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ORIGINAL RESEARCH

Immune checkpoint molecules performance in ANCA vasculitis

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ABSTRACT

Objective The PD-1 axis promotes protection against autoimmunity. Immune checkpoint (IC) molecules performance in anti-neutrophil cytoplasmic antibodyassociated vasculitis (AAV) remains unknown. This study aims to assess the IC pathway's role in the AAV's pathophysiology.

Methods We recruited 88 AAV from our centre as a discovery cohort (acute=42, remission=46) and 30 patients from another institution for external validation (acute=16, remission=14).

Serum, urine and peripheral blood mononuclear cells (PBMCs) were collected. In vitro IC molecules production by lymphocytes was studied with and without MPO/ PR3 antigen stimulus. Cell culture supernatant (SN) was obtained by centrifugation. PD-1, PD-L1 and PD-L2 concentrations were assessed in serum (s), urine (u) and SN of AAV and healthy controls (HC) using a multiplex assay. PD-1 and PD-L1's expression was analysed in six diagnostic kidney biopsies.

Results uPD-1 and uPD-L2's concentration was lower in AAV than HC (p<0.0001, p=0.0075). Acute patients exhibited lower uPD-L2 levels compared with those in remission (p=0.036). Similarly, PBMCs showed reduced PD-1 production than HC (stimulated group p=0.04, unstimulated p=0.0074). Furthermore, patients with inflammatory renal lesions had fewer PD-1-positive interstitial cells/staining intensity compared with those with sclerotic lesions. Contradictorily, sPD-1 and sPD-L1's concentration was higher in AAV than HC (p=0.007, p<0.0001) with acute patients exhibiting elevated sPD-1 levels compared with those in remission (p=0.0051). Serum and urine findings were confirmed in the validation cohort.

Conclusions Results in urine, SN and histology suggest IC pathway abolition during acute disease restored in remission and contribute to understand PD-1 axis's role in AAV proposing it as a new biomarker of disease activity.

INTRODUCTION

Anti-neutrophil cytoplasmic antibodies (ANCA)-associated vasculitis (AAV) is a multisystemic disease characterised by small vessel inflammation and frequent kidney

WHAT IS ALREADY KNOWN ON THIS TOPIC

⇒ The PD-1, PD-L1 and PD-L2 axis known as the immune checkpoint (IC) pathway promotes immunotolerance by providing protection against autoimmune phenomena. Dysregulation of IC molecules has been described in several autoimmune diseases; however, little is known about the role of ICs in antineutrophil cytoplasmic antibody (ANCA)-associated vasculitis (AAV).

WHAT THIS STUDY ADDS

⇒ Our results indicate the involvement of the immune checkpoint pathway in the pathophysiology of ANCA vasculitis. Results in urine, in vitro production and histology suggest IC pathway abolition during acute disease restored in remission phase.

HOW THIS STUDY MIGHT AFFECT RESEARCH, PRACTICE OR POLICY

⇒ PD-1, PD-L1 and PD-L2 could be used as a tool to detect and monitor disease activity in the clinical practice. PD-1 axis could be a therapeutic target in AAV patients.

involvement. These antibodies bind to specific components of neutrophils and monocytes (myeloperoxidase (MPO) and proteinase 3 (PR3)) and trigger their degranulation which cause the endothelial injury.¹

T lymphocytes are also involved in the pathophysiology of AAV and have been found infiltrating renal tissue in patients with ANCA vasculitis.² In fact, count and functional alterations of T lymphocyte subtypes have been related to disease activity: an increase in Th1 and Th17^{3 4} and a dysfunction of Treg in the acute phase of the disease.⁵

