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CD13 expression in B cell malignancies is a hallmark of plasmacytic differentiation

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Summary

The diagnosis of Waldenström Macroglobulinaemia (WM)/lymphoplasmacytic lymphoma (LPL) remains one of exclusion because other B-cell lymphoproliferative disorders (B-LPD), such as marginal zone lymphoma (MZL), can fulfil similar criteria, including *MYD88* L265P mutation. It has been suggested that expression of the myeloid marker CD13 (also termed ANPEP) is more frequent in LPL than in other B-LPD and has also been described on normal and malignant plasma cells. Here, CD13 expression was tested in a cohort of 1037 B-LPD patients from 3 centres by flow cytometry. The percentage of CD13-expressing cells was found to be variable among B-LPD but significantly higher in WM/LPL (median 31% vs. 0% in non-WM/LPL, $P < 0{\cdot}001$). In multivariate linear regression, CD13 expression remained significantly associated with a diagnosis of WM/LPL ($P < 0{\cdot}001$). A cut-off value of 2% of $CD19^+$ cells co-expressing CD13 yielded the best diagnostic performance for WM/LPL assertion. This was further improved by association with the presence or absence of IgM paraprotein. Finally, given that previously published transcriptomic data revealed no difference in *CD13* (also termed *ANPEP*) mRNA between normal and pathological B-cells, the hypothesis of some post-transcriptional regulation must be favoured. These results suggest that testing for CD13 expression in routine flow cytometry panels could help to discriminate WM/LPL from other B-LPD.

Keywords: CD13, flow cytometry, B-cell lymphoproliferative disorders, Waldenström macroglobulinaemia/lymphoplasmatic lymphoma.

According to the 2017 World Health Organization (WHO) Classification of Tumours of Haematopoietic and Lymphoid tissues, lymphoplasmacytic lymphoma (LPL) is defined as "a neoplasm of small B lymphocytes, plasmacytoid lymphocytes and plasma cells, involving bone marrow (BM), sometimes lymph nodes and spleen, which does not fulfil the criteria for any of the other small B cell neoplasms that may also have plasmacytic differentiation" (Swerdlow *et al*, 2017). Despite the high frequency (>90%) of *MYD88* L265P mutation in this disease (Treon *et al*, 2012), this abnormality is neither specific nor required for diagnosis (Swerdlow *et al*, 2017). Waldenström macroglobulinaemia (WM) is defined as a subset of LPL with BM infiltration and an IgM plasmatic paraprotein (Swerdlow *et al*, 2017). WM/LPL is a diagnosis of

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exclusion, since other small B-cell lymphoproliferative disorders (B-LPD) fulfil similar criteria, especially marginal zone lymphomas (MZLs). Moreover, the WHO 2017 classification stated that "no specific chromosomal abnormalities are recognized" (Swerdlow *et al*, 2017). Yet, 6q deletion has been described in more than half of BM-based LPL, and trisomy 4 in approximately 20% of LPL, although these cytogenetic anomalies are indeed not specific of WM/LPL (Cook *et al*, 2005; Terre *et al*, 2006). In addition to the *MYD88* mutation, approximately 30% of the cases have truncated *CXCR4* mutations (Swerdlow *et al*, 2017). From an immunophenotypic point of view, WM/LPL cells are characterized by surface expression of B-cell associated antigens (CD19, CD20, CD22, CD79a) and membrane immunoglobulins light chain restriction. CD5, CD10, CD103 and CD23 are usually absent while CD25 and CD38 are frequently expressed (Lin *et al*, 2011). However, a widely variable frequency of CD5, CD23 and CD10 positivity has been reported in WM/LPL (Hunter *et al*, 2005; Konoplev *et al*, 2005; Morice *et al*, 2009). Thus, WM/LPL do not display any truly specific immunophenotype and can also be misdiagnosed as another small B-LPD. Of course, morphological features, with the so-called "plasmacytic inflexion" may be of help but it requires the expertise of well-trained morphologists, which are not necessarily available in flow cytometry (FCM) core facilities.

As mentioned earlier, the *MYD88* L265P mutation has been recognized to be present in about 90% of WM/LPLs (Treon *et al*, 2012). However, this mutation is also present in a small proportion of IgM monoclonal gammopathies of unknown significance (MGUS), other small B-LPD (such as MZL) and, even more frequently, in diffuse large B-cell lymphomas (DLBCL) (Swerdlow *et al*, 2017). Although the 2017 revision of the WHO classification acknowledges the diagnostic importance of the *MYD88* L265P mutation in WM/LPL (Swerdlow *et al*, 2017), this does not exclude the need for other diagnostic markers of WM/LPL, because genetic testing is not readily available in all centres, and, when available, comes as a delayed result.

