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CD62L on blood basophils: a first pre-treatment predictor of remission in severe lupus nephritis

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ABSTRACT

Background. Basophils were recently shown to contribute to lupus nephritis (LN). This study assessed blood basophil activation markers (BAMs) for the diagnosis of LN severity and as pre-treatment prognostic markers of the response to treatment in patients with severe LN.

Method. The diagnostic study included all the patients of a monocentric prospective observational cohort built with consecutive patients diagnosed with LN on the basis of a renal biopsy. The prognostic study selected patients of this cohort according to the following inclusion criteria: ≥ 18 years old, Class III or IV A \pm C \pm Class V or pure Class V LN at the time of inclusion and treated with an induction treatment for LN. Clinical data and BAMs were obtained at the time of the kidney biopsy. LN remission status was recorded 12 months after induction therapy initiation. Associations between baseline data and histological severity of LN or LN remission were assessed using logistic regression.

Results. No significant association was found between BAMs and the histological severity of LN in 101 patients. Among the 83 patients included in the prognostic study, 64 reached renal remission. CD62L expression on blood basophils at baseline was independently negatively associated with remission at 12 months [odds ratio = 0.26, 95% confidence interval 0.08–0.82, $P = 0.02$ for quantitative CD62L expression > 105 (geometric fluorescent intensity) gMFI]. CD62L < 105 gMFI was associated with a probability of 0.87 of LN remission in the next 12 months after the start of induction therapy.

Conclusion. Pre-treatment CD62L expression on blood basophils could be a first predictive biomarker of renal response to induction therapy at 12 months in patients with severe LN.

Keywords: basophils, basophil activation markers, biomarkers, lupus nephritis, systemic lupus

INTRODUCTION

Thirty to sixty per cent of patients diagnosed with systemic lupus erythematosus (SLE) will develop lupus nephritis (LN) [1, 2]. LN is associated with significant morbidity in the most severe cases [3, 4]. The clinical significance of LN and its need for a specific and aggressive therapy depend on its histological severity, which is graded according to the current International Society of Nephrology and Renal Pathology Society (ISN/RPS) classification [5, 6]. It includes six main classes of LN depending on glomerular active and/or chronic lesions and the proportion of involved glomeruli. The most severe forms, Class III or Class IV with histological activity, and pure Class V LN with a high level of proteinuria, require induction therapy to reach remission and subsequent maintenance therapy to prevent relapses.

Therefore, it is of primary importance to adequately establish the LN class in an SLE patient with renal involvement. However, renal semiology and serum biomarkers are not reliable enough to predict or exclude an active proliferative LN in SLE patients with proteinuria [7–9]. Only the histological analysis of a renal biopsy is helpful for the diagnosis and the optimal treatment determination. Nevertheless, renal biopsy is invasive, with a substantial bleeding risk. To date, no reliable non-invasive biomarker of severe LN has been identified to replace renal biopsy [10–12].

Moreover, only two-thirds of patients with the most severe LN treated with an induction therapy will be recorded as being in a remission state after 12 months, which is associated with a reduced risk of ulterior chronic kidney disease [10, 11]. In an attempt to determine early predictors of the response to induction therapy, Dall'Era *et al.* showed that C3 and C4 normalization at 8 weeks of induction therapy was predictive of the renal response at 24 weeks in the Aspreva Management Lupus Study (ALMS) [12]. Nonetheless, there is no reliable indicator able to

KEY LEARNING POINTS

What is already known about this subject?

- basophils were recently shown to contribute to lupus nephritis (LN) and activation of blood basophils correlates with LN activity. There is no predictor of response to induction treatment given for severe LN.

What this study adds?

- CD62L expression measured on blood basophils at diagnosis of severe LN is shown to correlate with future response to standard therapy.

What impact this may have on practice?

- CD62L on blood basophils at diagnosis of severe LN may be a first reliable predictor of response to standard therapy and then a tool for treatment personalization.

predict the treatment response in individuals at the time of diagnosis. Such a predictor would undoubtedly help to personalize induction in those patients who eventually will experience treatment failure with the standard of care. Conversely, this predictor could avoid overimmunosuppression in the two-thirds of patients who do not need to be exposed to its adverse effects.

