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CLINICAL RESEARCH

Clinical-Pathological Characteristics of Renal Injuries Identify Different Clusters in Patients With Antiphospholipid Antibodies

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Introduction: Significant heterogeneity still exists in the nomenclature of renal involvement in antiphospholipid syndrome (APS).

Methods: We applied a hierarchical cluster analysis to determine subgroups of patients according to clinical, laboratory, and renal histology characteristics in a cohort of subjects with confirmed antiphospholipid antibodies (aPL) positivity and biopsy proven aPL-related renal injuries. Kidney outcomes were then assessed at 12 months.

Results: A total of 123 aPL-positive patients were included in the study (101 [82%] female, 109 [88.6%] with systemic lupus erythematosus [SLE], 14 (11.4%) with primary APS [PAPS]). Three clusters were identified. Twenty-three patients (18.7%) were included in the first cluster (cluster 1), characterized by a higher prevalence of glomerular capillary and arteriolar thrombi and fragmented red blood cells in the subendothelial space. Cluster 2 included 33 patients (26.8%) and showed a higher prevalence of fibromyointimal proliferative lesions as seen in hyperplastic vasculopathy. Cluster 3 was the largest (67 patients, mainly with SLE) and was characterized by higher prevalence of subendothelial edema, of both glomerular capillaries and arterioles.

Conclusion: Three different clusters of patients with aPL and renal injuries emerged from our study as follows: the first, with the worst renal prognosis, was associated with features of thrombotic microangiopathy (TMA), thrombosis, triple aPL positivity and higher adjusted Global APS Score (aGAPSS) values; the second, characterized by hyperplastic vasculopathy with an intermediate prognosis, was seen more frequently in patients with cerebrovascular manifestations; and the third, more benign in terms of outcomes and with no overt association with thrombotic features, was characterized by endothelial swelling in concomitant lupus nephritis (LN).

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APS is primarily characterized by thrombosis in both the venous and arterial systems, affecting all calibers and different organs.[1] Although less common, renal involvement in patients with detectable aPL significantly impacts morbidity and mortality. Clinical and histologic presentations are heterogeneous and have been reported with various prevalence in different studies.[2,3]

In the early 1990s, Amigo *et al.*[4] provided the first description of intrarenal vascular damage in 5 patients with PAPS. All patients included were hypertensive with a variable degree of proteinuria and kidney dysfunction. This also included histologic features suggesting TMA, along with fibrous intimal hyperplasia, subendothelial fibrosis, and eccentric narrowing of the interlobular arterioles. Typical features of LN, such as immune-complex deposits, proliferative glomerular lesions, crescents, and tubulo-reticular bodies were not found.[2] Those noninflammatory lesions appeared to be unrelated to SLE and were better characterized by Nochy and colleagues in 1999, by evaluating kidney biopsies from 16 patients with PAPS.[5] They reported vascular lesions, such as fibrotic intimal thickening, arterial and arteriolar fibrous and fibrocellular occlusions, and TMA. Along with vascular damage, they described glomerular mesangiolysis and focal cortical atrophy, findings though nonspecific, that likely reflected repeated ischemic damage, and "thyroidization" of the tubular component.[5] Since then, the description of the spectrum of aPL-related renal lesions has rapidly increased.[6,7] The term APS nephropathy (APSN) was coined to refer to a diverse group of renal injuries observed in patients persistently positive for aPL. However, to date, significant heterogeneity still exists in the nomenclature for this condition. This includes variations in defining the histologic features identified as belonging to APSN, variable accuracy of recognition of these renal lesions, and heterogenous clinico-pathological associations. More importantly, the prognostic value of the different types of aPL-related renal lesions remains to be fully elucidated.

The main aim of this multicenter study is to describe the clinico-pathological characteristics of aPL-related renal injuries (so-called APS-Nephropathy)[6] in a large cohort of patients investigated by renal biopsy who were persistently positive for aPL. Second, we aimed to evaluate if renal prognosis is associated with different clinico-pathological types of renal injuries. Third, we sought to perform a hierarchical clustering analysis to explore the aggregation of patients into different subgroups sharing common characteristics in terms of clinical and laboratory phenotypes.

METHODS

Data Source and Population

This multicenter retrospective cohort study was carried out at the following centers: (i) Center of Research of Immunopathology and Rare Diseases and Nephrology and Dialysis, S. Giovanni Bosco Hospital, Turin, Italy; (ii) Division of Rheumatology, Russell/Engleman Research Center, University of California, San Francisco, California, USA; (iii) Lupus Unit, Rheumatology Division, Guy's and St. Thomas NHS Foundation Trust, London, UK; (iv) Department of Experimental and Clinical Medicine, University of Florence, Italy; (v) Division of Nephrology and Dialysis, IRCCS Ca' Granda Ospedale Maggiore, Milano, Italy; and (vi) Rheumatology Division, São Paulo University, São Paulo, Brazil. The study was conducted in agreement with the Helsinki Declaration and written consent was obtained before performing renal biopsy.