The CD13 differentiation antigen, originally identified as MCS-2, was recognized as a "panmyeloid" marker in the early 1980s (Gregg *et al*, 1984) and has been extensively used as a granulocytic and monocytic marker ever since. CD13, also known as Aminopeptidase N (ANPEP), is widely expressed on mammalian cells. This ectodipeptidyl-peptidase is involved in a variety of normal and malignant functions (Wickstrom *et al*, 2011). Aberrant expression of CD13 has been described in acute lymphoblastic leukaemia (ALL) (Sobol *et al*, 1987; Lauria *et al*, 1994) and anaplastic large cell lymphomas (Bovio & Allan, 2008), however reports of CD13 expression in mature lymphoid neoplasms are scarce. One study showed CD13 expression in several cases of small B-cell neoplasms [chronic lymphoid leukaemia (CLL), variant hairy cell leukaemia (HCL), other unspecified B-cell non-Hodgkin lymphomas (NHL)] but not in mature T-cell neoplasms (Nakase *et al*, 1996). Another work, focusing only on CLL, reported aberrant CD13 expression in 22% of cases (Pinto *et al*, 1991). It has been suggested that aberrant expression of CD13 might be more frequent in LPL than in other small B-LPD (Craig & Foon, 2008). Conversely, CD13 expression had long been reported on normal (Terstappen *et al*, 1990) and monoclonal (San Miguel *et al*, 1991; Ruiz-Arguelles & San Miguel, 1994) plasma cells.

In the present work, the value of testing for CD13 expression by FCM in the diagnosis of small B-LPD was investigated. CD13 expression was indeed found to be more frequent in WM/LPL and could thus be used as a relevant diagnostic marker.

Patients and methods

Patients

A cohort of 1037 patients issued from 3 centres [Linz (Austria), Paris and Bordeaux (France)] was included in this multicentre retrospective study. All patients were referred to the respective laboratories for immunophenotypic study and had a detectable monotypic B-cell population. Morphological, immunophenotypic, cytogenetic and molecular analyses, as well as clinical data, were collected in order to establish consensual integrated diagnoses according to the criteria of the WHO classification (Swerdlow *et al*, 2017). The clinical and biological characteristics of the study population are reported in Table I.

All investigations were approved by local Ethics Committees and the procedures followed were in accordance with the Helsinki Declaration.

Flow cytometry (FCM)

All samples were immunophenotyped according to local procedures using 8- to 10-colour immunophenotyping panels on Navios (Beckman Coulter, Miami, FL, USA) or Canto II (BD Biosciences, San Jose, CA, USA) flow cytometers. B cells were gated according to CD19 expression, as illustrated in Fig 1. Details of the clones and fluorochromes used are given in Table I.

Transcriptomic studies

Previously published transcriptomic data available in the gene expression omnibus (GEO) repository (https://www.ncbi.nlm.nih.gov/geo/) for GSE6691 (Gutierrez *et al*, 2007) and GSE61597 (Paiva *et al*, 2015) were reanalysed using GEO2R (https://www.ncbi.nlm.nih.gov/geo/geo2r/).

Statistics

Categorical and continuous variables are reported as numbers and percentages, or median and percentiles, respectively. Group comparisons for dichotomic, categorical and

Table I. Basic characteristics of the study population.

