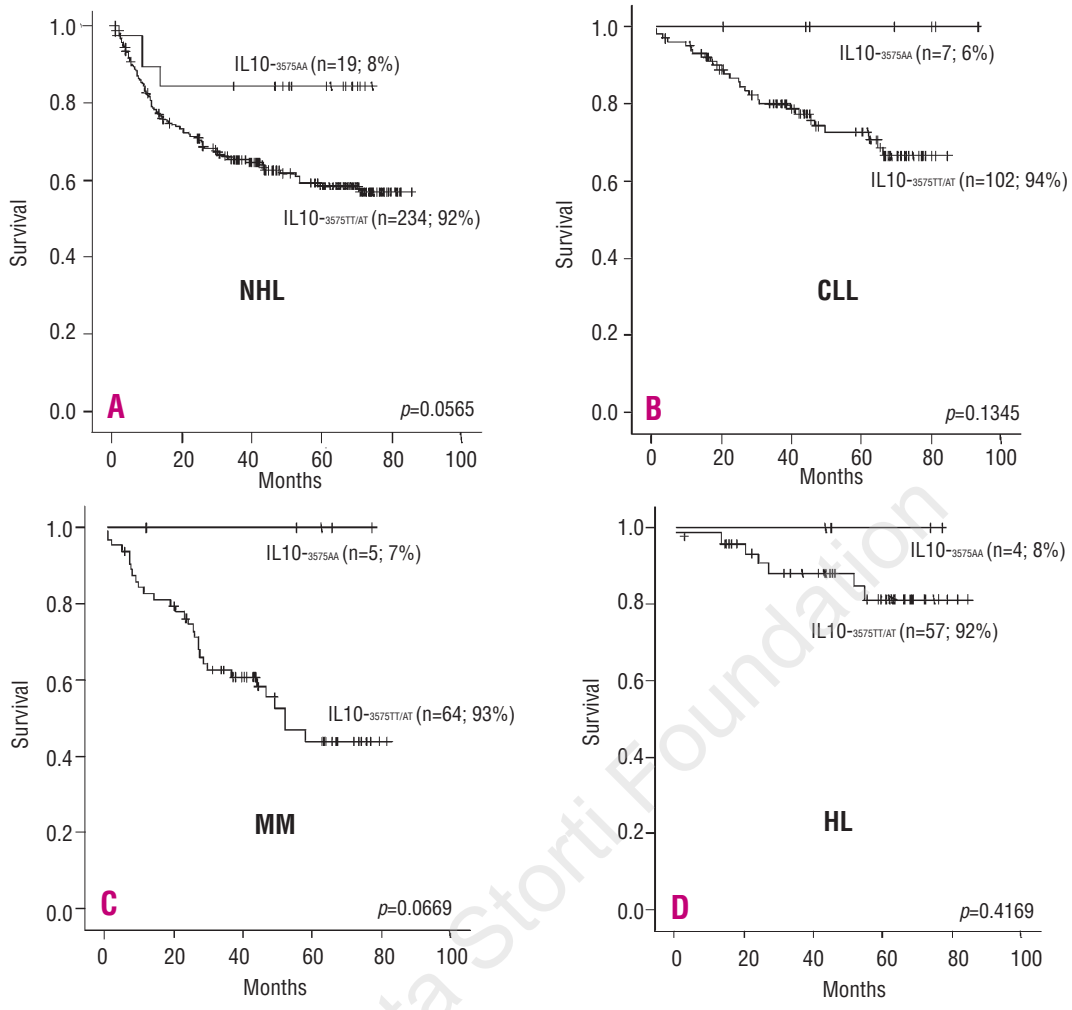

Figure 2. Kaplan-Meier overall survival estimate for lymphoma subtypes according to L10-3575 polymorphism. Kaplan-Meier analysis for the recessive model was performed for non-Hodgkin's lymphoma (NHL), chronic lymphocytic leukemia (CLL), multiple myeloma (MM) and Hodgkin's lymphoma (HL); (A) NHL (n=234) excluding CLL, MM and HL. (B) CLL (n=109). (C) MM (n=69). (D) HL (n=51); *p* refers to the log-rank test.

