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ORIGINAL ARTICLE

Anti-beta-2-glycoprotein I domain 1 identifies antiphospholipid antibodies-related injuries in patients with concomitant lupus nephritis

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Abstract

Background In this study we aimed to evaluate the usefulness of domain profiling of Beta-2-glycoprotein I(β2GPI)-Domain-1 (D1) antibodies in relation to antiphospholipid antibodies (aPL)-related nephropathy (aPL-N) in patients with biopsy-proven lupus nephritis (LN).

Methods Of 124 consecutive patients (96 women, mean age 45.5 ± 12.3 years, mean disease duration 14.7 ± 9.6 years) fulfilling the 1982 criteria for systemic lupus erythematosus (SLE), we identified 39 patients (mean age 39.84 ± 8.6 years, mean disease duration 11.3 ± 7.7 years) with the following characteristics: (a) biopsy-proven LN; (b) no previous diagnosis of antiphospholipid syndrome (APS) according to the current classification criteria.

Results Patients with both LN and aPL-N had higher median aβ2GPI-D1 antibody titres (220.1 CU, 25–75th IQ 29.1–334.2) as compared those with LN alone (46.5 CU, 25–75th IQ 12.5–75.1) ($p = 0.0087$). Median aβ2GPI-D1 antibody titres were higher in patients with acute thrombotic microangiopathy (aTMA) ($N = 7$) (250.1 CU, 25–75th IQ 61.2–334.2) vs. with LN alone (46.5 CU, 25–75th IQ 12.5–75.1 CU) ($p = 0.0009$). Having a Global Antiphospholipid Syndrome Score > 10 confers an increased probability of having acute features of aTMA (OR 6.25, 95%CI 1.2–31.8). As compared to other aPL, aβ2GPI-D1 antibodies have the best diagnostic accuracy for aTMA as evaluated by performances in Area Under the Curves in a ROC analysis.

Conclusions aβ2GPI-D1 antibodies detection might provide a second-line assay to be performed in aβ2GPI positive patients with LN, allowing more accurate stratification of the renal vascular involvement risk, thus potentially leading to a more tailored management.

Keywords Lupus nephritis · SLE · Systemic lupus erythematosus · Antiphosphospholipid syndrome · APS · aPL · Antiphosphospholipid antibodies

Introduction

Beta 2 glycoprotein I (β2GPI), a 50-kDa single-chain glycoprotein consisting of five domains, is the main autoantigen targeted by antiphospholipid antibodies (aPL) which are the

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biomarkers of the systemic autoimmune disease known as antiphospholipid syndrome (APS) [1].

Anti-β2GPI antibodies are therefore considered the main pathogenic aPL subset, mediating both thrombotic and obstetric complications [2]. These autoantibodies are usually polyclonal and recognize multiple linear peptides in the β2GPI molecule [3] with domain (D) 1, D4 and D5 being the most investigated epitopes [4]. Experimental evidence showed that antibodies targeting D1 support the most relevant immunogenic in patients with APS [5–9]. In particular, patients at greatest risk, i.e. those with triple aPL positivity [i.e., positive lupus anticoagulant (LA), anticardiolipin (aCL) and anti-β2GPI antibodies] [10], displayed the highest frequency and titres of aβ2GPI-D1 antibodies [11–13].

The kidney is a major target organ in APS and renal thrombosis can occur at any level within the vasculature of the kidney (renal arteries, intrarenal arteries, glomerular capillaries and renal veins) [14]. Renal involvement in patients with aPL, the so-called aPL-related nephropathy (aPL-N) reflects the site and size of the involved vessels. Histological findings vary widely, including ischaemic glomeruli and thrombotic lesions without glomerular or arterial immune deposits on immunofluorescence. Renal prognosis is affected by the presence of aPL in patients with lupus nephritis (LN) and can be poor [15].