We recently showed that basophils are key players in the pathogenesis of SLE and LN, notably by promoting an amplification loop of autoantibody production through their activation by prostaglandin D₂ (PGD₂) and immune complexes (CICs) containing autoreactive immunoglobulin E. These factors lead to basophil accumulation in secondary lymphoid organs (SLOs), where they support short-lived plasma cell survival both in human SLE and lupus-like mouse models [13–17]. Activated basophils are identified in the blood of SLE patients with active lupus by flow cytometry as they overexpress on their surface a number of basophil activation markers (BAMs), including CD203c, CD62L, HLA-DR and CXCR4 [14, 15, 17, 18]. Their migration to SLOs leads to a basopenia that correlates with disease activity [13, 14, 17]. Through their important role in the pathophysiology of LN, blood BAMs are good candidates to be evaluated as diagnostic and prognostic biomarkers of LN.

The objectives of this study, based on a prospective monocentric observational cohort, were to assess the clinical usefulness of BAMs as reliable non-invasive diagnostic biomarkers of active LN and as reliable non-invasive prognostic biomarkers of the response to treatment in severe LN.

MATERIALS AND METHODS

The study was approved by the *comité de protection des personnes* (Paris, France) under reference ID-RCB-2014-A00809-38. All patients provided written informed consent.

Participants

Eligible subjects were prospectively recruited during their hospitalization in the Nephrology Department of Bichat Hospital (Assistance Publique-Hôpitaux de Paris, France) for evaluation of kidney disease. All consecutive patients requiring a renal biopsy were recommended for BAMs evaluation. In parallel, clinical data at diagnosis and during the follow-up were prospectively collected. For the present analysis, two sub-cohorts were built with all consecutive patients who had a kidney biopsy with a diagnosis of LN, with all patients fulfilling the American College of Rheumatology classification criteria for SLE: Cohort A for the diagnostic assessment and Cohort B for the prognostic assessment of BAMs.

The inclusion criteria for Cohort A were being >18 years old and having a kidney biopsy with a diagnosis of LN at the time of inclusion. Excluded were patients with unavailable histological activity score or chronicity score.

The inclusion criteria for Cohort B were being >18 years old, having a kidney biopsy with a diagnosis of a Class III or Class IV A ± C ± Class V or pure Class V LN at the time of inclusion and being treated with an induction treatment for the LN. The exclusion criterion for Cohort B was having both an estimated glomerular filtration rate according to the Chronic Kidney Disease Epidemiology collaboration (CKD-EPI) formula of <30 mL/min/1.73 m² at the time of the biopsy AND >50% of glomeruli globally sclerosed on the kidney biopsy. Exclusion aimed to evaluate remission of LN in all patients treated with an induction therapy except those unable to reach LN remission criteria due to non-reversible kidney alterations.

Accordingly, Cohort A included all patients with a biopsy-proven LN except five with no activity and chronicity scores available, and Cohort B was built with patients who required an induction therapy for a severe LN. Cohort B was almost a sub-cohort of Cohort A except for five patients included in Cohort B but excluded from Cohort A due to unavailability of activity and chronicity score. Cohort B did not include patients with inactive LN by histology, except those with a severe pure Class V LN.

Data

The following data were prospectively collected for each patient: demographics (age and sex), non-renal SLE disease activity index (SLEDAI) and the SELENA-SLEDAI, disease duration from SLE diagnosis, time since first LN diagnosis for LN relapses, number of renal lupus flares, blood creatinine (D0 creatinine) and 24-h proteinuria (D0 proteinuria) at the time of the diagnostic kidney biopsy, class of the LN as well as activity and chronicity indexes according to the ISN/RPS 2003 classification [19]. In particular, chronicity and activity were graded as the proportion of glomeruli affected by chronic lesions (glomerular sclerosis, fibrous adhesions and fibrous crescents) and active lesions (endocapillary hypercellularity, karyorrhexis, fibrinoid necrosis, rupture of glomerular basement membrane, cellular or fibrocellular crescents, wire loops and hyaline thrombi). In this study, an active Class III and Class IV LN was defined as a Class III or Class IV LN with at least 10% of the glomeruli with active lesions on diagnostic renal biopsy. Conversely, a non-active Class III or Class IV LN was defined as a Class III or

Class IV LN with <10% of the glomeruli showing active lesions. Class I, Class II, non-active Class III or Class IV LN and pure Class V were classified as non-active LN.