Median [IQR] or N (%)	Linz n = 360 (35)	Paris n = 554 (53)	Bordeaux n = 123 (12)	All patients n = 1037	P value
Age (years)	74 [65–80]	71 [63–81]	70 [62–78]	72 [63–80]	0·1550
Gender (male/female)	156/204 (43/57)	275/279 (50/50)	60/63 (49/51)	491/546 (47/53)	0·167
Blood	261 (72)	365 (66)	123 (100)	749 (72)	$<10^{-4}$
Bone marrow	97 (27	107 (19)	0	204 (20)	
Lymph node	0	71 (13)	0	71 (7)	
Others	2 (1)	10 (2)	0	12 (1)	
Diagnosis					
Chronic lymphocytic leukaemia	221 (61)	255 (46)	5 (4)	481 (46)	$<10^{-4}$
Marginal zone lymphoma	42 (12)	89 (16)	24 (20)	155 (15)	
WM/LPL	35 (10)	65 (12)	11 (9)	111 (11)	0·540
Follicular lymphoma	12 (3)	41 (7)	11 (9)	64 (6)	
Mantle cell lymphoma	20 (5)	17 (3)	23 (19)	60 (6)	
Hairy cell leukaemia	6 (2)	12 (2)	3 (2)	21 (2)	
Multiple myeloma	2 (1)	4 (1)	1 (1)	7 (1)	
Other B-NHL	22 (6)	71 (13)	45 (36)	138 (13)	
Monoclonal paraprotein (presence/absence), n = 725	38/322 (11/89)	126/133 (49/51)	28/78 (26/74)	192/533 (26/74)	$<10^{-4}$
IgM paraprotein (presence/absence), n = 723	34/325 (9/91)	101/157 (39/61)	15/91 (14/86)	150/573 (21/79)	$<10^{-4}$
WBC count (10^9/l), n = 874	11·7 [8·73–18·2]	11·9 [7·9–17·2]	8·8 [6·1–13·3]	11·3 [7·7–17·2]	0·0002
Lymphocyte count (10^9/l), n = 868	5·8 [3–11·7]	5·2 [1·9–9·5]	2·8 [1·4–6·6]	5·1 [2·1–9·8]	0·0001
CD13 median, (5–95th)	0 (0–20)	0 (0–60)	0 (0–78)	0 (0–56)	0·0001
CD19 conjugate	PerCP	ECD	APC-AF750		
CD19 clone	SJ25-CI	J3-119	J3-119		
CD13 conjugate	APC	PE-Cy5.5	ECD		
CD13 clone	WM15	Immu103.44	WM15		

AF750, Alexa Fluor® 750; APC, allophycocyanin; B-NHL, B-cell non-Hodgkin lymphoma; Cy5.5, cyanin 5.5; ECD, electron coupled dye (PE + Texas Red); IQR, interquartile range; LPL, lymphoplasmacytic lymphoma; PE, phycoerythrin; PerCP, peridinin chlorophyll; WBC, white blood cell; WM, Waldenström macroglobulinaemia.

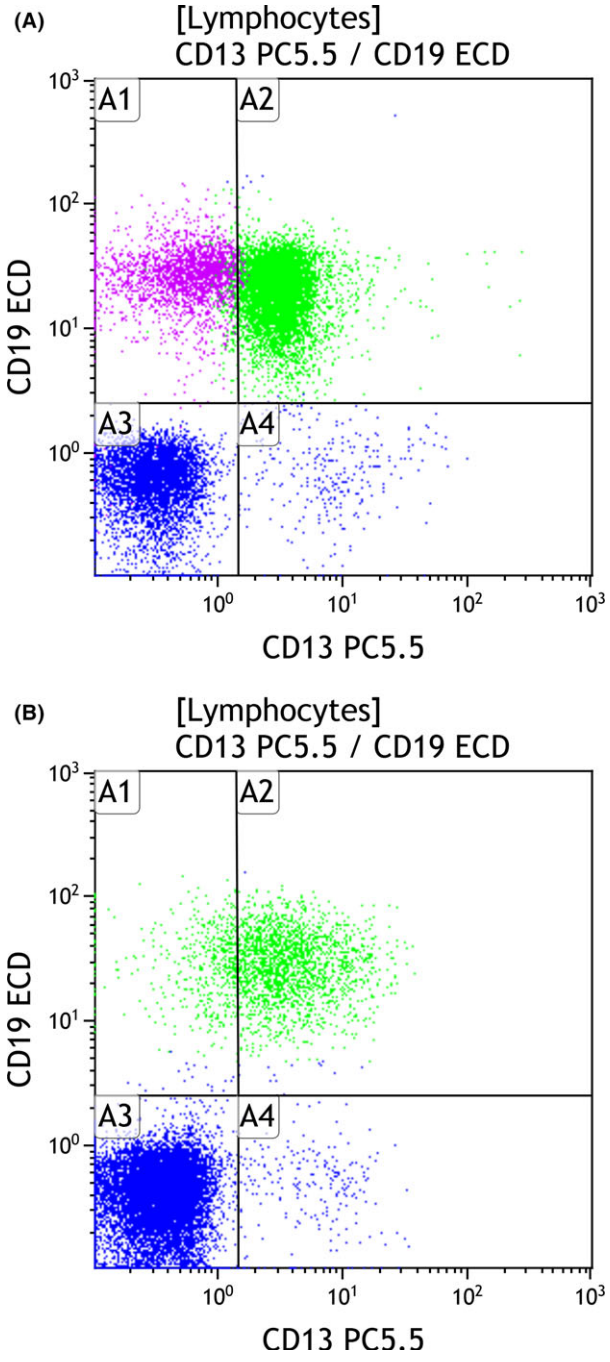

Fig 1. Typical expression patterns illustrated in two patients. (A) normal residual B cells (purple) are negative for CD13 and pathological B cells (green) show CD13 expression. (B) CD13 expression in pathological B cells (green). T cells (blue) are used as internal negative control.

continuous variables were carried out with Fisher's exact test, Mann-Whitney (two groups comparisons) or Kruskall-Wallis (three groups comparisons) tests, respectively.