Clinical and laboratory data of these were searched through the electronic medical record system of the hospitals (2011−2021) and eligible patients were identified according to the following inclusion criteria: (i) >18 years of age; (ii) aPL-positive testing using standard criteria, with confirmed positivity 12 weeks apart[8]; (iii) clinical renal involvement suspected for the presence of any of the following findings: (nephrotic-range proteinuria, active urinary sediment, urine abnormalities [hematuria and non-nephrotic proteinuria], acute worsening of kidney function), and subsequently confirmed by a kidney biopsy. Patients with incomplete records (e.g., incomplete follow-up data on outcomes of interest) were excluded from the study.

Data Collection

Demographic and Clinical Variables

Demographic data and clinical manifestations included age, sex, ethnicity (Caucasian, Black, Hispanic, Asian, Other), aPL profile (any of the following: [anti-cardiolipin antibodies IgG and/or IgM, anti-β2 Glycoprotein IgG and/or IgM, lupus anticoagulant]; single, triple positivity), concomitant APS diagnosis, aGAPSS (calculated as previously described,[9] cut off values: 10, 12), concomitant SLE diagnosis, low C3 or C4, anti-DNA positivity, LN class (proliferative vs. membranous LN) and TMA characteristics (acute vs. chronic features, as detailed in Supplementary Table S1).

Forms of immunosuppressant therapy was categorized by type of induction therapy (cyclophosphamide, either NIH[10] or EUROLUPUS[11] regimen), mycophenolate mofetil or other), type of maintenance therapy (mycophenolate mofetil, azathioprine, other)

Anticoagulant therapy included antiplatelets and/or anticoagulant agents (heparins, either low molecular weight heparin or unfractionated heparin), or vitamin K antagonists. For patients on vitamin K antagonists, satisfactory time in therapeutic range was defined at >65%.

Anticoagulant and Immunosuppressive therapies were considered if given for at least 6 consecutive months after aPL-related renal injury diagnosis.

Renal Histology

Renal involvement was classified according to the type of renal injury observed. Data on lesions observed by light microscopy involving interlobular artery, arteriole, and glomerular capillary lesions, as well as endothelial cell swelling; luminal narrowing or obliteration; and thrombi formation, fibro-intimal thickening, arteriolar organized thrombi with or without recanalization; and the extent of tubulo-interstitial disease were collected. The aPL-related injuries were divided into acute and chronic lesions. An *a priori* defined data collection approach was used to categorize renal histopathology lesions and to homogenize the defined nomenclature (Supplementary Table S1). Pathology reports were centrally rereviewed to improve consistency. In the case of concomitant LN, biopsy results were interpreted according to the classification of International Society of Nephrology and the Renal Pathology Society 2003 LN classification system and the revised version.[12]

Kidney Outcomes

Kidney outcomes were assessed at 12 months, according to KDIGO guidelines.[13] Complete renal response, defined as complete return of serum creatinine to previous baseline, plus a decline in the urine protein-to-creatinine ratio (uPCR) to <500 mg/g (<50 mg/mmol). Partial renal response defined as stabilization (±25%), or improvement of serum creatinine, but not to normal, plus a ≥50% decrease in uPCR. If there was nephrotic-range proteinuria (uPCR ≥3000 mg/g [≥300 mg/mmol]), improvement requires a ≥50% reduction in uPCR, and a uPCR ≥3000 mg/g [≥300 mg/mmol]. No renal response was defined as a sustained 25% increase in serum creatinine, an increase in proteinuria, or a reduction in proteinuria, but not to the extent of complete or partial response. Estimated glomerular filtration rate was assessed in all the patients by the Modification of Diet in Renal Disease equation.

Statistical Analysis

Numeric data with normal distribution were compared by independent samples t-test; numeric data with abnormal distribution of ranked data were compared by Mann-Whitney U-test. Categorical data were compared by $\chi 2$ test or Fisher exact test.

A hierarchical cluster analysis from the multiple correspondence analysis was used to determine subgroups of patients according to clinical and laboratory characteristics. This approach was chosen because it proceeds successively from less inclusive clusters through larger more inclusive clusters and continues until all variables are clustered in a single group. This technique differs from other forms of cluster analysis in which a single set of mutually exclusive and exhaustive clusters is formed.[14] Euclidean distance and the Ward agglomerative method were applied. Crude associations were performed between the different included variables that participated in the construction and those that were positioned with clusters identified by the hierarchical cluster analysis.