Remission or absence of remission of LN status was based on the blood creatinine level and the 24-h proteinuria recorded during the 12-month follow-up. For Class III/IV± V LN, complete remission was defined as a normal creatinine and no increase >15% when compared with D0 creatinine and proteinuria <0.5 g/day. Partial remission was defined as a normal creatinine and no increase in blood creatinine >15% when compared with D0 creatinine and proteinuria <1 g/day or <3 g/day with <50% of D0 proteinuria if D0 proteinuria was >3 g/day. For pure Class V LN, complete remission was defined as a normal creatinine and no increase >15% when compared with D0 creatinine and proteinuria <0.5 g/day. Partial remission of pure Class V LN was defined as a normal creatinine and no increase >15% when compared with D0 creatinine and a reduction of 50% of D0 proteinuria. The absence of remission was recorded in the absence of complete or partial remission or in the case of treatment failure. Treatment failure was defined as a new induction regimen decision based on the results of a new kidney biopsy. 'Absence of remission at 12 months' was considered in the absence of remission during the 12 months following the initiation of induction therapy. Conversely, 'remission' was recorded when complete or partial remission was recorded during the 12 months following induction therapy initiation.

Blood sample handling for basophil analyses

BAMs were evaluated extemporaneously on blood samples at the time of the diagnostic renal biopsy using flow cytometry as described elsewhere [14, 17]. Briefly, after plasma harvesting, red blood cells were lysed with ACK lysing solution (150 mM NH₄Cl, 12 mM NaHCO₃, 1 mM EDTA, pH 7.4). Cells were then resuspended in the original volume of blood in fluorescence-activated cell sorting (FACS) buffer (phosphate-buffered saline, 1% bovine serum albumin; 0.1% NaN₃). The number of leukocytes per millilitre and viability was assessed with trypan blue and a Malassez' haemocytometer. Viability was always >95%. Cells were then processed for extracellular staining by blocking unspecific binding sites with a saturation solution in FACS buffer [containing 100 µg/mL of immunoglobulin G (IgG) from human, goat, mouse and rat origins] and then adding specific antibodies to the indicated surface markers (see [Supplementary data, Table S1](#)). After 30 min of incubation at 4°C in the dark, cells were washed twice in FACS buffer before acquisition. Flow cytometry was performed with a BD LRSII Fortessa using Diva software. All data relative to marker expression levels are expressed as the ratio between the geometric mean fluorescent intensity (gMFI) of the indicated marker on the cells of interest and the gMFI of the corresponding control isotype.

Statistical analysis

The results are expressed as the number of patients (percentage) for categorical variables and as the median (interquartile range) for continuous variables. To analyse the diagnostic value of BAMs, we assessed whether BAMs were associated with an active LN in patients of Cohort A. To analyse the prognostic value of BAMs, we assessed whether BAMs at diagnosis of LN

were associated with remission at 12 months in patients who required an induction therapy for LN (Cohort B).

For both objectives (diagnostic and prognostic), BAMs and other potential predictors as disease characteristics (time since diagnosis, SLEDAI, creatinine, proteinuria, histological class, activity and chronicity index) were evaluated in bivariate analyses for each of the outcomes using a logistic regression model. Then, all variables with $P < 0.20$ and <15% missing values were used in a stepwise backward multivariable logistic analysis. No variable was forced into the model because there was no clinical justification for doing so. Type I error was 0.05. In the prognostic analysis, the discrimination of the model was measured using the area under curve (AUC) of the receiver operating characteristic (ROC) curve. The choice of the optimal cut-off in terms of sensitivity and specificity was computed using the Youden index.

Statistical analyses were conducted using R software.

RESULTS

BAMs as a diagnostic biomarker for active LN versus non-active LN

Cohort A included 101 patients ([Table 1](#)) with the following histological classification of their LN: 5 (5%) had a Class II, 17 (17%) had a pure Class V, 43 (42%) had a Class III/IV LN including 36 (85%) with activity, and 36 (36%) had a Class III/IV + V LN including 27 (75%) with activity. Sixty-three of the renal biopsies (62%) showed a LN with significant activity (active LN group) with a median activity of 30%. The median chronicity was 5%. The patients with an active LN had higher non-renal activity of lupus when compared with that of patients with non-active LN, as their median non-renal SLEDAI scores were 6 and 2, respectively ($P = 0.005$).