We recently provided new evidence supporting the potential role of anticoagulation in the management of concomitant thrombotic microangiopathy (TMA) and LN, especially in patients testing positive for aPL [16]. However, to date, identifying which patients are at higher risk of developing aPL-N among those with systemic lupus erythematosus (SLE) is still an unmet need and there is no experimental evidence on the clinical meaning of aβ2GPI-D1 antibodies positivity in LN. Due to the lack of available data, this study attempts to evaluate the usefulness of domain profiling of anti-β2GPI-D1 in relation to aPL-N in patients with biopsy-proven LN.

Patients and methods

Of 124 consecutive patients (96 women, mean age 45.5 ± 12.3 years, mean disease duration 14.7 ± 9.6 years) fulfilling the 1982 criteria for SLE [17] who presented at our out-patient clinics at the CMID-Center of Research of Immunopathology and Rare Diseases and the Division of Nephrology (S. Giovanni Bosco Hospital, Turin, IT), 39 were diagnosed with biopsy-proven LN (mean age 39.84 ± 8.6 years, mean disease duration 11.3 ± 7.7 years) defined according to the International Society of Nephrology/Renal Pathology Society Glomerulonephritis Classification [18].

No previous thrombotic nor pregnancy morbidity event according to the current classification criteria for APS [1] were reported in the 39 patients with LN. Demographic, clinical and laboratory characteristics were collected from their clinical charts and are summarized in Table 1 and 1S. Figure 1a includes the main characteristics of the patients with LN, sub-grouped by (1) LN and no aPL-N (27 patients), (2) LN and aPL-N (12 patients), (3) LN and acute TMA (aTMA) (7 patients of the 12 with LN and aPL-N).

Patients with biopsy proven LN received treatment according to treating physicians' opinion. In brief, 11 patients (28.2%) received induction therapy with mycophenolate, 13 Euro-cyclophosphamide protocol (33.3%) while the remaining 15 (38.5%) received rituximab-based regimens. Thirty-seven patients (94.9%) were on hydroxychloroquine (HCQ).

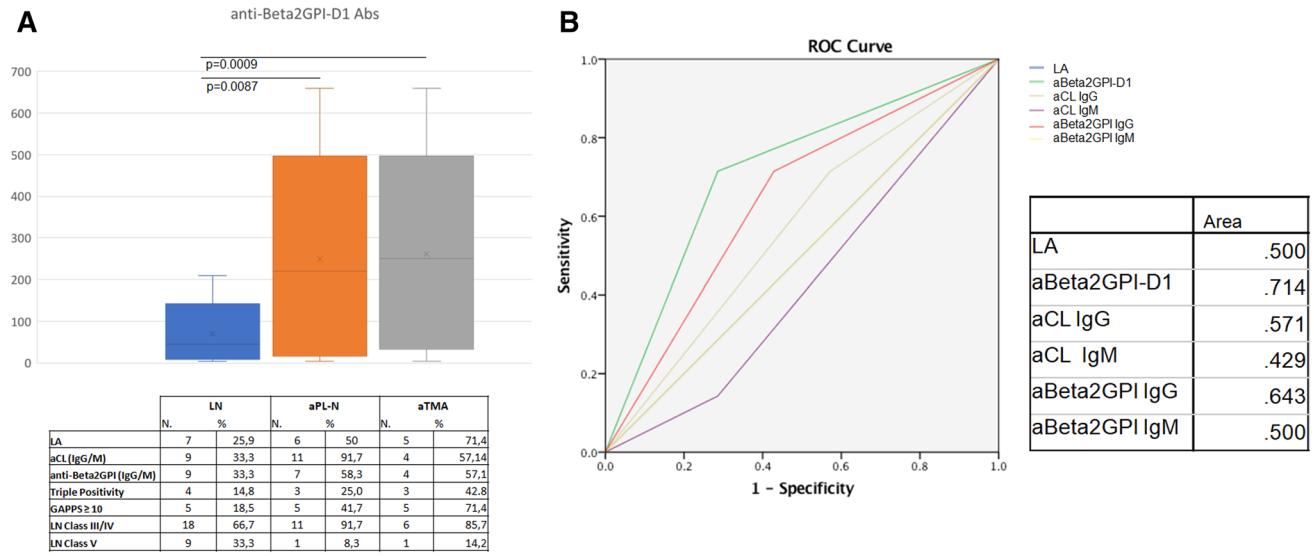
aPL-N has been defined as previously described [19]. In brief, aPL-N includes renal artery stenosis,