All statistical analyses were performed with the SPSS statistical package version 26.0 (SPSS Inc, Chicago, IL, USA). Missing data were included as an additional category in the analysis. *P* values less than 0.05 were considered significant in 2-sided tests. Additional details on data collection are provided in Supplementary Tables S2 and S3.

RESULTS

Demographic and Clinical Manifestations

A total of 123 patients, including 101 (82%) females, 109 (88.6%) with SLE, 14 (11.4%) with PAPS fulfilled the inclusion criteria. The main characteristics of the cohort are summarized in Table 1.

A total of 41 patients out of 123 had a diagnosis of APS; 14 cases had no features of other autoimmune disease, whereas 27 patients presented with a concomitant diagnosis of SLE according to the American College of Rheumatology classification criteria.[14] All the remaining 82 patients had SLE with aPL but did not meet the classification criteria for APS. In the group of patients with APS associated with SLE, 20 of

Table 1. Population characteristics

Characteristics	Total (123)	%
Female	101	82.1
SLE	109	88.6
Class III (III+V)	10	8.1
Class IV	87	70.7
Class V	8	6.5
Class IV-V	4	3.6
Secondary APS	27	22.0
Primary APS	14	11.4
Thrombocytopenia (<100.000 platelets/μl)	22	17.9
aPL antibody positivity	123	100.0
LA	45	43.7
aCL	41	39.8
anti-β2 GPI	31	30.1
Triple aPL positive	21	20.4
Creatinine > 3 mg/dl	31	30.1
Microscopic hematuria (>5 erythrocytes/HPF)	81	78.6
Proteinuria > 3.5 g/d	52	50.5
Arterial hypertension	72	69.9
Hyperlipemia	49	47.6
aGAPSS ≥ 10	37	35.9
aGAPSS ≥ 12	29	28.2

aCL, anticardiolipin antibodies; aGAPSS, adjusted Global APS Score; aPL, antiphospholipid antibodies; APS, antiphospholipid syndrome; HPF, high-power field; LA, lupus anticoagulant.

27 presented with thrombotic APS, 5 with the obstetrical manifestations, and 2 presented with both.

Among patients with concomitant LN, class IV was the most frequently observed (87/123, 70.73%).

Regarding the aPL profiles, a majority of the patients showed lupus anticoagulant (LA) positivity (43%), whereas triple positivity (defined as concomitant positivity for aCL, aβ2 GPI and LA) was found in 20.4% of patients. Arterial hypertension at the time of renal biopsy was frequently observed in this cohort (70%). Histopathological findings and their frequency are summarized in Table 2. Of note, 109 patients presented with a concomitant diagnosis of LN. Data on anticoagulation and different immunosuppressant regimens are detailed in the Supplementary Table S2.

Histopathological Findings

When considering histopathological findings found on kidney biopsy (all groups), subendothelial edema was the most common acute glomerular lesion (64%) by light microscopy, whereas capillary wall thickening with double contours was the most frequent chronic lesion (56%). Fragmented red blood cells in subendothelial space and intimal layers were the most frequent acute lesions seen in arteries and arterioles, whereas fibromyointimal proliferation with luminal narrowing was the most common chronic lesion in this compartment.

Table 2. Frequency of each histologic changes

Histologic changes	n	%
Glomerular acute lesions (any)	87	70,7
• Endothelial swelling with partial or complete occlusion of lumina	79	64,2
• Microthrombi. focal or global	33	26,8
• Fragmented RBC on glomerular subendothelial space and/or mesangial areas	17	13,8
• Mesangiolysis. focal. segmental/global	78	63,4
• Glomerular congestion with efferent arteriolar occlusion	27	22,0
Glomerular chronic lesions (any)	72	58,5
• Capillary wall thickening with double contours	69	56,1
• Organizing capillary thrombi	11	8,9
• Glomerular ischemic collapse with afferent arteriolar occlusion	9	7,3
• Segmental/global glomerulosclerosis	7	5,7
Arteriolar acute lesions		
• Endothelial swelling with partial or complete occlusion	5	4,1
• Fibrin/platelet thrombi. segmental/partial or occlusive	7	5,7
• Fragmented RBC in subendothelial space	21	17,1
Arteriolar chronic lesions		
• Organizing thrombi. partial or occlusive	9	7,3
• Fibromyointimal thickening and proliferation	47	38,2
Arterial acute lesions		
• Endothelial separation with intimal mucoid degeneration	3	2,4
• Intravascular thrombi. segmental/partial or occlusive	4	3,3
• Fragmented RBC in subendothelial space	7	5,7
Arterial chronic lesions		
• Organizing thrombi. partial or occlusive	7	5,7
• Fibromyointimal thickening and proliferation	44	35,8

RBC, red blood cell.

