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LETTER TO THE EDITOR

Distinctive IGHV gene usage and stereotyped receptors in South American patients with chronic lymphocytic leukemia

Running head: IGHV analysis in South American CLL patients

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To the Editor

The IGHV (*immunoglobulin heavy-chain variable region*) mutational status is one of the most important prognostic factors in chronic lymphocytic leukemia (CLL), the most frequent adult leukemia in the Western World. About 30% of CLL patients display stereotyped B-cell receptors (BCRs) assigned to different subsets, some of them with clinical relevance (1). There is limited information about IGHV repertoire and stereotyped BCRs from South American (SA) countries. Thus, we have evaluated the IGHV mutational status, gene usage and stereotyped BCRs in CLL patients from four SA countries, in the context of a multi-institutional collaborative study.

Our cohort included 900 unselected CLL patients from Argentina (362), Brazil (358), Uruguay (101) and Venezuela (79) (530 males; mean age: 65.6 years; Rai stages: 0: 38%, I-II: 43%, III-IV: 19%). The distribution of patients from each country by sex, age and clinical stages is detailed in Supplementary Table S1. Diagnosis was established according to the International Workshop on CLL Guidelines (2). For IGHV analysis, PCR and bidirectional sequencing was used. Cytogenetic and FISH analysis were performed. The methodology used is described in Supplementary Methods. The study was approved by the local Ethics Committees of each Institution. All individuals provided their informed consent.

Supplementary Tables S2 and S3 show the distribution of mutated (M) and unmutated (UM) patients and the IGHV gene rearrangement for each case, respectively. The analysis of IGHV families revealed the high frequency of IGHV3 (42.9%), followed by IGHV4 and IGHV1 with the same frequency (24%) (Figure 1A; Supplementary Table S4). This distribution is similar to those observed by Bomben et al (4) in Italian CLL patients, but differs from both Western and Asian series (2, 5). Among genes, IGHV1-69 was the most frequently used (12.2%), followed by IGHV4-34 (9.6%) and IGHV3-23 (7.6%), but interesting differences among SA countries were found (Figure 1B; Supplementary Table S5). IGHV1-69 was the most frequent rearrangement in Brazilian patients, with differences compared to Argentina ($p=0.03$). Uruguayan series showed the highest frequencies of IGHV3-7 and IGHV3-23 rearrangements, being the highest observed in the literature (2, 5), with statistical differences with respect to Argentina ($p=0.01$) for IGHV3-7, and Venezuela and Brazil ($p=0.02$) for IGHV3-23. CLL patients from Venezuela showed the highest IGHV3-21 rates, similar to those observed in Scandinavian series (6), with differences compared to Brazil

and Uruguay ($p<0.02$). As a whole, our data were close to those from USA and European cohorts (2), with IGHV1-69 as the most frequently represented but very far from of Asian series, particularly for IGHV1-2 and IGHV1-69 genes (5) (Supplementary Table S6).

Stereotyped BCRs were present in 14.1% of cases, mostly associated with an UM state (69%); 66% of them belong to major subsets. Supplementary Table S7 showed the distribution of stereotyped BCRs in CLL patients from each country. In the whole series, subset #4 was the most frequently found, followed by subsets #1 and #2. This distribution is quite different from those observed in large international series, in which subset #2 is the most frequently found, followed by subset #1, while subset #4 accounts for about 1% of patients (2). Subset #1 was the largest in Brazil, subset #2 in Argentina, whereas subset #4 was the most frequently found in Uruguay and Venezuela. A similar proportion of cases with subset #8 was found in our whole series and international data (2), but it was absent in Venezuelan patients and highly represented in Brazilian and Uruguayan series. Simultaneously, except for subset #8, frequencies of Argentinean cohort were similar to international data. Remarkable, Venezuelan patients showed a very low frequency of major subsets, making an interesting difference compared to the other SA countries evaluated (Figure 1D; Supplementary Table S8). Interestingly, one novel potential subset composed by two patients from Uruguay and one from Argentina was observed (Figure 1C; Supplementary Table S9); they exhibited VH CDR3 of 10 aa length, homology >94%, and used IGHV1-18, D2-15 and J1, J4 or J6.

We also compared the distribution between stereotyped and heterogeneous (not stereotyped) receptors in different genes (Figure 2AI-IV). IGHV1-69 showed a similar distribution in Argentinean and Brazilian cohorts, while a higher number of stereotyped rearrangements were observed in Venezuelan CLL patients. IGHV3-21 was always included in the heterogeneous group in Brazilian and Venezuelan cohorts contrary to Argentinean and Uruguayan series that showed ~50% of IGHV3-21 stereotyped BCRs. These findings results of importance considering that only those IGHV3-21 belonging to subset #2 are uniformly aggressive (7).