Table 1 Demographic and clinical characteristics of the SLE cohort

Total $N = 124$		N	%
Female		96	77.4
Clinical features	–		
Skin involvement		33	26.6
Hematological involvement		50	40.3
Joint involvement		59	47.6
NPSLE		4	3.2
LN		39	31.5
Thrombotic APS[a]		29	23.4
Obstetric APS		2	1.6
aPL-N[b]		12	9.7
Acute TMA		7	5.6
Renal vein thrombosis		1	0.8
Laboratory profile	–		
aPL antibody positivity			
LA		34	27.4
aCL (IgG/M)		29	23.4
Anti-β2GPI (IgG/M)		32	25.8
Triple positivity		19	15.3
Anti-dsDNA		66	53.2
Creatinine > 3 mg/dL		10	8.1
Low C3 levels		49	39.5
Low C4 levels		41	33.1
Proteinuria > 3.5 mg/day		16	12.9
Cardiovascular profile			
Arterial hypertension		32	25.8
Hyperlipemia		14	11.3
GAPPS ≥ 10		26	21.0
GAPPS ≥ 12		19	15.3
Smoking		9	7.3

[a]None of which with APSN

[b]features of aPL-N associated to LN



	LN		aPL-N		aTMA	
	N.	%	N.	%	N.	%
LA	7	25,9	6	50	5	71,4
aCL (IgG/M)	9	33,3	11	91,7	4	57,14
anti-Beta2GPI (IgG/M)	9	33,3	7	58,3	4	57,1
Triple Positivity	4	14,8	3	25,0	3	42.8
GAPPS ≥ 10	5	18,5	5	41,7	5	71,4
LN Class III/IV	18	66,7	11	91,7	6	85,7
LN Class V	9	33,3	1	8,3	1	14,2

	Area
LA	.500
aBeta2GPI-D1	.714
aCL IgG	.571
aCL IgM	.429
aBeta2GPI IgG	.643
aBeta2GPI IgM	.500

Fig. 1 **a** Upper panel: distribution of anti-β2GPI-D1 antibodies expressed as box-and-whisker plots. Lower panel: demographic, clinical and laboratory characteristics in the three subgroups. aTMA group includes 7 out 12 patients with aPL-N. **b** Receiver operating characteristic (ROC) curves of the various aPL. Sensitivity and specificity were calculated according to the presence of acute features of TMA

renal infarction, renal vein thrombosis and TMA. Renal TMA was defined as interlobular artery, arteriole, and/or glomerular capillary lesions, including endothelial cell swelling, lumen narrowing or obliteration, and thrombi formation by light microscopy. TMA manifestations were divided into aTMA and chronic lesions (cTMA), as previously described [16]. In brief, out of the 7 patients with aTMA, 3 presented mainly with features of glomerular acute lesions (to include: endothelial swelling with partial/complete occlusion of lumina; microthrombi-focal or global-; fragmented red blood cells on glomerular subendothelial space and/or mesangial areas; mesangiolysis-focal/segmental/global-; glomerular congestion with efferent arteriolar occlusion); 2 patients showed predominant arteriolar acute lesions in TMA (endothelial swelling with partial or complete occlusion; fibrin/platelet thrombi) while the remaining 2 patients presented mixed features of acute glomerular and arteriolar involvement.