Cluster Analysis and Prognostic Value

To identify clusters of patients with similar renal involvement (both clinical and pathological), a hierarchical clustering was performed. The hierarchical tree identified a division into 3 clusters. These variables included in the model are detailed as Supplementary Table S1. Results of the analysis are shown in Table 3.

Out of 123 patients who tested positive for aPL included in the study, 23 (18.7%) were included in the first cluster (cluster 1). Cluster 1 was characterized by a higher prevalence of capillary and arteriolar thrombi and fragmented red blood cells in the subendothelial space (as seen typically in acute TMA). This included capillary wall thickening with double contours, organizing thrombi, and ischemic collapse of the glomerular tuft with afferent arterial occlusion (as typically seen in chronic TMA). Cluster 1 was therefore named "TMA". The prevalence of thrombotic events, aGAPSS (>12) and triple positive aPL tests were higher in this cluster. This cluster comprise 18 patients with SLE and 5 with PAPS

Cluster 2 included 33 patients (26.8%) and showed a higher prevalence of fibromyointimal proliferative lesions when compared to the other clusters. This type of injury is usually considered a chronic lesion with hyperplasia of myointimal cells and progressive narrowing of the vascular lumen. Cluster 2 was named "hyperplastic vasculopathy". This cluster comprised 24 patients with SLE and 9 with PAPS

Cluster 3 was the largest (67 patients) and was characterized by higher prevalence of subendothelial edema, both of glomerular capillaries and arterioles. This lesion is considered an acute reaction to endothelial injury. We named this cluster "subendothelial edema". This cluster comprised only patients with SLE and none with PAPS.

The distribution of complete renal response, partial renal response, and no renal response in the whole cohort is shown in Figure 1, whereas the distribution across the 3 clusters, with the TMA cluster associated with a lower complete renal response and partial renal response when compared to the other subgroups is shown in Figure 2 and Table 4. Examples of aPL-kidney injuries at the histologic level are shown in Figures 3, 4, and 5. No statistically significant difference was observed when the use of different immunosuppressant regimens across the 3 clusters were compared.

DISCUSSION

Over the years, the term APSN has been used to cover a heterogeneous group of aPL-related renal lesions with

Table 3. Analysis of clusters

Items	Total	Cluster 1 $n = 23$	% Total	% cluster 1	Cluster 2 $N = 33$	% Total	% cluster 2	Cluster 3 $n = 67$	% Total	% cluster 3	*P* cluster 1 vs. 2	*P* cluster 1. vs.3	*P* cluster 2 vs.3
Endothelial swelling with partial or complete occlusion of lumina	79	13	10.6	56.5	5	4.1	15.2	61	49.6	91.0	.001[a]	<.001[a]	.867
Fibromyointimal thickening and proliferation	61	4	3.3	17.4	29	23.6	87.9	28	22.8	41.8	.579	.035[a]	<.001[a]
Capillary wall thickening with double contours	69	19	15.4	82.6	30	24.4	90.9	20	16.3	29.9	.335	<.001[a]	<.001[a]
Organizing thrombi, partial or occlusive	16	13	10.6	56.5	0	0.0	0.0	3	2.4	4.5	<.001[a]	<.001[a]	.728
Glomerular ischemic collapse with afferent arteriolar occlusion	10	9	7.3	39.1	0	0.0	0.0	1	0.8	1.5	<.001[a]	<.001[a]	.606
Fibrin/platelet thrombi, segmental/partial or occlusive; Microthrombi, focal or global	33	23	18.7	100.0	5	4.1	15.2	5	4.1	7.5	<.001[a]	<.001[a]	.207
Fragmented RBC in subendothelial space	28	17	13.8	73.9	5	4.1	15.2	6	4.9	9.0	<.001[a]	<.001[a]	.352
Thrombotic APS	26	21	17.1	91.3	2	1.6	6.1	3	2.4	4.5	<.001[a]	<.001[a]	.733
SLE	109	18	14.6	78.3	24	19.5	72.7	67	54.4	100.0	.221	<.001[a]	<.001[a]
Cerebrovascular Events (Stroke and TIA)	10	2	1.6	8.7	7	5.7	21.2	1	0.8	1.5	.210	.108	<.001[a]
Triple positivity	21	15	12.2	65.2	3	2.4	9.1	3	2.4	4.5	<.001[a]	<.001[a]	.361
aGAPSS>12	29	13	10.6	56.5	12	9.8	36.4	4	3.3	6.0	.135	<.001[a]	<.001[a]

APS, antiphospholipid syndrome; aGAPSS, adjusted Global APS Score; RBC, red blood cell; SLE, systemic lupus erythematosus; TIA, transient ischaemic attack.
[a]statistically significant at $P < 0.05$.
Cluster 1 (thrombotic microangiopathy); cluster 2 (hyperplastic vasculopathy); cluster 3 (subendothelial edema).