Immune T-cell responses are modulated by co-stimulatory and co-inhibitory stimuli.⁶ From this perspective, immune checkpoint (IC) molecules play a crucial role in maintaining immunotolerance and protecting against autoimmunity, thus, inducing lymphocyte anergy. Programmed cell death 1 (PD-1) regulates immune system responses by binding to its ligands programmed cell death ligand 1 (PD-L1) and programmed cell death ligand 2 (PD-L2). PD-1 is usually present on the membrane of T, B and NK cells; whereas, PD-L1 and PD-L2 are usually expressed on antigen-presenting cells but also on other non-professional immune cells (such as endothelial cells or tubular cells).⁷ The blockade of the PD-1 pathway and its ligands has been extensively studied in the oncology field. Immune check point inhibitors (ICIs) block the PD-1 pathway by stimulating the immune system to eliminate cancer cells.⁸ As a collateral effect of ICI, relapses and debut of autoimmune diseases, including ANCA vasculitis, have been described in patients undergoing treatment with ICIs, which strongly suggests a role of PD-1 pathway in the pathophysiology of autoimmune diseases.9¹⁰

PD-1, PD-L1 and PD-L2 are transmembrane molecules. However, recent studies have identified soluble forms of these proteins generated by alternative splicing or by the direct action of proteases on the transmembrane form. The regulatory mechanism of production of the soluble particles or their function remains controversial.⁷

In human cancer studies, soluble PD-L1 was found to play an immunosuppressive role by inhibiting T-cell activation and promoting T-cell apoptosis, mimicking the effect of transmembrane PD-L1. Beyond cancer, soluble PD-1 axis molecules associated with markers of inflammation have been detected in inflammatory diseases.¹¹ Dysregulation of PD-1, PD-L1 and PD-L2 have also been described in autoimmune diseases such as systemic lupus erythematosus¹² and rheumatoid arthritis.¹³ Bailly et al^{11} describe that elevated levels of soluble PD-L1 were observed in patients with acute pancreatitis, and both soluble PD-1 and PD-L1 were found to be negatively correlated with circulating lymphocyte levels, contributing to immunosuppression. The report also details elevated levels of sPD-1 and sPD-L1 in patients with oral lichen planus when compared with control groups and describes a negative correlation between sPD-L1 expression and CD4⁺ T-cells.

Levels of sPD-1 have also been found to be elevated in patients with rheumatoid arthritis suggesting suppression of T-cell action.^{13 14}

Concerning ANCA vasculitis, it has been reported that both the soluble and transmembrane forms of PD-1 are overexpressed in AAV patients compared with healthy controls (HC). In addition, the transmembrane form correlated with Birmingham Vasculitis Activity (BVAS).¹⁵ Gamerith *et al*¹⁶ investigated the association of soluble immune checkpoints with CD4⁺ and CD8⁺ T-cells and performed a Spearman correlation matrix showing a negative correlation of sPD-1 with CD8 T-cells and a positive correlation for regulatory CD4 T-cells in AAV. However, the mechanisms by which these soluble forms of the proteins act remain poorly understood.⁷ This study aims to shed light on the possible dysregulation of the PD1, PD-L1 and PD-L2 immunoregulatory pathway in AAV, its role in the pathophysiology of the disease and its possible utility as biomarker.

MATERIAL AND METHODS Study population

This is a two-centre observational study performed in Bellvitge University Hospital (HUB), as a discovery cohort, and Navarra University Hospital (HUN), as a validation cohort. A total of 118 patients diagnosed with AAV were recruited, both in acute and in remission phase. Chapel Hill consensus criteria were established to determine the AAV diagnosis¹⁷ and BVAS¹⁸ was used to determine disease activity. BVAS=0 defined patients in remission status. Patients in the acute phase were treatment-naïve patients included at the moment of their initial diagnosis or patients on a disease flare. Patients in remission were AAV patients previously diagnosed who had achieved remission after induction treatment. The study population was followed prospectively and periodically at HUB and HUN and was under a similar immunosuppressive scheme. Remission was reached after intravenous methylprednisolone bolus and/or plasmapheresis treatment followed by rituximab or cyclophosphamide. Maintenance treatment consisted of oral prednisone, mycophenolate, azathioprine or rituximab, as specified in the EULAR/ERA-EDTA guidelines.¹⁹

Other concomitant autoimmune illnesses, active neoplasm or infection during the recruitment and endstage kidney disease were contraindications for participation in the study.