LA testing was performed according to international guidelines [20]. Solid-phase aPL testing was performed by a chemiluminescent immunoassay exploiting the BIOFLASH® technology (QUANTA Flash® and QUANTA Flash® β2GPI Domain 1 IgG; Inova Diagnostics, San Diego, CA, USA) [21]. The cut-off values for anti-β2GPI-D1 IgG positivity were 20 chemiluminescent units (CU) as previously determined [11].

Global APS Score (GAPSS) was calculated according to Sciascia et al. [22].

The study was conducted according to the declaration of Helsinki.

Statistical analysis

Data were expressed as a percentage for categorical variables and as median (interquartile range [IQR]) for continuous variables. Between-groups comparisons were performed by Chi-squared or Fisher's exact tests for categorical variables and by Mann–Whitney test or Kruskal–Wallis with Dunn's post hoc test for continuous variables. The diagnostic accuracy of anti-β2GPI-D1 in identifying aPL-N and aTMA was set using ROC curves. Logistic regression analyses were performed to investigate the relationship between binary outcomes and clinically/biologically meaningful risk factors. A p-value < 0.05 was considered statistically significant.

All statistical analyses were performed using SPSS version 19.0 (IBM, Armonk, NY, USA).

Results

As shown in Fig. 1a, we observed that patients with both LN and aPL-N had higher median anti-β2GPI-D1 antibody titres (220.1 CU, IQR 29.1–334.2 CU) as compared to those with LN alone (46.5 CU, IQR 12.5–75.1 CU) ($p = 0.0087$). Similarly, when we identified the 7 patients with aTMA among the 12 aPL-N patients, we found that median anti-β2GPI-D1 antibodies titres were higher in patients with aTMA than in

those with LN alone [250.1 CU (IQR 61.2–334.2) vs. 46.5 CU (IQR 12.5–75.1 CU), $p = 0.0009$].

Besides, we observed that patients with both LN and aPL-N had higher median anti-β2GPI-D1 antibodies titres (220.1 CU, IQR 29.1–334.2 CU) as compared to those with SLE alone (41.4 CU, IQR 11.3–91.3 CU) ($p = 0.0093$). When focusing on the 7 patients with aTMA, we found that median anti-β2GPI-D1 antibodies titres were higher than in patients with SLE with no renal involvement [250.1 CU (IQR 61.2–334.2) vs. (41.4 CU, IQR 11.3–91.3 CU), $p = 0.0007$].

Although we observed a trend towards a higher prevalence of triple positivity (LA, aCL and anti-β2GPI antibodies) in patients with aPL-N and aTMA when compared to LN alone [3/12 (25%), 2/7 (29%), and 4/27(15%), respectively], it failed to reach a statistically significant difference. Conversely, patients with aPL-N and aTMA showed higher values of GAPSS than patients with LN alone [GAPSS > 10 observed in 5/12(42%), 5/7 (71%) and 5/27 (19%), respectively, reaching a statistically significant level of difference when comparing aTMA and LN alone ($p = 0.02$). Having GAPSS > 10 confers an increased probability of having aTMA (OR 6.25, 95%CI 1.2–31.8).

The level of diagnostic accuracy for aTMA among the tested aPL is presented in Fig. 1b, showing that anti-β2GPI-D1 antibodies have the best performance in terms of Area Under the Curves.

No statistical difference was observed in terms of aPL status, levels of anti-β2GPI-D1 or aTMA when stratifying patients for LN-induction regimen or HCQ use.

Discussion

To our knowledge, this is the first study to specifically characterize the domain profiling of anti-β2GPI antibodies in relation to renal vascular involvement in patients with LN. In a cohort of patients with LN and no previous diagnosis of APS or thrombotic events, we observed significantly higher anti-β2GPI-D1 antibodies titres in patients with aPL-N associated with LN as compared to LN alone. Similarly, higher median titres of the anti-β2GPI-D1 antibodies were found when comparing patients with aTMA to those with LN alone.