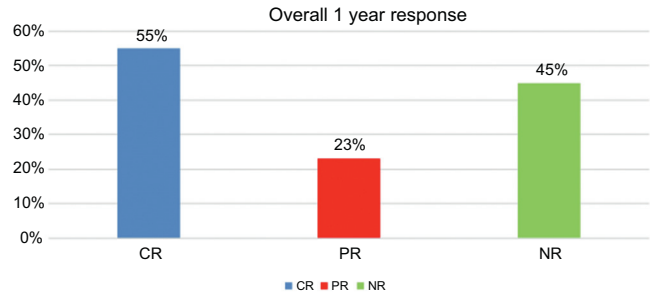

Figure 1. One year follow up response. CR, complete response; PR, partial response; NR, no response.

potentially different prognostic significance and clinical presentations. Although the 2015 APS Task Force on "non-criteria" manifestations of APS found a higher grade of evidence to support the inclusion of the APSN among the main clinical manifestations of the syndrome, the overall grade of evidence was then lowered because of the lack of well-designed studies.[15] Indeed, the authors concluded that larger, multicenter studies were needed to address the prognostic significance of the pathologic changes in APSN. To respond to that international call, we investigated the clinical and pathological features observed in a large multicenter cohort of confirmed aPL postitive patients with renal lesions suggestive of APSN. When considering the totality of the observed renal injuries patterns, some considerations are worth noting.

In this study, we attempted to investigate if different subgroups of aPL-positive patients exist among those presenting with renal dysfunction in terms of clinical presentation, histopathologic features, and renal outcomes.

Our data showed that the TMA cluster presented a higher frequency of patients with thrombotic APS. This observation is consistent with the higher rate of triple positivity and elevated aGAPSS (>12) patients observed in this cluster compared to the others. One could speculate that this cluster might be the most representative for what could be considered APSN.

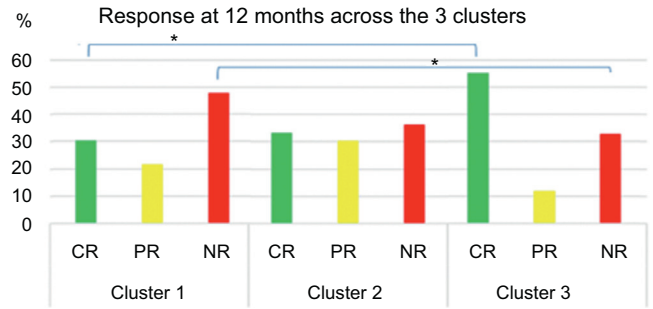

Figure 2. Renal response by clusters.
Cluster 1 (thrombotic microangiopathy); Cluster 2 (hyperplastic vasculopathy); Cluster 3 (subendothelial edema). CR, complete response; PR, partial response; NR, no response.

Table 4. Renal response by clusters. Cluster 1(TMA); cluster 2 (hyperplastic vasculopathy); cluster 3 (subendothelial edema)

	Cluster 1 TMA			Cluster 2 Hyperplastic vasculopathy			Cluster 3 Subendothelial edema		
Renal response	CR	PR	NR	CR	PR	NR	CR	PR	NR
n	7	5	11	11	10	12	37	8	22
%	30.4	21.7	47.8	33.3	30.3	36.4	55.2	11.9	32.8

CR (complete response), PR (partial response), NR (no response); TMA, thrombotic microangiopathy.

A predominance of glomerular lesions, both acute and chronic, was observed in our cohort (70, 7% and 58, 5% respectively). Among acute lesions, glomerular capillary subendothelial edema and mesangiolysis were the most frequently observed with a smaller frequency of capillary microthrombi. Among chronic lesions, we found glomerular basement membrane thickening with double contours were the most commonly occurring feature. Although the latter lesion is traditionally considered as resulting from healed endothelial injury with glomerular basement membrane remodeling, a chronic change, it could appear quite early in the disease course according to some authors, suggesting prior subclinical injury.[3,16] Fibrous intimal hyperplasia was the main change observed in both arterioles and small arteries (49, 5%). These observations are in line with previous reports.[3] When focusing on single histopathological findings, we found fibrin thrombi in up to 26 (8%) of the cases, with a similar rate as observed by Nochy *et al*. (31%).[5] Conversely, myointimal hyperplasia with luminal narrowing was less prevalent in our cohort when compared to the previous study (75% vs. 49.5%).[5] This difference might be explained by the fact that only PAPS patients were enrolled in the French cohort. In fact, when these authors analyzed a cohort of SLE patients, a frequency of 18% of TMA and 24% of chronic lesions (including fibrous intimal hyperplasia) was found.[16]

Because of the existing overlap of different pathological findings, we performed further analyses to investigate if different clusters of renal injuries exist, and if they have prognostic significance. Three main clusters of pathological findings were identified.