Bivariate analyses were performed to determine which clinical characteristics or BAMs were associated with active LN. Results are given in [Table 1](#). Among the patients' clinical characteristics, the SLEDAI score recorded at the time of the renal biopsy [odds ratio (OR) = 1.20, 95% confidence interval (CI) 1.09–1.34, $P < 0.001$], the non-renal SLEDAI score recorded at the time of the renal biopsy (OR = 1.18, 95% CI 1.06–1.34, $P = 0.005$) and the daily dosage of prednisone >10 mg at the time of biopsy were found to be associated with active LN by histology. As the non-renal SLEDAI and the SLEDAI scores are strongly correlated (Pearson correlation test: $P < 0.0001$), only the non-renal SLEDAI score was introduced in the multivariable analysis.

The following covariates were included in the multivariable analyses: non-renal SLEDAI at renal biopsy, corticoids dosage >10 mg, immunosuppressive treatment, and HLA-DR, CD123 and CXCR4 expression on blood basophils at the time of the renal biopsy. The non-renal SLEDAI and immunosuppressive treatment at the time of biopsy remained in the final model. Non-renal SLEDAI and immunosuppressive treatment were correlated to the findings of an active LN by renal histology (OR = 1.24, 95% CI 1.10–1.42, $P = 0.001$ and OR = 2.74, 95% CI 1.08–7.45, $P = 0.04$, respectively). The AUC of the ROC curve of the logistic model including only non-renal SLEDAI score was 0.69. Therefore, an increased non-renal SLEDAI

Table 1. Characteristics of patients included in Cohort A dedicated to the diagnosis evaluation of BAMs and ORs of the association with active LN

	All subjects (n = 101)	Non-active LN (n = 38; 37%)	Active LN (n = 63; 63%)	OR (95% CI)	P-value
Clinical, histological and biological features					
Age (years)	35 (26–42)	35 (37–43)	35 (24–42)	1.00 (0.96–1.04)	0.96
Females	91 (90%)	34 (89%)	57 (90%)	1.12 (0.27–4.16)	0.87
Number of renal lupus flares	1 (1–2) (11 missing data)	1 (1–2.3) (6 missing data)	1 (1–2) (5 missing data)	0.98 (0.65–1.50)	0.92
Time since SLE diagnosis (years)	7.6 (1.7–13.2) (9 missing data)	5.4 (2.3–13.2) (3 missing data)	8.2 (1.3–12.5) (6 missing data)	1.02 (0.96–1.09)	0.48
Time since first LN diagnosis (years)	0.9 (0–8.3) (7 missing data)	2.1 (0–9.7) (3 missing data)	0.7 (0–7.0) (4 missing data)	0.98 (0.91–1.04)	0.47
SLEDAI	11 (8–15) (1 missing data)	8 (6–12)	13 (9–16) (1 missing data)	1.20 (1.09–1.34)	<0.001
Non-renal SLEDAI	6 (2–8) (1 missing data)	2 (0.3–6)	6 (4–9) (1 missing data)	1.18 (1.06–1.34)	0.005
Creatinine (µmol/L)	75 (60–113)	66 (53–89)	77 (64–137)	1.00 (1.00–1.01)	0.67
Proteinuria (g/day)	1.6 (0.9–2.9)	1.4 (0.8–3)	1.6 (0.9–2.9)	1.01 (0.83–1.24)	0.91
Prednisone >10 mg/day at the time of biopsy	23 (23%)	4 (11%)	19 (30%)	3.67 (1.24–13.56)	0.03
Immunosuppressive treatment at the time of biopsy	36 (36%) (2 missing data)	10 (27%) (1 missing data)	26 (42%) (1 missing data)	1.95 (0.82–4.87)	0.14
Class II LN	5 (5%)	5 (13%)	0	NA	NA
Class III/IV LN	43 (43%)	7 (18%)	36 (57%)	NA	NA
Class III/IV + V LN	36 (36%)	9 (24%)	27 (43%)	NA	NA
Pure Class V LN	17 (17%)	17 (45%)	0	NA	NA
Per cent of glomeruli with activity lesions	16 (0–33)	0 (0–0)	30 (18–62)	NA	NA
Per cent of glomeruli with chronicity lesions	5 (0–17)	0 (0–11)	6 (0–26)	1.98 (0.36–13.77)	0.45
IgG anti-dsDNA (EU)	3.8 (2.1–10.6) (45 missing data)	2.3 (1.1–3.4) (19 missing data)	8.8 (2.1–11.6) (26 missing data)	1.22 (1.00–1.66)	0.09
BAMs					
Basophil count (/µL of blood)	7.8 (3.2–16.0) (2 missing data)	9.3 (4.0–15.3)	6.7 (2.4–16.2) (2 missing data)	0.99 (0.95–1.03)	0.50
Per cent of basophils among leukocytes	0.21 (0.07–0.38)	0.23 (0.08–0.44)	0.20 (0.05–0.34)	0.44 (0.09–1.87)	0.27
Per cent of HLA-DR ⁺ basophils ^a	8.4 (6.3–12.7) (2 missing data)	7.8 (5.8–10.3)	8.7 (6.7–14.7) (2 missing data)	1.03 (0.99–1.07)	0.18
HLA-DR ^a	1.6 (1.2–2.6) (2 missing data)	1.5 (1.1–2.3)	1.8 (1.2–2.8) (2 missing data)	1.21 (0.93–1.69)	0.19
CD62L ^a	84.4 (44.6–124.5) (11 missing data)	75.9 (43.2–124.5) (6 missing data)	90.0 (46.5–136.2) (5 missing data)	1.00 (1.00–1.01)	0.26
FcεRIα ^a	101.3 (54.3–156.2) (1 missing data)	112.0 (78.3–154.8)	96.9 (49.1–157.9) (1 missing data)	1.00 (0.99–1.00)	0.37
CD203c ^a	7.7 (4.8–13.7) (1 missing data)	9.3 (4.6–16.5)	7.1 (4.8–13.0) (1 missing data)	0.98 (0.95–1.02)	0.34
CD123 ^a	20.2 (14.5–58.3) (1 missing data)	19.2 (10.5–66.8)	21.1 (14.8–44.9) (1 missing data)	0.99 (0.99–1.00)	0.10
CRTH2 ^a	13.7 (9.0–20.1) (8 missing data)	14.4 (9.6–20.8) (1 missing data)	13.2 (9.0–19.3) (7 missing data)	1.00 (0.98–1.02)	0.92
CXCR4 ^a	3.5 (2.5–5.3) (12 missing data)	3.1 (2.0–4.7) (2 missing data)	3.7 (2.8–5.7) (10 missing data)	1.16 (1.02–1.40)	0.06