Cytogenetic analysis was performed in 152 cases, 102 showed normal karyotypes and 50 had clonal chromosome abnormalities, 13 of them with complex karyotypes (CK). The last group was mostly associated to the UM state (69% of cases) ($p=0.032$). Interphase FISH

was performed on 263 patients; 169 cases were evaluated with the complete CLL FISH panel. A significant association between trisomy 12 (p=0.019), del11q22 (p=0.038), del17p13, ≥2 FISH alterations (p=0.025) and the UM-IGHV state was observed. M-CLL had higher frequency of isolated del13q14 (p=0.004) (Figure 2BI-III). A significant association between the use of IGHV1-69 gene with the presence of del11q22 (35.7% of cases) compared to the remaining used genes (13.3%) (p=0.042) was found. Del Giudice et al (8) found del11q22 as a distinctive association with subset #1, (mostly composed by IGHV1-69 rearrangements), particularly in comparison with other stereotyped UM-CLL. Furthermore, and in concordance with the literature (9), 55.5% of cases expressing IGHV4-34 did not show FISH alterations compared to 34.7% of those using other IGHV genes, and 58.3% of patients expressing IGHV3-21 had del13q14.

Analysis of clinical parameters showed a significant association between the UM state and advanced clinical stages (p=0.001), higher whole blood cell count and increase of β2 microglobulin level (p=0.037 and p=0.012, respectively) (Supplementary Table S10) as well as a shorter time to first treatment (TTFT) compared to M-CLL patients (p=0.0001) (Figure 2C). We also found an inverse association between the increase of the IGHV percent levels and the TTFT (p=0.0001), supporting recently findings (10).

To our knowledge, this is the first multi-institutional study of SA CLL patients. Our results showed differences in both the IGHV gen usage and the distribution of stereotyped BCRs among countries, reinforcing the hypothesis that the genetic background and environmental factors could have a role in the origin and pathogenesis of CLL.

References

1. Baliakas P, Moysiadis T, Hadzidimitriou A, et al. Tailored approaches grounded on immunogenetic features for refined prognostication in chronic lymphocytic leukemia. Haematologica 2019; 104(2):360-369.

2. Hallek M, Cheson BD, Catovsky D, et al. iwCLL guidelines for diagnosis, indications for treatment, response assessment, and supportive management of CLL. Blood 2018; 131(25):2745-2760.

3. Agathangelidis A, Darzentas N, Hadzidimitriou A, et al. Stereotyped B-cell receptors in one-third of chronic lymphocytic leukemia: a molecular classification with implications for targeted therapies. Blood 2012;119(19):4467-4475.

4. Bomben R, Dal Bo M, Capello D, et al. Molecular and clinical features of chronic lymphocytic leukaemia with stereotyped B cell receptors: results from an Italian multicentre study. Br J Haematol 2009;144(4) :492-506.

5. Marinelli M, Ilari C**,** Xia Y, et al. Immunoglobulin gene rearrangements in Chinese and Italian patients with chronic lymphocytic leukemia. Oncotarget 2016;7(15):20520-20531.

6. Cahill N, Sutton LA, Jansson M, et al. IGHV3-21 gene frequency in a Swedish cohort of patients with newly diagnosed chronic lymphocytic leukemia. Clin Lymph Myeloma Leuk 2012;12(3):201-206.

7. Baliakas P, Agathangelidis A, Hadzidimitriou A, et al. Not all IGHV3-21 chronic lymphocytic leukemias are equal: prognostic considerations. Blood 2015;125(5):856-859.

8. Del Giudice I, Chiaretti S, Santangelo S, et al. Stereotyped subset #1 chronic lymphocytic leukemia: a direct link between B-cell receptor structure, function, and patients' prognosis. Am J Hematol 2014;89(1):74-82.

9. Marincevic M, Cahill N, Gunnarsson R, et al. High-density screening reveals a different spectrum of genomic aberrations in chronic lymphocytic leukemia patients with 'stereotyped' *IGHV3-21* and *IGHV4-34* B-cell receptors. Haematologica 2010; 95(9):1519-1525.

10. Jain P, Nogueras González GM, et al. The absolute percent deviation of IGHV mutation rather than a 98% cut-off predicts survival of chronic lymphocytic leukaemia patients treated with fludarabine, cyclophosphamide and rituximab. Br J Haematol. 2018;180(1):33-40.