Correlations between continuous variables were assessed by Spearman's correlation, using log-transformed values for white blood cell (WBC) and lymphocyte counts. The diagnostic performance of CD13 expression was assessed by non-parametric Receiver-Operator Characteristic (ROC) analysis. The optimal cut-off was defined by the maximum of Youden index, computed as (J = sensitivity + specificity−1). A multivariate diagnostic model for WM/LPL was then developed by logistic regression including all variables significantly associated with WM/LPL diagnosis in univariate analysis ($P < 0{\cdot}05$). Specification errors were checked by a link test and a Box-Tidwell model. Goodness of fit was inspected by the Hosmer and Lemeshow's test (Hosmer *et al*, 2013). Collinearity was estimated with variance inflation factors (VIF), retaining the conventional VIF threshold of 4 as indicative of unacceptable collinearity (O'Brien, 2007). Inspection of Pearson residuals, deviance and leverage identified a single observation with excessive influence corresponding to a patient with 100% of $CD13^+$ B cells. Removing this observation did not affect the model. The 95% confidence intervals (CIs) for dichotomic outcomes (sensitivity, specificity, positive predictive value [PPV] and negative predictive value [NPV]) were computed with binomial distributions. All analyses were performed using STATA 12 (STATA Corp LLC, College Station, TX, USA).

Results

Characteristics of the cohort

Demographic characteristics of the patients studied were globally similar across the 3 centres, with no significant differences in gender distribution nor age range (median age, 72 years; Table I). A monoclonal paraprotein was present in 192 cases (26% of 725 patients with available data on immunofixation and/or immunoelectrophoresis), and was an IgM paraprotein in 150 of these cases (78%).

CD13 expression is highly variable among B-cell malignancies

The percentage of CD13-positive B cells expressed as median (5–95th percentiles) was 31% (0–100) in WM/LPL, and 0% in all other conditions, with variable ranges: 0–70 in MZL, 0–3 in CLL, 0–0 in follicular lymphoma (FL), 0–6 in mantle cell lymphoma (MCL), 0–1 in multiple myeloma (MM), 0–53 in HCL and 0–50 in other B-cell NHL (Table II and Fig 2). Using the arbitrary positivity cut-off of 20%, commonly used in FCM, the number of patients with positive CD13 expression on B cells in each group was as follows: 71/111 (64%) in WM/LPL, 25/155 (16%) in MZL, 8/481 (1·6%) in CLL, 1/64 (1·5%) in FL, 2/60 (3%) in MCL, 0/7 (0%) in MM, 3/21 (14%) in HCL and 12/138 (8·7%) in other B-cell NHL. These differences were statistically significantly different between groups ($P < 0{\cdot}001$, Table II and Fig 2). The subgroup with the largest overlap to WM/LPL was that of MZL. Examination of the characteristics of these 25 $CD13^+$ MZL patients disclosed that only 7 also had an IgM peak, but without low lymphocyte counts.

Table II. Association of study parameters with CD13 expression in univariate analysis.

Continuous parameters	Cases (*n*)	Correlation coefficient	*P* value
Total WBC count	675	−0·06	0·11
Lymphocyte count	671	−0·08	0·03
Total M paraprotein levels	67	0·06	0·64

Categorical parameters	Cases (*n*)	CD13%, Median (5–95th)	*P* value
Gender			
Male	546	0 (0–55)	0·8244
Female	491	0 (0–61)	
Presence of a monoclonal paraprotein			
Yes	192	7 (0–90)	$<10^{-4}$
No	533	0 (0–25)	
Presence of IgM paraprotein			
Yes	150	10 (0–90)	$<10^{-4}$
No	573	0 (0–31)	
Sample source			
Blood	749	0 (0–58)	$<10^{-4}$ BM vs. other
BM	204	0 (0–61)	
Lymph nodes	60	0 (0–0)	
Other	24	0 (0–30)	
Diagnosis			
CLL	481	0 (0–3)	$<10^{-4}$ WM vs. other
MZL	155	0 (0–70)	
WM/LPL	111	31 (0–100)	
FL	64	0 (0–0)	
MCL	60	0 (0–6)	
HCL	21	0 (0–53)	
MM	7	0 (0–1)	
Other B-NHL	138	0 (0–50)	