The smaller cluster (23 patients) was defined as the TMA cluster and patients belonging to this group were invariably found to have glomerular fibrin thrombi, along with trapped fragmented red blood cells and organized thrombi. Hyperplastic vasculopathy was the main characteristic in a cluster including 33 patients. The largest cluster (67 patients) showed a high frequency of subendothelial edema and a lower frequency of thrombi and myointimal hyperplasia when compared to the other subgroups.

The TMA cluster showed the poorest renal outcome. This result is consistent with other studies where the presence of TMA is reported to negatively impact overall renal prognosis when compared to other vascular lesions. Similarly, in patients with LN, the coexistence of TMA is a well-recognized negative prognostic factor.[16-20]

In the hyperplastic vasculopathy cluster, we observed an intermediate renal outcome profile between those observed in the TMA cluster and the "subendothelial edema" cluster (the latter representing a majority of the patients). Interestingly, in the hyperplastic vasculopathy cluster, we did not find a higher rate of thrombotic events, nor a strong association with overt APS. These findings are consistent with recent studies suggesting that hyperplastic lesions in APS could have a different pathophysiology from thrombosis.[21] Among others, a study by Canaud showed that, *in vitro*, aPLs can interact with the m-TOR complex, stimulating the growth and proliferation of intimal and probably endothelial cells,[22] indicating a prospect for consideration of new therapeutic options beyond anticoagulation in this setting.[23]

The patients in the so-called subendothelial edema cluster were found to have the highest rate of renal response at 1 year. This cluster included solely patients with SLE and LN and only a few cases of concomitant APS. One could speculate that the histologic lesions characterizing this cluster might be considered an expression of early or a milder form of TMA or could be nonspecific for aPL-related injuries and part of the LN spectrum, which could have a potential for reversibility without significant sequelae following treatment. This raises the possibility that any appropriate intervention including immunosuppressive therapy could be more effective in these cases because of the potential early phase nature of these lesions. Nevertheless, although investigating the mechanisms underlying these lesions was outside the scope of this study, one could question whether the inclusion of these injuries should be still part of the spectrum of APSN. Similarly, the presence of mesangiolysis, the most frequently reported lesions of our cohort, is equally distributed among the 3 clusters having no prognostic value in this cohort.

In summary, our study confirms that different patterns of renal lesions with different prognoses and treatment responses do exist, emphasizing the importance of a renal biopsy when renal involvement is suspected in patients with aPL. In line with available data, our findings support the notion that renal histopathological lesions highly suggestive of TMA, as observed in the first cluster, are associated with worse renal outcomes and systemic thrombotic events, and