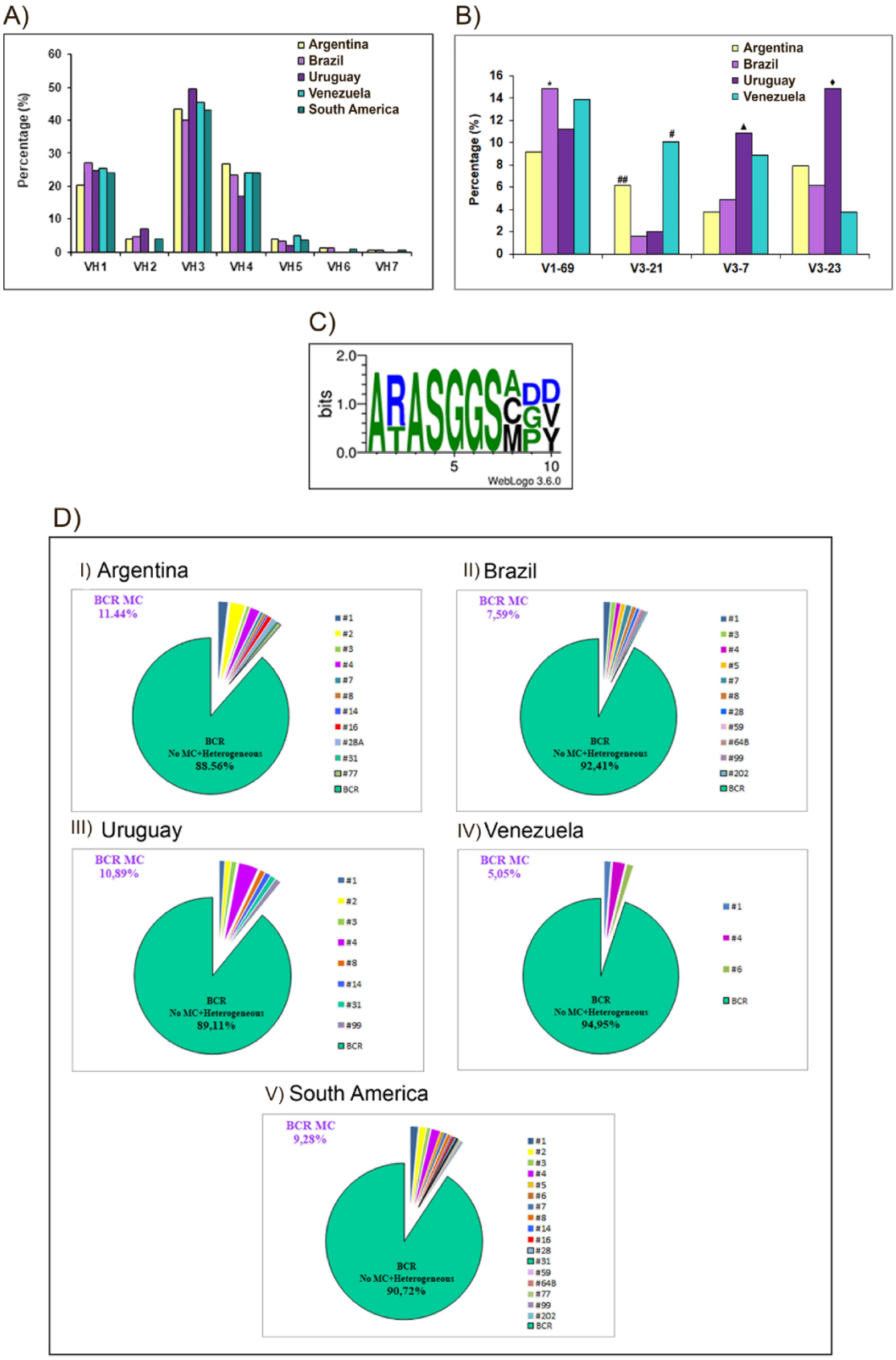

Figure 1: A) VH family distribution in South American countries. Argentinean CLL patients show an IGHV3>IGHV4>IGHV1 usage, while the remaining cohorts and the whole series

exhibited an IGHV3>IGHV1>IGHV4 distribution. B) Frequency of IGHV genes in CLL patients from South american countries. *VH1-69: Significant differences with respect to Argentina (p=0.03); VH3-21: #Significant differences compared to Brazilian (p=0.0008) and Uruguayan (p=0.02) cohorts; ##Significant differences compared to Brazil (p=0.001); ▲VH3-7: Significant differences compared to Argentina (p=0.01); ♦VH3-23: Significant differences with respect to Venezuela (p=0.022) and Brazil (p= 0.012). C) Sequence logo of probable new subset. D) Graphics showing the relative size of each major subset in: I) Argentina, II) Brazil, III) Uruguay, IV) Venezuela and, V) Whole cohort.