^aOnly BAMs with <15% missing values are given in the table; BAMs are expressed as the ratio between the gMFI of the indicated marker on the basophils and the gMFI of the corresponding control isotype.

Data are presented as the *n* (%) or median with interquartile range (Q1–Q3). Per cent calculation was based on the ratio of the number of patients who presented with the characteristic of interest over the total number of patients of each column, i.e. all subjects, patients with non-active LN, patients with active LN. In case of missing data, the number of patients with missing data is given for each variable, and per cent of patients was calculated on the number of patients with data available.

Anti-dsDNA, anti-double-stranded DNA; EU, Elisa unit; NA, not applicable.

score and the use of immunosuppressive treatment at the time of renal biopsy—but no BAMs—were independently associated with an active LN.

BAMs as pre-treatment prognostic biomarkers of severe LN

We evaluated the association of BAMs and other clinical features recorded at the time of the diagnostic renal biopsy with

LN remission at 12 months. The analyses were only performed in patients who required an induction therapy for an active Class III or an active Class IV LN or a pure Class V LN with nephrotic syndrome.

Cohort B included 83 patients (Table 2). Of them, 34 (41%) had a Class III/IV LN with activity, 35 (42%) had a Class III/IV + V LN with activity and 14 (17%) had a pure Class V LN. The median time from lupus diagnosis was 8.5 years at the

Table 2. Characteristics of patients included in Cohort B dedicated to the prognostic evaluation of BAMs and ORs of the association with LN remission in the 12 months following induction therapy