B-NHL, B-cell non-Hodgkin lymphoma; BM, bone marrow; CLL, chronic lymphocytic leukaemia; FL, follicular lymphoma; HCL, hairy cell leukaemia; LPL, lymphoplasmacytic lymphoma; MCL, mantle cell lymphoma; MM, multiple myeloma; MZL, marginal zone lymphoma; WBC, white blood cell; WM, Waldenström macroglobulinaemia.

Surprisingly, the percentage of $CD13^+$ B cells was also found to be significantly different according to the sample source, regardless of the diagnosis, with a higher expression in BM samples: the percentage ranges were 0–58% in blood, 0–61% in BM, 0–0% in lymph nodes and 0–30% in other sources ($P < 0·001$, Table II and Fig 3).

CD13 expression is highly associated with a WM/LPL diagnosis

Screening baseline characteristics of the 1037 patients of the cohort revealed five variables associated with CD13 expression (Table II). When analysed as continuous parameter, CD13 expression was higher in patients with WM/LPL diagnosis ($P < 0·001$), BM vs. other sample source ($P < 0·001$), presence of a monoclonal paraprotein ($n = 725$, $P < 0·001$), IgM paraprotein ($n = 723$, $P < 0·001$) and lower lymphocyte count ($n = 671$ for patients who had CD13 expression measured in a blood sample, $P = 0·03$). There was no significant

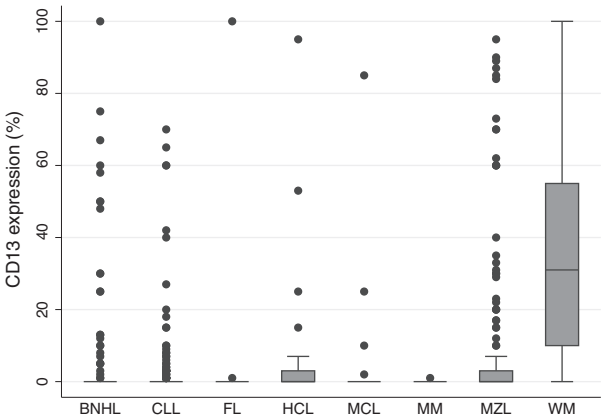

Fig 2. Box plots showing CD13 expression percentages in different B-cell malignancies. B-NHL, B-cell non-Hodgkin lymphoma; CLL, chronic lymphocytic leukaemia; FL, follicular lymphoma; HCL, hairy cell leukaemia; MCL, mantle cell lymphoma; MM, multiple myeloma; MZL, marginal zone lymphoma; WM, Waldenström macroglobulinaemia.

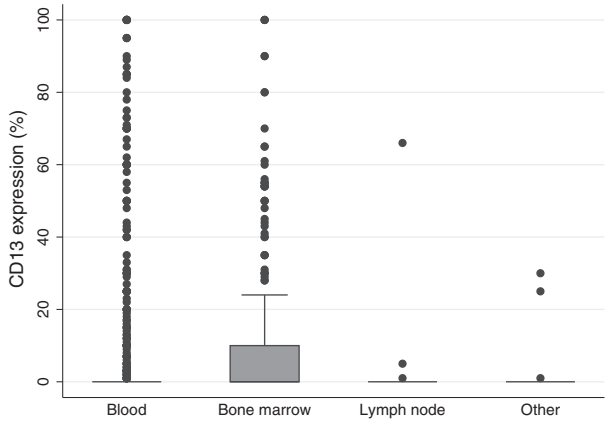

Fig 3. Box plots showing CD13 expression percentages according to the sample source in the whole patient cohort.