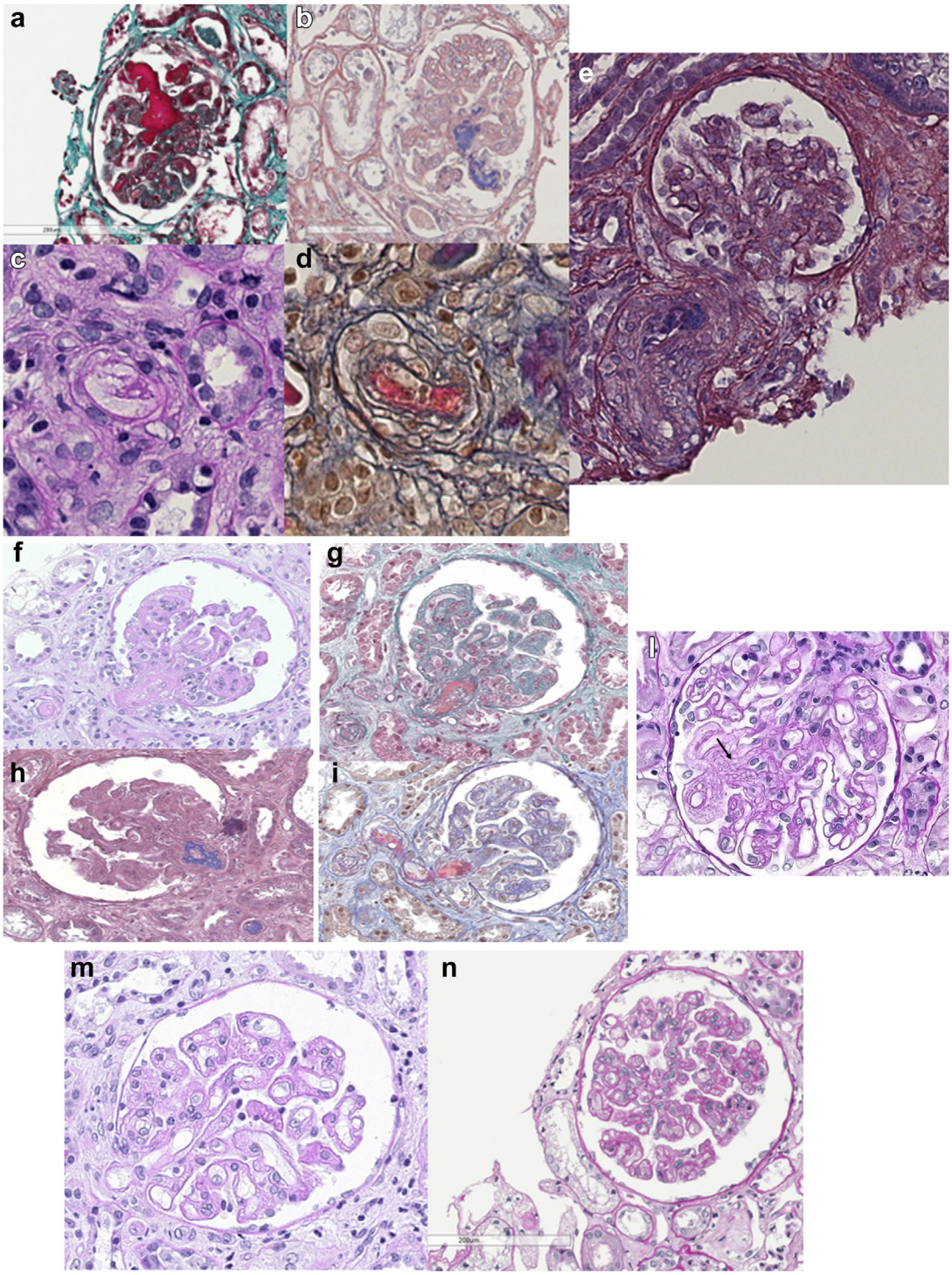

Figure 3. Examples of lesions identified in Cluster 1: (a) Trichrome staining demonstrates a thrombotic lesion involving glomerular capillary lumina and vascular hilum, that appears in blue in (b) phosphotungstic acid haematoxylin (PTAH) stain. (c) Periodic acid–Schiff (PAS) stain demonstrates a small early arteriolar thrombotic lesion that stains orange in (d) AFOG because of fibrin positivity. (e) PTAH stain showing one glomerulus with ischemic changes. The afferent arteriole is occluded by a thrombus that tipically stains in blue for fibrin positivity with PTAH (white arrow). (f) PAS showing a glomerulus with ischemic collapsing features. The afferent arteriole is entirely occluded by a thrombus that stains (g) red in trichrome, (h) blue in PTAH and (i) orange in AFOG because of fibrin positivity. (l) PAS staining showing a glomerulus with mesangiolysis. (m, n) PAS stain showing capillary wall thickening with double contours.

therefore could be referred to as APSN. This interpretation would also help to classify those patients within the spectrum of APS in line with the current classification criteria.[9]

The identification of predominantly hyperplastic vasculopathy may have prognostic and therapeutic significance. Data from available literature suggest that renal TMA and hyperplastic vasculopathy diverge on

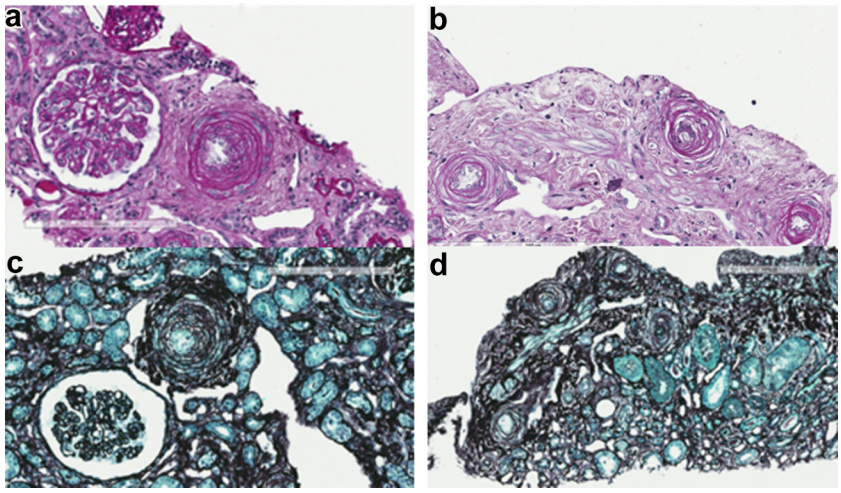

Figure 4. Examples of lesions identified in Cluster 2: PAS stain demonstrates onion-skin layering as seen in chronic lesions in a (a) small artery and in (b) some arterioles. (c, d) Jones stain highlights concentric lamination of intimal fibrosis.

pathophysiological and clinical grounds, even if they could coexist, especially in long-term APS patients.