A total of 15 HCs volunteers were also recruited for the study. All HCs were required to have no known medical conditions and not be undergoing any pharmacological treatment.

The patient's samples included in this study were stored by the Biobank HUB-ICO-IDIBELL (PT20/00171) and by Biobank Navarrabiomed (PT13/0010/0051). Biological products were handled in accordance with established protocols and the approval consent of the Ethics and Scientific Committees from both institutions. All patients signed an informed consent form prior to recruitment.

This study was approved by HUB and HUN ethical review boards (PR096/17).

Clinical and laboratory data

Clinical and analytical data were acquired from electronic medical records. We recorded the main demographical data: age, gender and date of diagnosis. We gathered routine analytical data both at the time of diagnosis and recruitment: ANCA titres and specificity (MPO and/or PR3), C reactive protein, and the presence of proteinuria and haematuria. Glomerular filtration rate was determined using the Chronic Kidney Disease Epidemiology Collaboration formula (CKD-EPI). Histopathological assessment was performed by a specialised pathologist

Baseline characteristics of the discovery conort				
N=88	Acute phase, n=42	Remission phase, n=46	P value	
Gender (%male)	42.86	39.13	0.72	
Mean age±SD (years)	67.98±14.37	67.39±10.91	0.36	
ANCA type (%MPO)	76.19	80.43	0.62	
Mean ANCA titres±SD (karbU/L)	534.8±1029	39.36±102	<0.0001	
Mean serum creatinine±SD (umol/L)	295.9±210.5	169.5±91.28	0.0007	
Haematuria (%)	88.10	15.22	<0.0001	
Mean proteinuria±SD (gr/day)	1.109±0.91	0.36±0.45	<0.0001	
Mean CRP±SD (mg/L)	40.62±41.75	9.68±35.47	<0.0001	
Berden histopathological classification at the moment of the	diagnostic (%)			
Focal	9.52	13.04	0.74	
Crescentic	21.43	23.91	0.78	
Mixed	40.48	30.43	0.32	
Sclerotic	9.52	10.87	>0.99	
Induction therapy:				
Methylprednisolone EV (%)	46.34	38.64	0.47	
Cyclophosphamide (%)	47.62	54.35	0.52	
Rituximab (%)	45.24	26.09	0.06	
Plasma exchange (%)	38.10	28.26	0.32	
Maintenance therapy in remission patients (%)				
Without treatment		2.17		
Oral steroids		72.72 (low 75.26, moderate 20.39, high 4.35)		
Mycophenolate mofetil		78.26		
Azathioprine		10.86		
Rituximab		6.52		

Intravenous doses of corticosteroids are considered high dose (more than 250 mg methylprednisolone). With regard to oral steroid dosage, the table differentiates between low dose (5 mg or less of prednisone), moderate dose (6–20 mg of prednisone) and high dose (more than 20 mg of prednisone per day).

ANCA, anti-neutrophil cytoplasmic antibodies; CRP, C reactive protein; EV, endovenous; MPO, myeloperoxidase.

according to Berden's classification.²⁰ The induction, the maintenance treatment, and the patient and renal survival were also recorded.

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Sampling and measurement of immune checkpoint molecules We obtained peripheral blood and urine samples from patients at recruitment. We separated peripheral blood mononuclear cells (PBMC) by Ficoll-Paque density gradient.²¹ We cultured PBMC for 48 hours at a

Table 2 Baseline characteristics of healthy controls		
Healthy controls (n=15)		
Male/female gender (%)	46.66/53.33	
Age (years)	36±11.3	
Body Mass Index (kg/m ²)	26.6±2.2	

concentration of 106 PBMC/400 μ L HyClone RPMI-1640 under three conditions: (1) non-supplemented, (2) with a specific stimulus (supplemented with specific MPO or PR3 antigens 10 μ g/mL according to the specificity of the ANCA antibodies of the patient (Human Leucocyte Myeloperoxidase, Merk and Human non-recombinant Proteinase 3, Diarect, respectively)) and (3) with a nonspecific stimulus (supplemented with phytohemagglutinin (PHA) 30 μ g/mL as a polyclonal lymphocyte stimulus)) to verify the viability of PBMCs culture. Thereafter, supernatant was obtained by centrifugation of the cell suspension. Serum, urine and supernatant were stored at -80° C until utilisation.