Some considerations are worth noting: (i) aPL positivity correctly identified patients with a diagnosis of aPL-N, although some heterogeneity among different antibody specificities does exist; (ii) anti-β2GPI-D1 antibodies were associated with acute features of TMA; (iii) aPL-N and aTMA are more frequently seen in patients with more severe risk profiles as expressed by GAPSS or "triple aPL positivity".

These findings are in accordance with previous data [5–9] and overall are in line with the concept that anti-β2GPI-D1 antibodies are a strong risk factor for vascular thrombosis [23]. Some considerations are noteworthy. Firstly, as shown in Fig. 1b, we observed broad heterogeneity in the diagnostic accuracy of different aPL specificities identifying patients with aPL-N as expressed by ROC analysis. anti-β2GPI-D1 antibodies and anti-β2GPI IgG antibodies are those with the best diagnostic performance, thus supporting the concept that the β2GPI represents the main autoantigen targeted by aPL. Moreover, in a cohort of patients at high risk for microvasculature involvement per se (active LN, concomitant systemic disease) it is not surprising that a test with higher specificity might have better performance as compared to those with higher sensitivity for clinical events (e.g. aCL). If confirmed, these observations might support the use of anti-β2GPI-D1 antibodies as second-line testing in an attempt to identify patients at higher risk of clinical events even among those who already tested positive for aPL.

Secondly, while several studies support the possibility that the presence of TMA, or renal vascular involvement in general, are independent risk factors for poorer clinical outcome in subjects with LN [15, 24], translating this concept in term of clinical choices is still challenging and requires further investigation. From these perspectives, anti-β2GPI-D1 antibodies may represent an additional tool to guide both primary and secondary thrombo-prophylactic strategies.

Thirdly, it has been assumed that endothelial β2GPI represents the most important antigenic target for anti-β2GPI antibodies because of the role of the endothelium on coagulation [25]. According to the "two-hit theory", it has been suggested that the inflammatory second hit may affect β2GPI expression on the endothelium [2]. Animal pre-treatment with small amounts of lipopolysaccharide increases the presence of injected labelled β2GPI in vascular tissues and eventually allows antibody binding and complement fixation [26]. Similarly, Meroni and co-workers recently described a case report that apparently also supports such a cascade of events in patients [27]. In fact, β2GPI was found by indirect immunofluorescence staining on the wall of a popliteal artery after thrombosis in a primary APS patient, unlike the negative staining in normal arterial vessels. More importantly, it co-localized with IgG and complement deposits. They hypothesized that a local inflammatory insult can be responsible for the increased β2GPI presence on the vessel wall, followed by antibody binding in an amount large enough to trigger complement activation and clotting. To participate in this intriguing debate, we performed immunohistochemistry staining on kidney biopsy tissue with anti-β2GPI-D1 antibodies (kindly provided by INOVA Diagnostic, San Diego, CA, USA), and showed that immune-positive cells are situated exclusively within the endothelial layer of the blood