for the IL10-$_{3575T\rightarrow A}$ polymorphism. Subjects homozygous for the minor allele showed a better, albeit not statistically significant, overall survival, suggesting a recessive inheritance model. A fully adjusted multivariate model that included the two polymorphisms showed that the minor allele of IL10-$_{3575T\rightarrow A}$ was associated with a better prognosis, with a recessive effect (HR:0.20; 95%CI 0.05-0.92). The same model was also used for the group of patients with non-Hodgkin's lymphomas (excluding patients with CLL, MM and Hodgkin's lymphoma), and IL10-$_{3575AA}$ genotype was also associated with a better prognosis although not statistically significant, likely due to the small number of deaths that occurred (HR: 0.4; 95% CI 0.08-2.0) (*no further data shown*).

To further evaluate the association of IL-10 polymorphisms, haplotypes were reconstructed. In total, four distinct haplotypes were found, all of which had a frequency higher than 1% (Table 3). No differences in survival were identified by the haplotype analysis. However, patients homozygous for the G-A haplotype, i.e. the G-A/G-A diplotype, had a better overall survival compared to those carrying the A-T/G-A diplotype (HR:0.26; 95%CI 0.08-0.83) and to those with the other diplotypes considered as a group. Figure 3 shows the survival curves of patients with the G-A/G-A diplotype compared to those of patients with the remaining haplotypes (p=0.003).

Discussion

In this study, two genotypes, IL-10-$_{1082GG}$ and IL-10-$_{3575AA}$, were identified as being associated with a better overall survival of patients with lymphoma, together with the known prognostic factors. However, the IL-10-$_{3575AA}$ genotype was found to be the strongest prognostic factor for lymphoma survival, when both polymorphisms were included in a multivariate analysis.

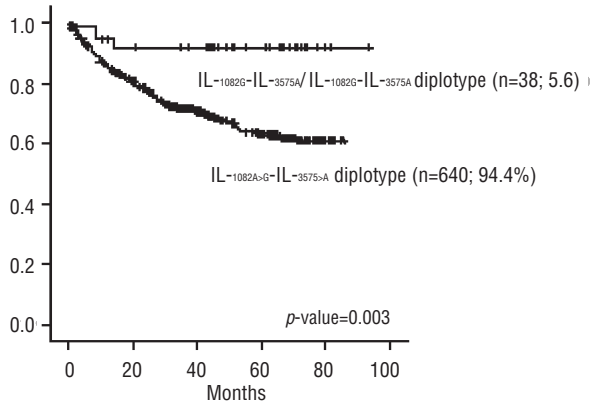

Figure 3. Kaplan-Meier overall survival estimate according to diplotype. Kaplan-Meier overall survival estimates of patients with IL-10-1082 and -3575 G-A/G-A diplotype vs. patients with all other diplotypes. Differences between individuals curves were evaluated by means of the log-rank test.

Table 3. Cox models for haplotypes and diplotypes of IL10-1082A>G and IL10-3575T>A polymorphisms adjusted for prognostic factors.

		Haplotype			
IL10-1082A>G	*IL10-3575T>A*	*Frequency*	*HR*	*95% CI*	*p*
A	T	59.33	1		
A	A	0.39	1.86	(0.30-11.50)	0.505
G	T	11.48	1.00	(0.67-1.50)	0.994
G	A	28.81	1.03	(0.77-1.37)	0.838

				Diplotype			
IL10-1082A>G	*IL10-3575T>A*	*IL10-1082A>G*	*IL10-3575T>A*	*Frequency*	*HR*	*95% CI*	*p*
A	T	G	A	36.03	1		
A	T	A	T	34.36	0.70	(0.47-1.05)	0.085
A	T	G	T	12.68	0.80	(0.45-1.44)	0.465
G	A	G	A	7.97	0.26	(0.08-0.83)	0.024
G	T	G	A	6.61	1.22	(0.63-2.37)	0.560
G	T	G	T	1.73	0.24	(0.03-1.77)	0.162
Rare				0.62	1.47	(0.23-9.27)	0.683

Frequency refers to the estimated frequency of the haplotype. HR indicates the hazard ratio obtained by the multivariate Cox model adjusted for age, sex, center of recruitment, LDH, ECOG status and stage; p value refers to the Wald test.