We measured the concentration of soluble PD-1, PD-L1 and PD-L2 in serum (s), urine (u) and cell culture supernatant (SN) using a commercial customised multiplex



Figure 1 (A) Serum levels of PD-1 in AAV and HC. (B) Mean of serum PD-1 in acute and remission patients and in HC. (C) Serum concentration of PD-L1 in AAV and HC. Values correspond to discovery cohort.

assay (3-plex Human ProcartaPlexTM) according to the manufacturer's protocol.

Immunostaining

For PD-1 and PD-L1 immunostaining, 2.5 µm paraffinembedded kidney sections were deparaffinized and rehydrated. Tissue was incubated with the primary antibodies-mouse anti-human PD-1 or rabbit anti-human PD-L1 antibodies (1:500, ab52587, Abcam, Cambridge, UK and 1:100, ab205921, Abcam, Cambridge, UK, respectively)-according to the manufacturers' protocols. Thereafter, sections were incubated with a conjugated secondary antibody, and the binding was revealed using 3.3'-diaminobenzidine as a chromogen with avidin-biotinperoxidase complex (EnVision Flex K8002 visualisation System). Kidney biopsies were blindly evaluated by light microscopy. The presence of PD-1 and PD-L1 staining was assessed at 400× magnification on each compartment (glomerulus, tubule, interstitium and vascular compartment). The intensity was scored semiquantitatively (0: no staining, 1: weak staining, 2: moderate staining, 3: strong staining). Using the mean values of 10 randomly chosen cortical visual areas at 400× magnification, interstitial cells positive for PD-1 were assessed and graded semiquantitatively (0: no cell per visual field, 1: 1-3 cells per visual field, 2: 3-6 cells per visual field, 3: >6 cells per visual field), according to the methodology of a previous study.²²

Statistical analysis

Data were analysed using GraphPad Prism V.8.00 (GraphPad Software, La Jolla, CA, USA) and IBM Statistics



Figure 2 (A) Urine levels of PD-1 in AAV and HC. (B) Concentration of uPD-1 in acute, remission patients, and HC. (C) Mean of uPD-L2 in AAV and HC. (D) Concentration of uPD-L2 in acute, remission patients, and HC. Values correspond to discovery cohort.

V.26.0 (IBM Corp., Armonk, NY, USA). To determine the Gaussian distribution of the variables, Shapiro Wilk or Kolmogorov-Smirnov test was applied. Categorical variables were expressed as total number (n) and percentage (%) and continuous variables as mean \pm SD. For continuous variables, Student's t-test or Mann-Whitney U was performed. x² test was applied for categorical variables and Fisher test was used when the number of cases were less than 5. Depending on the distribution of the variables, Spearman's or Pearson's correlation was performed. P values were two-tailed and p<0.05 was considered significant.

RESULTS

Baseline characteristics

88 patients recruited in HUB were included in the discovery cohort. 42 were in acute phase (diagnostic=31, relapse=11) and 46 were in remission stage. Baseline characteristics of the discovery cohort are summarised in table 1.

Table 2 presents the baseline characteristics of the HC.

Increased levels of sPD-1, sPD-L1, and sPD-L2 in serum from patients with AAV

We determined the concentration of the soluble fraction of the IC molecules PD-1, PD-L1 and PD-L2 in serum of patients with AAV and in HC. We observed that sPD-1 was significantly higher in AAV patients compared with HC (92.16±76.25 pg/mL and 35.65±24.07 pg/mL, p=0.007) (figure 1A). Besides, we observed a greater sPD-1 concentration in acute compared with remission patients (118.5±85.84 pg/mL and 67.66±56.87 pg/mL, p=0.0051) (figure 1B).