	All subjects (n = 83)	Patients without remission (n = 19; 23%)	Patients with LN remission (n = 64; 77%)	OR (95% CI)	P-value
Clinical, histological and biological features					
Age (years)	35.5 (26–43)	38.3 (27–43)	34.6 (26–43)	0.97 (0.92–1.02)	0.21
Female	76 (92%)	16 (84%)	60 (94%)	2.77 (0.51–14.29)	0.20
Number of renal lupus flares	1.0 (1.0–2.0) (10 missing data)	1.0 (1.0–2.8) (1 missing data)	1.0 (1.0–2.0) (9 missing data)	0.96 (0.61–1.59)	0.86
Time since SLE diagnosis (years)	8.5 (1.1–13.4) (8 missing data)	8.2 (0.8–13.3) (2 missing data)	8.7 (1.4–14.0) (6 missing data)	1.01 (0.94–1.09)	0.76
Time since first LN diagnosis (years)	0.3 (0.0–7.2) (6 missing data)	0.8 (0.0–7.9)	0.3 (0.0–6.6) (6 missing data)	1.00 (0.92–1.09)	0.93
SLEDAI	12 (8–15) (1 missing data)	11 (8–15)	12 (8–16) (1 missing data)	1.02 (0.93–1.12)	0.73
Non-renal SLEDAI	6 (3–9) (1 missing data)	6 (2–8)	6 (4–9) (1 missing data)	1.04 (0.92–1.18)	0.53
Creatinine (µmol/L)	75 (61–101)	113 (61–163)	73 (62–84)	0.99 (0.98–1.00)	0.02
Proteinuria (g/day)	1.7 (0.9–3.2)	1.9 (1.0–3.3)	1.6 (0.9–3.1)	0.99 (0.79–1.27)	0.91
Prednisone dosage >10 mg/day at the time of biopsy	24 (29%) (1 missing data)	7 (39%) (1 missing data)	17 (27%)	0.57 (0.19–1.76)	0.31
Immunosuppressive treatment at the time of biopsy	29 (35%)	9 (50%)	20 (31%)	0.45 (0.15–1.33)	0.15
Euro-lupus (induction)	33 (40%)	6 (32%)	27 (42%)	1.45 (0.49–4.76)	0.21
Steroids + MMF (induction)	25 (30%)	4 (21%)	21 (33%)	1.69 (0.53–6.67)	
Other induction ^a	25 (30%)	9 (47%)	16 (25%)	0.37 (0.13–1.09)	
Class III A ± C or Class IV A ± C LN	34 (41%)	9 (47%)	25 (39%)	1 Reference	0.54
Class III A ± C + V or Class IV A ± C + V LN	35 (42%)	6 (32%)	29 (45%)	1.74 (0.55–5.84)	
Pure Class V LN	14 (17%)	4 (21%)	10 (16%)	0.90 (0.23–3.93)	
Per cent of glomeruli with activity lesions	25 (12–56) (5 missing data)	33 (16–69) (1 missing data)	21 (11–51) (4 missing data)	0.36 (0.06–2.41)	0.28
Per cent of glomeruli with chronicity lesions	6 (0–15) (5 missing data)	5 (3–39) (1 missing data)	6 (0–15) (4 missing data)	0.05 (0.002–1.15)	0.06
IgG anti-dsDNA (EU)	7.0 (2.4–11.8) (36 missing data)	2.3 (2.2–2.6) (7 missing data)	9.4 (3.1–12.0) (29 missing data)	1.50 (1.01–4.35)	0.21
BAMs					
Basophil count (/µL of blood) ^b	8.3 (3.2–16.6) (2 missing data)	3.7 (2.0–9.7)	9.2 (4.0–16.8) (2 missing data)	1.03 (0.98–1.10)	0.27
Per cent of basophils among leukocytes ^b	0.21 (0.11–0.37)	0.14 (0.07–0.25)	0.23 (0.13–0.37)	1.15 (0.19–10.99)	0.89
Percent of HLA-DR ⁺ basophils ^b	8.3 (5.9–12.0) (3 missing data)	10.6 (7.4–14.5) (1 missing data)	7.8 (5.7–10.9) (2 missing data)	1.01 (0.97–1.06)	0.73
HLA-DR ^b	1.6 (1.2–2.5) (3 missing data)	2.1 (1.2–2.9) (1 missing data)	1.6 (1.2–2.3) (2 missing data)	1.05 (0.79–1.53)	0.78
CD62L ^b	82.4 (44.8–126.5) (8 missing data)	108.5 (76.9–241.3) (3 missing data)	79.8 (41.5–114.1) (5 missing data)	0.996 (0.993–0.999)	0.04
CD62L ≥105 gMFI	28 (37%) (8 missing data)	10 (63%) (3 missing data)	18 (31%) (5 missing data)	0.26 (0.08–0.82)	0.02
FcεRIα ^b	107.6 (56.9–156.4) (2 missing data)	95.2 (74.1–132.5) (1 missing data)	111.9 (54.0–156.4) (1 missing data)	1.00 (0.99–1.01)	0.83
CD203c ^b	8.8 (5.4–13.6) (2 missing data)	9.9 (5.0–16.5) (1 missing data)	8.6 (5.4–13.2) (1 missing data)	0.98 (0.92–1.04)	0.43
CD123 ^b	19.3 (14.5–50.0) (2 missing data)	17.3 (11.2–64.3) (1 missing data)	19.6 (14.7–44.5) (1 missing data)	1.00 (0.98–1.01)	0.70
CRT2 ^b	13.5 (8.6–18.1) (8 missing data)	15.8 (8.2–25.1) (3 missing data)	13.3 (9.2–16.7) (5 missing data)	0.98 (0.94–1.02)	0.27
CXCR4 ^b	3.5 (2.5–5.2) (12 missing data)	4.7 (3.0–6.2) (4 missing data)	3.3 (2.4–5.0) (8 missing data)	0.98 (0.89–1.09)	0.60