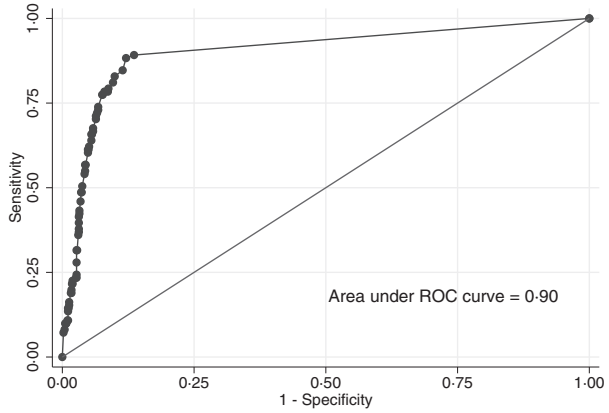

Fig 4. Receiver-operator-characteristic (ROC) curve for CD13. Area under the curve = 0·9012, 95% confidence interval (CI) = (0·87–0·93). With a threshold of 2%, sensitivity and specificity are 88·3% (95% CI: 80·8–93·6%) and 87·9% (95% CI: 85·6–89·9%), respectively.

association between $CD13^+$ percentage and gender, total WBC count or total M paraprotein level (Table II).

Using ROC curves (Fig 4) and Youden index, the discriminating CD13-positivity threshold was defined as 2% $CD13^+$ B cells [AUC = 0·9012 (95% CI = 0·87–0·93)]. With this cut-off, the sensitivity for the diagnosis of WM/LPL was 88·3% (95% CI = 80·8–93·6) and specificity was 87·9% (95% CI = 85·6–90·0). The PPVwas 46·7% (95% CI = 39·8–53·7) and NPV was 98·4% (95% CI = 97·3–99·2). In the following analyses, CD13 positivity refers to samples with ≥2% $CD13^+$ B cells.

Parameters associated with a WM/LPL diagnosis were tested using univariate analysis (Table III). A WM/LPL diagnosis was found to be significantly associated with CD13 expression ≥2% ($P < 10^{-4}$), presence of a monoclonal paraprotein ($P < 10^{-4}$), IgM paraprotein ($P < 10^{-4}$), higher WBC ($P = 0{\cdot}0002$) and lymphocyte counts ($P < 10^{-4}$) and higher total M paraprotein level ($P = 0{\cdot}0032$). We next performed a multivariate logistic regression model considering all variables significantly associated with WM/LPL diagnosis ($P < 0{\cdot}05$), i.e. CD13 positivity, presence of an IgM paraprotein, lymphocyte count (but not WBC count for obvious collinearity issues) in the 667 patients with available information on paraproteins, adjusting for sample source (BM *versus* others) and centre. CD13 ≥ 2% remained a predictor of WM/LPL diagnosis [odds ratio (OR) = 57·55; $P < 10^{-4}$], independently of the presence of an IgM paraprotein (OR = 18·33; $P < 10^{-4}$) while lymphocyte counts no longer predicted WM/LPL ($P = 0{\cdot}429$, Table IV). Importantly, the predictive role of CD13 positivity was also independent of sampling in the BM *versus* other tissues, and there was no significant collinearity between the presence of an IgM paraprotein, BM sampling nor CD13 positivity.

Table III. Association of study parameters with WM/LPL diagnosis using univariate analysis.

	Mean ± SD of parameter		
Quantitative parameter	WM/LPL diagnosis	Other B-cell malignancies	*P* value
Age (years)	72·9 ± 11·9 (n = 111)	70·9 ± 12·4 (n = 926)	0·1327
Mean WBC count (10^9/l) (n = 874)	2·24 ± 0·63 (n = 84)	2·51 ± 0·73 (n = 790)	0·0002
Mean lymphocyte count (10^9/l) (n = 868)	1·04 ± 1·02 (n = 83)	1·64 ± 1·18 (n = 785)	$<10^{-4}$
Total M paraprotein level (n = 87)	11·67 ± 9·94 (n = 45)	6·77 ± 6·84 (n = 42)	0·0032
	Number of positive cases		
Qualitative parameter	WM/LPL diagnosis	Other B-cell malignancies	*P* value
CD13 ≥ 2%	98/111	112/926	$<10^{-4}$
Male gender	57/111	489/926	0·424
Presence of a monoclonal paraprotein	99/105	93/620	$<10^{-4}$
Presence of an IgM paraprotein	88/105	62/618	$<10^{-4}$
Centre (Linz-Paris-Bordeaux)	35-65-11/111	325-489-112/926	0·540
Sample source (Blood-BM-LN-Other)	39-71-1-0/111	710-133-70-13/926	$<10^{-4}$

BM, bone marrow; LN, lymph nodes; LPL, lymphoplasmacytic lymphoma; SD, standard deviation; WBC, white blood cell; WM, Waldenström macroglobulinaemia.

Table IV. Association of study parameters with WM/LPL diagnosis using multivariate analysis.