Only 27% of AAV patients had detectable sPD-L1 levels. Comparing the different phases of the disease, we found higher proportion of patients presenting detectable levels of sPD-L1 among those in the acute phase compared with remission (p=0.02). Among the individuals presenting detectable levels, sPD-L1 concentrations in AAV patients were higher compared with HC (p<0.0001) (figure 1C).

sPD-L2 tended to be higher in AAV patients compared with HC but differences did not reach statistical significance (data not shown).

Interestingly, the neutrophil count in peripheral blood correlated to the serum concentration of sPD-1 (r=0.22, p=0.04).

Soluble PD-1 and PD-L2 decrease in the urine from individuals with vasculitis

uPD-1 levels were significantly lower in patients with AAV compared with HC (128.4±202.7 pg/mL and 433.5±287 pg/mL, p<0.0001) (figure 2A). No differences in uPD-1 concentration were found depending on disease phase (figure 2B). However, we studied 14 patients with serial samples available from both the acute and remission phases. We observed a significant increase in uPD-1 levels during remission compared with those measured in the same patients at the time of



Figure 3 Urine levels of PD-1 in the same group of patients in acute and then in remission phase. Values correspond to discovery cohort.

diagnosis (p=0.02) (figure 3). uPD-L2 in AAV patients showed significantly lower levels compared with healthy individuals ($588.9\pm837.8 \text{ pg/mL}$ and $3198\pm3430 \text{ pg/mL}$, p=0.0075) (figure 2C). We also found lower uPD-L2 in acute compared with remission phase ($415.8\pm792.3 \text{ pg/mL}$ and $739.5\pm859.6 \text{ pg/mL}$, p=0.036) (figure 2D).

We found no differences in uPD-L1 between AAV and HC or the different disease phases.

The levels of urinary biomarkers did not correlate to serum creatinine, and we did not observe differences in biomarker levels according to the stage of chronic kidney disease.

External validation of PD-1, PD-L1, and PD-L2 in serum and urine

An independent cohort from HUN comprising 30 patients (16 in diagnostic and 14 in remission phase) was recruited to validate our findings. Online supplemental table 1 shows the baseline characteristics of the validation cohort.

The analysis of the HUN cohort confirmed the results obtained in the discovery cohort. sPD-1 in AAV was significantly higher compared with HC (3227±2537 pg/mL and 35.65±24.07 pg/mL, p<0.0001), with the highest levels noticed in acute patients compared with remission (3531±2512 pg/mL and 2944±2557 pg/mL, p=0.0013) (online supplemental figure 1). We also found higher concentration of sPD-L2 in AAV compared with HC (7608 ± 3108pg/mL and 1951± 882.4 pg/mL, p<0.0001) (online supplemental figure 2). As regards to PD-L2, there were no differences between acute and remission stages.

As for the urine, we observed lower uPD-1 and uPD-L2 in patients with vasculitis compared with HC (190.8±115.5 pg/mL and 433.5±287 pg/mL, p=0.011 and 339.4±580.2 pg/mL and 3198±3430 pg/mL, p=0.044) (online supplemental figure 3). Neither uPD-1 nor uPD-L2 differences were found depending on disease stage.

No differences in PD-L1 were detected in either serum or urine.

In vitro PBMCs production of IC molecules is reduced in AAV

As stated in the methods, we evaluated the in vitro production of IC molecules by PBMCs in cell culture by measuring



Figure 4 (A) Mean of PD-1 in supernatant cell culture of PBMCs (SNPD-1) in AAV and HC (unestimulated group). (B) Concentration of SNPD-1 in acute, remission patients, and in HC (unestimulated group). (C) Mean of SNPD-1 in AAV and HC in the MPO/PR3 antigen-stimulated group. (D) Concentration of SNPD-1 in acute, remission patients, and in HC in the MPO/PR3 antigen-stimulated group. Values correspond to discovery cohort. PBMCs, peripheral blood mononuclear cells; MPO, myeloperoxidase; PR3, proteinase 3.

the concentration of the molecules in the supernatant. PBMCs were cultured in a non-supplemented medium (baseline production) and in a medium supplemented with MPO or PR3 (specific stimuli).