There is only one study, published by Lech-Maranda E *et al.*,[2] which has shown a better survival in patients with diffuse large B-cell lymphoma carrying the IL-10-1082G allele. In this study it was observed that patients with the IL-10-1082G allele (IL-10-1082GG/GA genotypes) had better a overall survival and 5-year freedom from progression, as well as a higher rate of complete remissions (not statistically significant; p=0.07). When the IL-10-1082G allele was included with IPI prognostic factors in a multivariate analysis, the former remained an independent prognostic variable for both overall survival and 5-year freedom from progression. A recent report by the same group[19] described an association between detectable levels of IL-10 in diffuse large B-cell lymphoma patients and IPI prognostic factors, as well as a lower complete remission rate and shorter progression-free survival. However, these results were not replicated in the series of 244 patients with diffuse large B-cell lymphoma published by Berglund M *et al.*[20] among whom no association between IL-10-1082A→G polymorphisms and survival was found.

In our analysis, patients with the IL-10-1082GG genotype had a better overall survival, but the genotype was not an independent prognostic factor. However, we did find that the IL-10-3575A/A genotype and the IL-10-1082 and -3575 G-A/G-A diplotype were independent prognostic factors. Although the association with the diplotype is likely driven largely by the IL-10-3575A/A genotype, the potential role of IL10-1082GG in the context of the diplotype cannot be excluded.

There is some evidence that the IL-10-3575A allele results in a lower production of IL-10 compared with the –3575T allele,[8] and it is known that patients with normal or low IL-10 levels have a better survival.[2,3,5,6] This is in agreement with our observations and can help to explain why patients with the IL10-3575AA genotype have a better overall survival. However, we did not have measurements of IL-10 production and a more detailed statistical analysis was limited by the small number of cases in each lymphoma category. At the same time, we would note that several studies[10,21] have shown that the IL10-3575AA genotype increases the risk of lymphoma, particularly of diffuse large B-cell lymphoma.

The estimated haplotype frequencies in our study population were consistent with those described by Gibson *et al.* and Lin *et al.*[8,22] The A/G haplotype (IL-10-3575AA allele and IL-10-1082GG allele) has previously been described to be associated with a decreased production of IL-10 *in vitro* among patients with lupus erythematosus.[8] Several reports have identified the G allele of IL-10-1082A→G to be associated with a better survival in melanoma patients,[23] and a decreased risk of graft-versus-host disease in allogeneic stem cell transplant recipients.[24]

Haplotype evaluation of IL-10-1082A→G and IL-10-3575T→A showed that, among our lymphoma patients, those with the G-A/ G-A diplotype had a better overall survival. In this sense, and following what we have previously mentioned, patients with this diplotype would probably have a low production of IL-10 and a better overall survival, concordant with what is described in the literature.

In conclusion, our study of common single-nucleotide polymorphisms of IL-10 identified two genetic variants of probable importance in overall survival of lymphoma patients. Our hypothesis is that the IL10-3575AA genotype is the more important of the two polymorphisms evaluated here, although we cannot rule out an additive protective effect of the IL-10-1082GG genotype as we demonstrate with the G-A/G-A diplotype. A larger number of patients with lymphoma are needed in order to confirm our findings and further investigate associations in different subtypes of non-Hodgkin's lymphoma.

Authors' contributions

EDD: collected, analyzed and interpreted data and wrote the paper; YB: analyzed and interpreted data; EG-B, CM, JG, RB

and AFS: collected data on patients' survival and reviewed the manuscript; SSW, QL and DW: performed research and reviewed the manuscript; NR, SdS: designed research, reviewed the manuscript and gave final approval of the version to be published.

Conflicts of Interest

The authors reported no potential conflicts of interest.

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