Parameter	ORR	95% CI	*P* value
CD13 ≥ 2%	57·55	20·75–159·59	$<10^{-4}$
Presence of an IgM paraprotein	18·33	8·00–42·02	$<10^{-4}$
Centre (Linz-Paris-Bordeaux)	0·66	0·41–1·05	0·540
Sample source* (BM–other)	10·28	3·61–29·26	$<10^{-4}$
Lymphocyte count†	1·17	0·80–1·71	0·429

95% CI, 95% confidence interval; LPL, lymphoplasmacytic lymphoma; ORR, overall response rate; SD, standard deviation; WM, Waldenström macroglobulinaemia.
*Considered as a factor variable.
†Log-transformed.

The performance of the association of CD13 ≥ 2% and IgM paraprotein as diagnostic markers was then tested. Expression of CD13 above 2% was significantly associated with a diagnosis of WM/LPL whether or not the patients presented with an IgM paraprotein. In patients with an IgM paraprotein (n = 150), CD13 ≥ 2% had a PPV of 79·2% (95% CI: 70·0–86·8%) and an NPV of 77·8% (64·4–88·0%), while in patients without an IgM paraprotein (n = 573), the PPV was only 21·2% (12·9–31·8%) yet the NPV was 100% (99·2–100%).

CD13 expression could be regulated at a post-transcriptional level

Retrospective analysis of previously published transcriptomic data (Gutierrez *et al*, 2007; Paiva *et al*, 2015) revealed no significant difference between CD13 expression in normal or pathological B cells at the mRNA level (Fig 5). *CD13* (*ANPEP*) mRNA expression was not significantly different in plasma cells compared to lymphocytes in healthy donors (Fig 5A), although CD13 expression can be observed on normal plasma cells by FCM at various levels (0–70% in our experience, unpublished personal data) but not on normal or reactive B cells (unpublished personal data). *CD13* mRNA expression was strictly comparable between normal BM cells from healthy donors and B cells from WM patients (Fig 5B). These observations indicate that *CD13* transcripts can be produced at various stages of B-cell differentiation but only result in protein expression in a selected cluster of B-lineage malignancies. Indeed, with the methodology used for mRNA analysis, similar levels would yield a "neutral" signal. This is therefore highly suggestive of an unexpected significant level of transcription of the *CD13* gene, regulated at later stages in most B cells so as not to translate in surface expression of the protein.

Discussion

This study reports that CD13 surface expression is significantly higher in WM/LPL compared to other small B-LPDs.

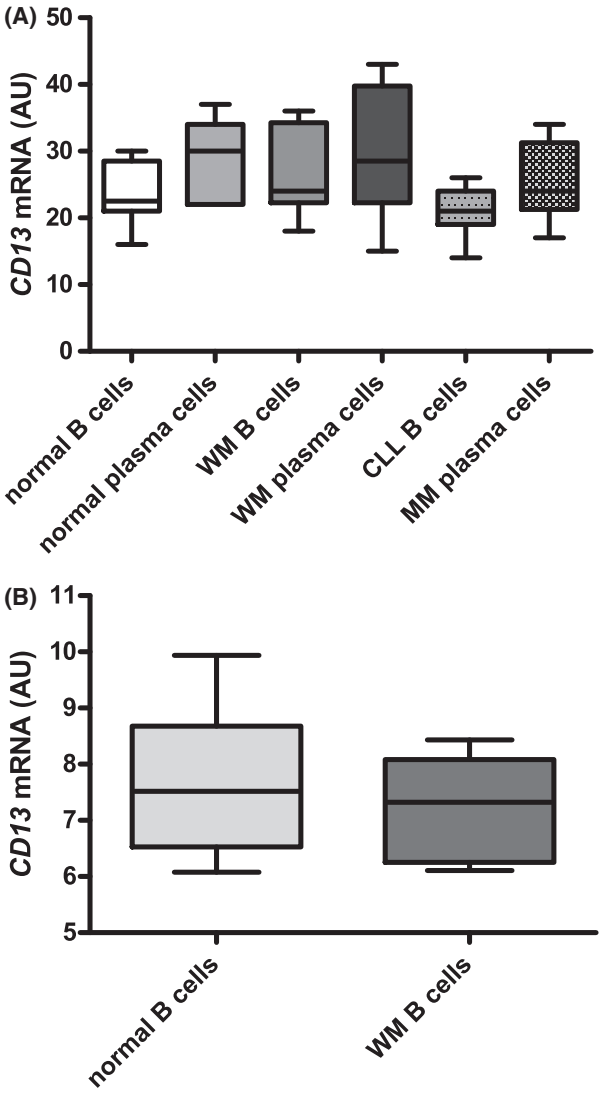

Fig 5. (A) *CD13* mRNA expression in sorted B cells or plasma cells from healthy donors (n = 8) and patients with Waldenström macroglobulinaemia (WM, n = 10), chronic lymphocytic leukaemia (CLL, n = 11) or multiple myeloma (MM, n = 12) (GSE 6691). (B) *CD13* mRNA expression in sorted bone marrow B cells from healthy donors (n = 17) and clonal B cells from WM patients (n = 14) (GSE 61597). AU, arbitrary units.