^aOther induction regimens were high-dose cyclophosphamide + steroids according to the 'short-NIH' protocol, MMF + rituximab according to the Rituxilup protocol, Euro-lupus + rituximab, MMF + steroids + rituximab, Euro-lupus + eculizumab, steroid + azathioprine, rituximab alone (for pure Class V LN).

^bOnly BAMs with <15% missing values are given in the table; BAMs are expressed as the ratio between the geometric gMFI of the indicated marker on the basophils and the gMFI of the corresponding control isotype.

Data are presented as the n (%) or median with interquartile range (Q1–Q3). Per cent calculation was based on the ratio of the number of patients who presented with the characteristic of interest over the total number of patients of each column, i.e. all subjects, patients without remission, patients with remission of LN. In case of missing data, the number of patients with missing data is given for each variable, and per cent of patients were calculated on the number of patients with data available.

Anti-dsDNA, anti-double-stranded DNA; EU, Elisa unit; MMF, mycophenolate mofetil.

moment of LN flare diagnosis. The current renal flare was the first one for 47 patients, and 36 patients were relapsing. Thirty-three patients (40%) were treated according to the Euro-lupus induction therapy, 25 (30%) with mycophenolate mofetil plus high-dose steroids and 25 (30%) with other induction regimen (Table 2). Sixty-four (77%) patients achieved LN remission during the 12 months following the initiation of induction therapy.

In bivariate analyses, only baseline blood creatinine (OR = 0.99, 95% CI 0.98–1.00, $P = 0.02$) and CD62L expression on blood basophils measured at the time of renal biopsy (OR = 0.996, 95% CI 0.993–0.999, $P = 0.04$) were associated with renal remission at 12 months.

In multivariable analyses, only the CD62L expression on blood basophils at diagnosis remained in the final model. The AUC of the ROC curve value was 0.68 (95% CI 0.53–0.82) for the logistic model including only CD62L. A Youden index was used to determine the best cut-off value for CD62L in terms of sensitivity and specificity: a basophil expression of CD62L below a gMFI ratio value of 105 was shown to discriminate the remission of LN with a specificity of 63% (35–85%) and a sensitivity of 69% (56–80%). A low basophil CD62L expression (<105 gMFI) had a positive predictive value of 87% (69–93%) for remission at 12 months (see [Supplementary data, Table S2](#)).

In conclusion, a basophil expression of CD62L below a gMFI ratio value of 105 at the time of diagnosis was associated with a probability of 0.87 of LN remission in the next 12 months after the initiation of an induction therapy given for severe LN.

DISCUSSION

In this study, CD62L quantitative expression on blood basophils measured at the time of kidney biopsy is shown to be a potential non-invasive biomarker of response to induction therapy in patients with the most severe LN, that is, Class IIIA LN, Class IVA LN and pure Class V LN associated with nephrotic syndrome. In contrast, we did not demonstrate that BAMs are a substitute for renal biopsy in the diagnosis process of LN severity.