PBMCs from AAV patients showed a lower baseline sPD-1 production compared with HC (15.90 ± 11.78 pg/mL and 25.16 ± 7.50 pg/mL, p=0.0074) (figure 4A). In vitro production of sPD-1 in response to MPO or PR3 was also lower in PBMCs from patients with AAV compared with HC (17.35 ± 14.40 pg/mL and 25.11 ± 10.77 pg/mL, p=0.04) (figure 4C). The in vitro production of sPD-1 was higher in HC and AAV in remission stage than in acute patients (figure 4B,D).

No differences were found in PD-L1 and PD-L2 production (data not shown).

Interstitial PD-1 decrease in active histology in ANCA vasculitis

Six AAV diagnostic kidney biopsies (three corresponding to the crescentic category and three corresponding to the sclerotic category according to the Berden histopathological classification²⁰) were stained to reveal the expression of PD-1 and PD-L1. PD-1 and PD-L1 presence/absence in glomerular, tubulointerstitial and vascular compartments was systematically analysed. PD-1 was mostly expressed in the interstitial compartment (figure 5). Biopsies classified as *sclerotic* tended to show higher interstitial PD-1 expression compared with those classified as *crescentic*, although differences did not reach statistical signification (data not shown) (figure 6A,B).

The number of PD-1 interstitial positive cells significantly correlated to proteinuria (r=0.81, p=0.04).

We did not observe positivity of PD-L1 in any renal compartment (data not shown).

DISCUSSION

Although PD-1 axis dysregulation has been observed in different autoimmune disorders, little is known about the function of these ICs in ANCA vasculitis.



Figure 5 This figure shows the presence of PD-1 in interstitial compartment in both sclerotic (A) and crescentic (B) biopsies.

Prior investigations suggest a function of PD-1 and its ligands in autoimmune diseases. Nishimura *et al*²³ described the development of glomerulonephritis and arthritis in a PD-1 knock-out murine model. Du *et al*²⁴ found elevated levels of sPD-1 and sPD-L1 in lupus individuals compared with HC and correlated these with disease activity parameters. Regarding ANCA vasculitis, the group of Gamerith *et al*¹⁶ observed that high baseline values of Tim-3 (>1200 pg/mL), sCD27 (>1250 pg/ mL) and sBTLA (>1000 pg/mL) were associated with sustained remission in PR3-positive AAV. Therefore, these results suggest that dysregulation of these immunomodulatory pathways may promote disease activation.

Our results support the hypothesis of PD-1 axis dysfunction in ANCA vasculitis patients. Regarding urine, we found that AAV patients had lower concentration of uPD-1 and uPD-L2 than HC and then, suggesting that the renal production of the molecules is abolished during the acute phase of the disease and, when remission is reached, urinary PD-1 secretion is restored. Thus, our results suggest the restoration of the uPD-1 levels as a marker of remission following treatment: the urine levels of PD-1 increase during remission phase compared with the levels measured in the same patients in the acute stage. This restoration was independent of the recovery of the kidney function, given the lack of correlation between the levels of urinary biomarkers and serum creatinine or the different stages of CKD.

Similarly in urine, PBMCs from AAV patients produced less soluble PD-1, even in response to stimulation with MPO and PR3. Likewise, Kristjansdottir *et al*²⁵ demonstrated lower frequency of PD-1+ cells and PD-1 expression on T-cells in lupus patients compared with controls. The authors suggested that downregulation of PD-1 in regulatory T-cells abrogates the immunosuppressive



Figure 6 Positivity of PD-1 interstitial cell and intensity PD-1 staining is shown as % of total patients.

activity of these cells leading to the activation of autoreactive T-cells. Further studies are needed to assess the PD-1producing T-cell phenotype in ANCA vasculitis.