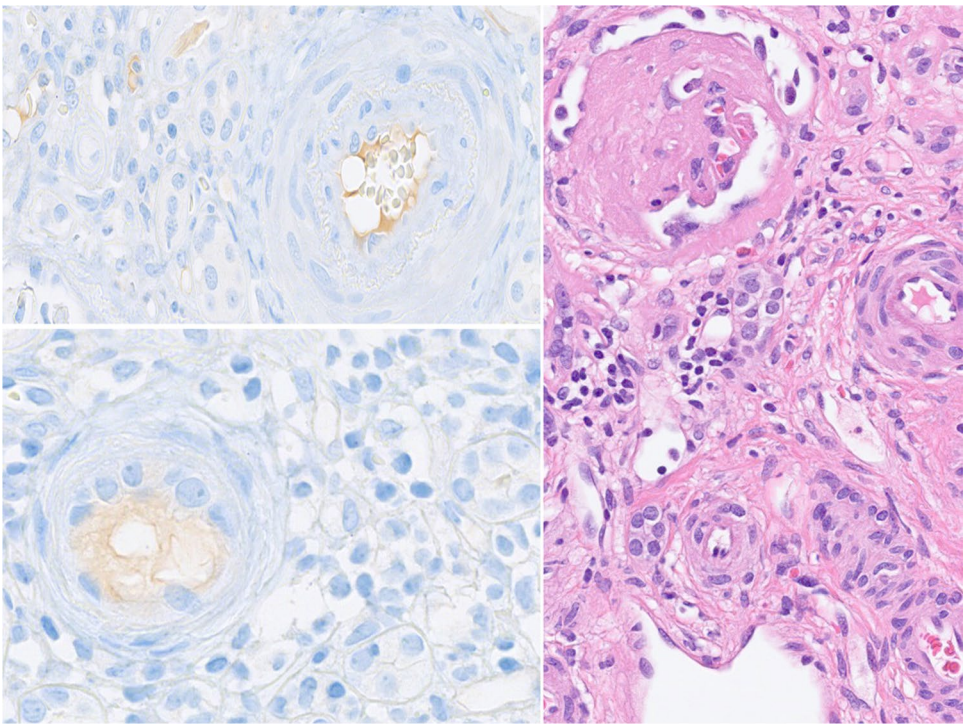


Fig. 2 Kidney biopsy showing luminal narrowing of arteriole and glomerulus exhibiting ischemic features (HE, right panel). An ethanol-fixed human kidney tissue labelled with anti-anti-β2GPI-D1 monoclonal (kindly provided by INOVA Diagnostic, San Diego, CA, USA) showed immunopositive cells are situated exclusively within the endothelial layer of the blood vessels (left panels)

vessels (Fig. 2). On the basis of the above-mentioned findings, the inflammatory microenvironment related to concomitant LN might have triggered the increased β2GPI presence and the exposure of the pathogenic domain 1, which in the presence of circulating anti-β2GPI-D1 antibodies may have triggered the microangiothrombotic complications. This preliminary analysis needs to be confirmed in a controlled fashion.

Even though not large, the sample size was relevant given the strict inclusion criteria we adopted, allowing us to adequately pursue the aim of this study. We acknowledge that the retrospective design should be regarded as a study limitation.

As a whole, our findings suggest that the relevance of the anti-domain reactivity goes beyond the association with thrombotic events. Anti-β2GPI-D1 antibodies detection might provide a second-line assay to be performed in anti-β2GPI positive patients with LN, allowing a more accurate stratification of the renal vascular involvement risk. Despite some limitations of the study, we found that anti-β2GPI-D1 antibodies are associated with aPL-N in patients with LN and that their positivity confers an increased risk of developing aTMA. The usefulness of anti-β2GPI-D1 antibodies testing to identify subjects at risk of aPL-N should be confirmed in well-designed prospective studies, hopefully leading to tailored therapeutic management and ultimately to an improvement in renal outcome in SLE.

Author contributions SS, MR, DRoc: research idea and study design; DRoc, LB, DRos, RF, ADM: data acquisition; SS, MR, IC, ER, DRoc: data analysis/interpretation; MR, SS, PM, SB: statistical analysis; DRoc, SS supervision or mentorship. SS: takes responsibility for all aspects of the reliability and freedom from bias of the data presented and their discussed interpretation. Each author contributed important intellectual content during manuscript drafting or revision, accepts personal accountability for the author's own contributions, and agrees to ensure that questions pertaining to the accuracy or integrity of any portion of the work are appropriately investigated and resolved.

Compliance with ethical standards

Conflict of interest The authors report no conflict of interest.

Research involving human participants and/or animals and informed consent The study was conducted according to the declaration of Helsinki and informed consent was given to all patients that participated in the study.

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