Because hyperplastic vasculopathy does not seem to be directly associated with the presence of overt APS (at least as currently classified), one could consider referring to these changes as aPLs-related vasculopathy rather than as part of the spectrum of APSN.

We acknowledge this study has some limitations. First, APSN is a rare condition that has a wide spectrum of manifestations. Considering that this is a retrospective analysis, we cannot exclude some inclusion bias when identifying or selecting patients. Furthermore, APSN may occur in the context of coexisting LN. To address this heterogeneity, whereas an *a priori* defined data collection approach was used to categorize renal histopathology lesions and to homogenize the defined nomenclature, it was impossible to investigate the independent effect of the presence of LN on the aPL-related pattern of injuries.

In addition, therapy was given according to treating physicians' judgment and was not controlled. The large majority of SLE patients received either cyclophosphamide or mycophenolate, and patients with previous diagnosis of thrombotic APS received therapy with a vitamin K antagonist. However, treatment heterogeneity in doses, durations of therapy, and adherence is likely. The role of anticoagulation in patients with concomitant LN and TMA was investigated elsewhere.[24] Lastly, renal response to therapy at 1 year was described according to KDIGO guidelines for LN and other glomerulopathies, because no specific consensus exists for assessing the renal response specifically in patients with aPL.[25–27]

In conclusion, we evaluated the frequency of the histologic changes in a large multicenter cohort of

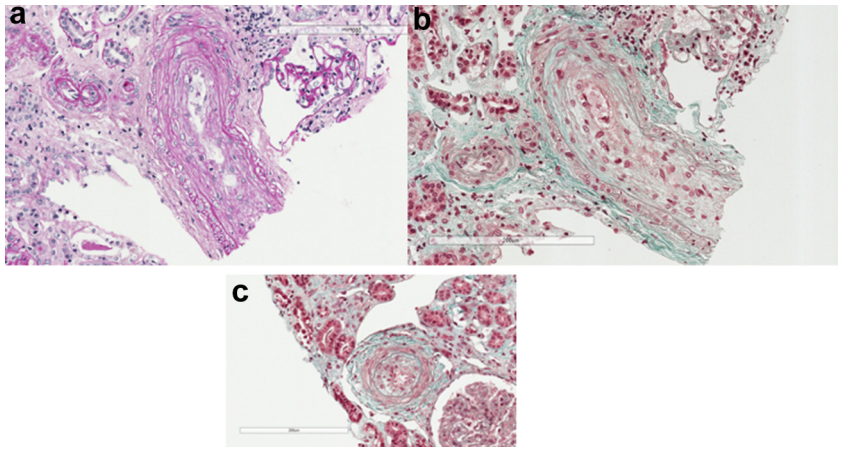

Figure 5. Examples of lesions identified in cluster 3: Myxoid intimal thickening of an artery with prominent subendothelial edema/mucoid degeneration and narrowed lumen (a, PAS stain; b, Trichrome stain). Another example of subendothelial edema in an arteriole (c, Trichrome stain).

APSN patients and we investigated their associations with clinical manifestations and outcomes. Three different clusters of patients with aPL and renal injuries emerged from our study as follows: the first, with the worst renal prognosis, was associated with features of TMA; the second, characterized by hyperplastic vasculopathy with an intermediate prognosis, was seen more frequently in patients with cerebrovascular manifestations; the third, more benign in term of outcomes and with no overt association with thrombotic features, was characterized by endothelial swelling and LN.

Although the results of this study need to be confirmed in future prospective studies, we identified different clusters of renal injury patterns that were associated with prognosis and presentation. This could potentially contribute toward an updated understanding of aPL-related renal disease.

DISCLOSURE

The authors declare no conflict of interest and declare: no support from any organization for the submitted work; no financial relationships with any organizations that might have an interest in the submitted work in the previous 3 years; no other relationships or activities that could appear to have influenced the submitted work.

SUPPLEMENTARY MATERIAL

Supplementary File (PDF)

Table S1. Shared and agreed terminology used to describe histologic changes.

Table S2. Therapeutic strategies applied in the 123 patients.

Table S3. Variables computed in the model.

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