The observation of lower uPD-1 during the acute phase of the disease—interpreted as a reflection of a lower local production in the kidney—is in line with the finding of a lower degree of PD-1 expression in the kidney biopsies with the most inflammatory lesions. These patients had fewer PD-1-positive cells and lower staining intensity than patients with more chronic biopsy damage. Hakroush *et al*²⁶ also described a predominant expression of PD-1 in the interstitial compartment and the loss of PD-1 positivity in active lesions and they further correlated the positivity of PD-1 with PD-L1 positivity in glomerulus and tubule. Unfortunately, we found no PD-L1 positivity in renal slides. Future studies are needed with more histology samples.

Contrary to the results we observed in urine, in the supernatant of the in vitro culture of PBMCs and in histology, we found in serum higher levels of sPD-1 and sPD-L1 in patients compared with HC, and higher levels of sPD-1 in acute compared with remission phase of the disease. These results are comparable with those of Yoon et at^{27} who also observed higher sPD-1 levels in patients with higher disease activity. PD-1 plays a crucial role in the regulation of the T-cell receptor (TCR). The more severe the disease, the more T lymphocytes participate by stimulating TCR activation and consequently increasing PD-1 transcription. Some authors have reported that increased transcription of transmembrane PD-1 can lead to an increase in soluble PD-1 molecules through alternative splicing and thus counteract the inhibitory effects of transmembrane PD-1 to stop the damage of the inflammatory disease. So, an increase of soluble PD-1 molecules can be found through the action of alternative splicing or the direct action of proteases on the transmembrane form to counteract the inhibitory effects of transmembrane PD-1 to stop the injury of the disease²⁸ and as an attempt to control systemic inflammation.

Given the potential role of these molecules in the disease pathogenesis, they could be considered as novel therapeutic targets in the future. In fact, there are clinical trials using PD-1 agonists to treat autoimmune diseases: the use of peresolimab²⁹ in patients with rheumatoid arthritis or CC-90006³⁰ as a treatment in patients with psoriasis. This new approach represents an exciting mechanism for the treatment of ANCA vasculitis and potentially other autoimmune diseases where lymphocytes play a pathogenic role.

This study presents some limitations. First, different soluble forms of PD-1, PD-L1 and PD-L2 have been described implying a biochemical and functional diversity of these molecules. The structural heterogeneity may make detection challenging using ELISA kits or Luminex assays.¹¹ Second, patients in remission do not have a renal biopsy to verify the lack of histological activity. Third, the control group was younger than AAV patients. Finally, the majority of the samples included in our study

were collected prior to the publication of the 2022 ACR/ EULAR classification criteria for AAV.^{31–33} Consequently, the new criteria were not employed in the selection of patients. We have some strengths in our study: despite the fact that ANCA vasculitis is a rare disease and collecting large cohorts is challenging, we were still able to enrol a sizeable number of patients, and we validated our results with a validation cohort. Then, to our knowledge, our group is the first study to examine the concentration of soluble PD-1 axis molecules in urine and the in vitro synthesis of these biomarkers in PMBCs cell culture.

In summary, our results suggest the involvement of the immune checkpoint pathway in the pathophysiology of ANCA vasculitis. PD-1, PD-L1 and PD-L2 could be used as a tool to detect and monitor disease activity in the clinical practice. If the involvement of the PD-1 axis in ANCA vasculitis is verified, this pathway could be a therapeutic target. To this end, we propose future studies conducted with a larger sample size, a longer follow-up of the patients, and with new methodology, such as flow cytometry, in order to facilitate a comparison of the mechanical properties of soluble and transmembrane PD-1, PD-L1 and PD-L2 molecules.

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Vasculitis

ORCID iDs

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