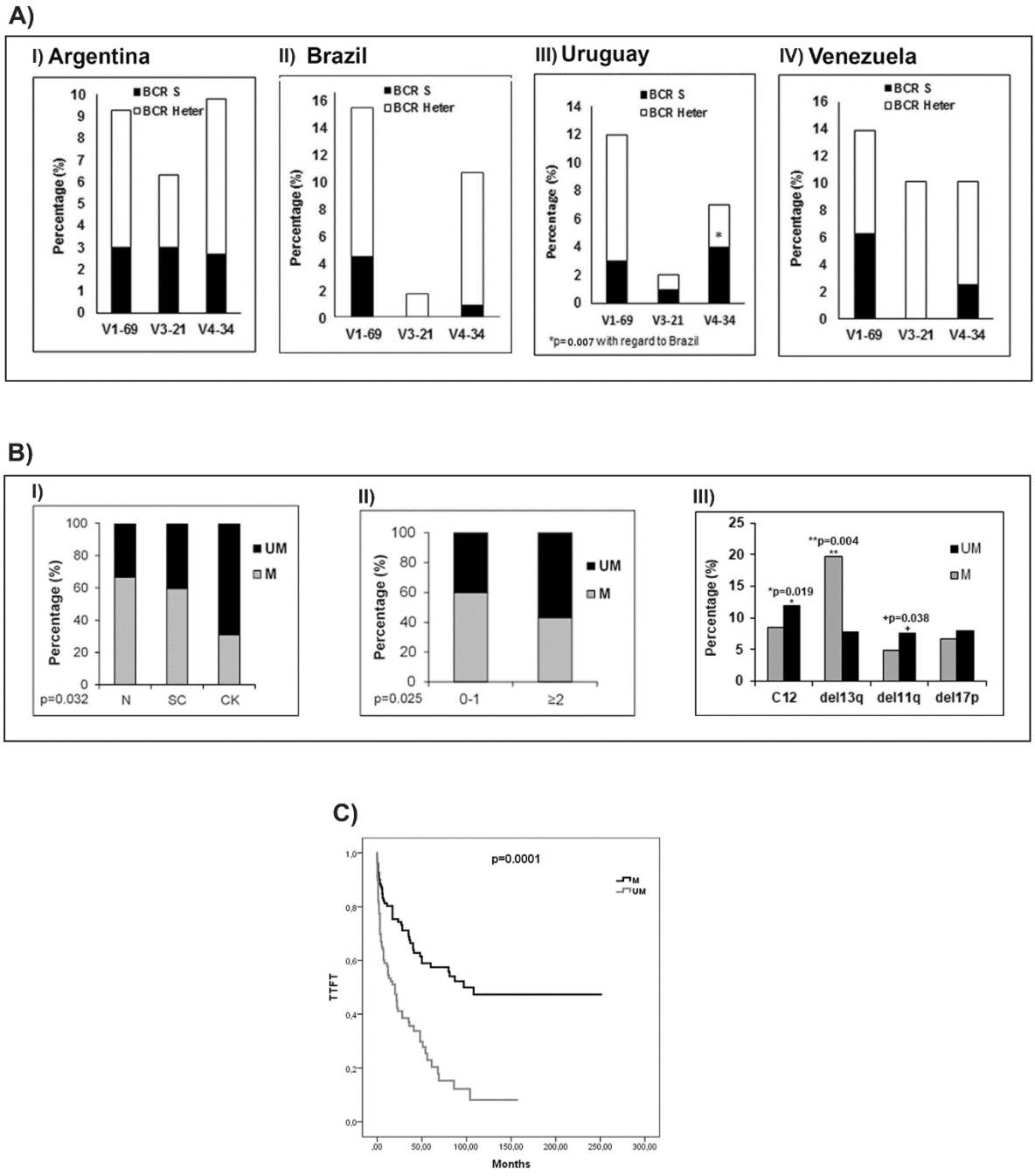

Figure 2: A) Frequency of stereotyped and heterogeneous BCRs for selected genes. I) Argentina, II) Brazil, III) Uruguay and, IV) Venezuela. * Significant differences between Brazil and Uruguay (p=0.007). B) Distribution of cytogenetics and FISH alterations according to IGHV mutational status. I) Distribution of normal (N) and abnormal karyotypes according to M- and UM-IGHV. Significant differences for complex karyotypes (CK) (p=0.032); II) Distribution of CLL patients with 0-1 FISH alterations compared to those with ≥2 FISH alterations. Significant association between ≥2 FISH alterations and UM-IGHV

(p=0.025); III) Distribution of M- and UM-IGHV patients according to different cytogenetic risk groups. Significant association between trisomy 12 (p=0.019) and 11q22 deletions and UM-IGHV (p=0.038). Significant association between 13q14 deletion isolated and M-IGHV (p=0.004). SC: simple karyotype. C) Kaplan-Meir plots showing estimated Time to First Treatment (TTFT) in CLL patients with M- and UM-IGHV (p=0.0001).