Our previous studies showed the contribution of basophils to LN development and SLE disease activity. Indeed, independent of treatment, blood basopenia is positively correlated with SLEDAI and with their accumulation in SLOs [14, 17]. This blood extravasation of basophils and their migration into SLOs is triggered by CXCR4 expression on the surface of basophils, a feature induced by PGD2. Since increased expression of BAMs is correlated with lupus activity and basopenia [13, 14, 17], we hypothesized that these activation markers could be related to LN severity. However, in this study, BAM analysis could not discriminate the class of LN that a given patient had. Interestingly, CXCR4 basophil expression—one of the BAMs we evaluated—could be a more sensitive marker, as we found a trend for its positive correlation to an active LN by bivariate analysis. However, this was not confirmed by multivariable analyses, where only the non-renal SLEDAI and the use of an immunosuppressive drug at the time of the renal biopsy remained in the final model for active LN prediction, indicating that, to this end, BAMs do not have a better diagnostic value

than clinical evaluation of lupus. The absence of correlation between BAMs and LN histological activity could be due to the lack of sensitivity to discriminate patients with high lupus activity. Indeed, in this study, all analysed patients had a high activity of the disease since their median non-renal SLEDAI score was 6. In addition, considering that at the time of renal biopsy and of basophils sampling, 30 and 42% of patients in the Cohort A received >10 mg of prednisone per day and an immunosuppressive drug, respectively, we cannot rule out the possibility that baseline immunosuppression may have interfered with the expression of an association between LN activity and BAMs.

According to published cohort studies and randomized clinical trials, almost 60–70% of SLE patients with severe LN achieve remission at 12 months with conventional induction therapies [20, 21]. Therefore, the benefit of adding new drugs to this standard of care to improve the results [20, 22, 23] should be balanced with the notion that 60–70% of patients, that is, those who will ultimately experience a renal response to standard of care, should not be challenged with additional drugs and their related risk for side-effects, provided physicians are able to predict renal outcome before treatment at LN diagnosis. A better patient selection is therefore mandatory to help the design of new clinical trials and future standard of care strategies. To date, the best predictor of the renal response to the induction therapy prescribed for severe LN is the normalization of the C3 and C4 complement compounds 8 weeks after the initiation of induction therapy [12]. In our cohort, having a CD62L below a gMFI ratio value of 105 at baseline was predictive of achieving remission during the first 12 months. CD62L is better known as L-selectin. It is highly expressed on activated basophils and is implicated in the mechanisms leading to basophil extravasation from the blood to the SLOs [24]. Therefore, CD62L might be an indirect surrogate of the amount of basophils in SLOs where they amplify the production of CICs. If validated, the measure of CD62L expression on circulating basophils at baseline could then be the first marker allowing the discrimination of high-risk and low-risk patients and could be useful for personalizing induction therapies and improving their benefit over risk ratios. In addition, future works should also investigate whether longitudinal monitoring of CD62L on blood basophils could help further the follow-up of LN patients.

Our study suffered from several limitations. There was a low number of patients. The single-centre design of the study does not allow the generalization of its results. This is explained by the current specificity of the basophil tests and the need for extemporaneous evaluation of blood basophils that did not allow us to expand our evaluation to other centres. A validation cohort is now mandatory.

In conclusion, CD62L expression on circulating basophils measured at the time of the diagnosis of severe LN could be predictive of renal response to induction therapy, a low CD62L expression being associated with a higher probability of remission. This is the first prognostic biomarker that will allow personalized medicine in patients with severe LN, provided these results are reproduced in a validation cohort.

SUPPLEMENTARY DATA

Supplementary data are available at [ndt online](https://ndt.online).

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AUTHORS' CONTRIBUTIONS

N.C. and E.D. conceptualized this project; M.H., C.L., P.M., D.B., N.C. and E.D. contributed to the methodology; M.H., G.H., C.P., B.D., P.M., D.B. and C.L. contributed to data collection, investigation and analysis; N.C. and E.D. contributed to the supervision of the study; C.L., N.C. and E.D. contributed to the validation; M.H., D.B., N.C. and E.D. contributed to the writing of the article.

CONFLICT OF INTEREST STATEMENT

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DATA AVAILABILITY STATEMENT

The anonymized data are available upon reasonable request for a collaborative research project. Please contact the corresponding author for all requests regarding data sharing.

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