CD13 expression is also higher in BM compared to other sample sources. CD13 expression was found to be significantly associated with the diagnosis of WM/LPL, the presence of a monoclonal paraprotein of IgM isotype and a lower total lymphocyte count. These criteria can be used to consolidate a diagnosis of WM/LPL and, perhaps more importantly, to rule out the diagnosis of WM /LPL when both CD13 expression and IgM paraprotein are absent (NPV 100%). Of note, although 7 MZL patients with CD13 expression also had an IgM paraprotein, none of them had lowered lymphocyte counts and morphological characteristics would have also helped to rule out a diagnosis of WM/LPL in these 4·5% of MZL cases.

In the past few years, the *MYD88* L265P mutation has emerged as a strong characteristic feature of WM/LPL (Swerdlow *et al*, 2017) that could be used for diagnosis. In the literature, *MYD88* mutation detection has been reported to have a sensitivity of 96–100%, a specificity of 92–97%, a PPV of 86–96% and a NPV of 99–100% for WM diagnosis, depending on the series and the detection techniques used (Xu *et al*, 2013; Hunter *et al*, 2014; Insuasti-Beltran *et al*, 2015). By comparison, the threshold of 2% of $CD19^+$ cells for CD13 positivity in the more rapid method of FCM that we propose, although showing a slightly lower discrimination, retains strong performance. Moreover, this study included all types of B cell neoplasms whereas the diagnostic power of *MYD88* L265P mutation has mainly been studied in IgM gammopathies with a large majority of WM. It must be considered that CD13 testing is readily available in all FCM core facilities, where lymphoproliferative disorders and acute leukaemia are routinely diagnosed, and can be easily added to FCM panels for mature lymphoid neoplasms. By contrast, as mentioned above, *MYD88* mutational status assessment is not available everywhere and the results of this molecular test are delayed compared to the rapid assessment of FCM evaluation on fresh cells (within half a day).

The aminopeptidase-N CD13 molecule is an ubiquitous protein involved in a variety of functions in physiological or malignant contexts, such as peptide cleavage, solid tumour cell invasion, differentiation, proliferation and apoptosis, cell motility, chemotaxis, antigen presentation, phagocytosis, angiogenesis and cell adhesion (Mina-Osorio, 2008; Wickstrom *et al*, 2011). However, its role in lymphoid cells remains elusive. CD13 expression is observed on haematopoietic stem cells and at the earliest stages of both B- and T-lymphoid maturation, but is then lost as lymphoid maturation progresses (Syrjala *et al*, 1994; Spits *et al*, 1995). Interestingly, data observed in CLL patients showed a correlation between CD13 expression on neoplastic lymphocytes and the pattern of BM infiltration and prognosis (Pinto *et al*, 1991). Moreover, CD13 has been implicated in the adhesion of B- and T-lymphocytes to fibroblasts and other cell types (Riemann *et al*, 1997). Given the importance of the BM microenvironment in the pathogenesis of WM (Agarwal & Ghobrial, 2013), CD13 could play a major role in the interaction of lymphoplasmacytic cells with the stroma. Our results also show a link between CD13 expression and the presence of a paraprotein (IgM or other isotypes). It can therefore be hypothesized that CD13 might play a role in paraprotein secretion, although no data in the literature suggest such an implication of CD13 in secretory functions. Finally, given that similar mRNA levels of *CD13* transcripts are observed in a variety of normal or pathological B cells, it can be suggested that the mechanisms impairing translation of these transcripts are altered in WM/LPL. The fact that MYD88 is a protein involved in mRNA stabilization (Sun & Ding, 2006) might not be alien to this observation.

Conflict of interest

The authors declare no conflict of interest.

Author contributions

AR and SMS performed the research, analysed the data and wrote the paper. RI and SC analysed the data and wrote the paper. NC, SM, JL, ASL, BB, OK and MF performed the research and analysed the data. MCB, FD, PB and VB designed the research study, analysed the data and wrote the paper.

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