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ORIGINAL ARTICLE

Monitoring of Soluble Forms of BAFF System (BAFF, APRIL, sR-BAFF, sTACI and sBCMA) in Kidney Transplantation

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Abstract

BAFF system plays an essential role in B cells homeostasis and tolerance, although it has widely not been tested in transplantation with doubtful results. The main purpose was to study the BAFF soluble forms and their correlation with acute rejection (AR) and donor-specific antibodies production. Serum levels of BAFF, APRIL, and soluble forms of their receptors were analyzed in renal recipients with and without acute rejection (AR/NAR) appearance. All molecules were evaluated at pre- and post-transplantation. sTACI showed a significant correlation with BAFF and sR-BAFF levels, and sBCMA also showed a positive correlation with sAPRIL levels. A significant increase in sAPRIL levels in patients suffering AR was also found, and ROC curves analysis showed an AUC = 0.724, a concentration of 6.05 ng/ml (sensitivity: 66.7%; specificity: 73.3%), the best cutoff point for predicting AR. In the post-transplant dynamics of sAPRIL levels in the longitudinal cohort, we observed a significant decrease at 3 and 6 month post-transplantation compared to pretransplantation status. We also observed that recipients with high pre-transplant levels of sAPRIL generated antibodies earlier than those with lower sAPRIL levels, although their long-term post-transplantation was not different. Our results show that elevated serum levels of APRIL may be helpful as a biomarker for the diagnosis of AR, although the longitudinal study shows that it is not helpful as a prognostic biomarker.

Keywords BAFF system soluble forms · Acute rejection · Kidney transplant · Forensic medicine

Introduction

B cells play an essential role in the biological processes that mediate renal graft rejection and tolerance through various effector mechanisms, mainly the production of alloantibodies, but also through the antigen presentation to T cells and the secretion of cytokines (Halloran and Halloran 2010; Luu et al. 2014; Nelson and Krensky 2001). During the development and proliferation of B cells, the availability of cytokines that provide survival signals, such as the IL-7 or the B cell-activating factor of the tumor necrosis factor family (BAFF), are crucial for the correct immune response mediated by B cells (Harris et al. 2000).

BAFF and APRIL (a proliferation-inducing ligand) receptors are cytokines that belong to the tumor necrosis factor family and that possess a homotrimeric type II transmembrane structure mainly expressed on B cells (Luu et al. 2014; Nelson and Krensky 2001; Ng et al. 2004). BAFF signaling plays an essential role in the homeostasis of B cells and

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their effector functions, playing a fundamental influence in maintaining tolerance. There are three receptors in the BAFF system, the BAFF receptor (R-BAFF), the transmembrane activator and calcium modulator, and cyclophilin ligand interactor (TACI), and the B-cell maturation antigen (BCMA) (Bossen and Schneider 2006; Daridon et al. 2008). R-BAFF has been reported to increase its expression in T cells upon activation and is constitutively expressed by regulatory T lymphocytes (Tregs) (Bloom et al. 2018; Chen et al. 2014). TACI is an inducible receptor in humans and is expressed by memory B cells, plasma cells, a small population of activated CD27-negative B cells, monocytes, and dendritic cells in addition to B cells (Darce et al. 2007; Sakai and Akkoyunlu 2017). BCMA expression is restricted to plasmablasts, plasma cells, and germinal center B cells (Calame 2001).

The soluble forms of the receptors are still poorly studied, but they could be potential biomarkers in several biological processes and pathologies (Davidson 2010; Sakai and Akkoyunlu 2017). In kidney transplantation, most studies have focused on studying mRNA from peripheral blood leukocytes, kidney biopsies, or urine samples (Newell et al. 2015). There are still no studies in transplantation regarding these soluble receptors, but they could be potential biomarkers of humoral responses to grafting (Thibault-Espitia et al. 2012), may be helpful as a post-transplant prognostic value, and even influence changes in immunosuppression (Alfaro et al. 2021c; Galián et al. 2016; Thibault-Espitia et al. 2012). B cells depletion by agents such as alemtuzumab or rituximab could increase de novo donor-specific antibodies (DSA) development and acute antibody-mediated rejection (AMR) appearance (Kwun et al. 2015; Shimizu et al. 2016).

Thus, we aimed to study the soluble forms of the BAFF system in patients undergoing kidney transplantation, analyzing their role in the de novo DSA production and later acute rejection (AR). All of our study is aimed to help discover expression profiles that allow stratifying the risks in renal recipients (RRs).

Patients and Methods

Demographic Data, Clinical Characteristics, and Study Design

A total of 42 consecutive adult kidney transplant recipients were analyzed at the University Clinic Hospital "Virgen de la Arrixaca" (Spain) retrospectively. Only patients whose kidney graft was in operation for at least 1-month post-transplantation presented DSA Luminex determinations, storage DNA, and follow-up over 5 year post-transplantation, and complete clinical data were included. Allograft loss was estimated as a return to dialysis. Estimated glomerular filtration rate (eGFR) and creatinine were analyzed in all recipients (normal values between brackets): creatine (0.7–1.2 mg/dl) and eGFR (> 90 ml/min/1.73 m^2). Our patients showed the following values pretransplant; creatinine (2.9 ± 2.1 mg/dl; mean ± SD) and an eGFR less than 60 mL/min/1.73 m^2 for more than 3 months suggests a chronic kidney disease.

The study of the serum forms of molecules of the BAFF system was divided into two parts (Fig. 1).

In the first phase, we studied the serum levels of BAFF, APRIL, and the soluble forms of their receptors (sR-BAFF, sTACI, and sBMCA) in 42 kidney transplant recipients (KTRs), 15 KTR with AR, and 27 KTRs without AR (NAR) during the first 2 years after transplant. Subsequently, those molecules with statistical significance in the first phase were evaluated in a second longitudinal cohort of 42 KTRs with samples obtained at pre-, 3, and 6 month post-transplantation. The clinical and demographic characteristics of both cohorts are shown in Table 1. No significant differences were observed in any of the variables evaluated.

All patients gave their informed consent for inclusion before participating in the study. This study was conducted in accordance with the Declaration of Helsinki, and

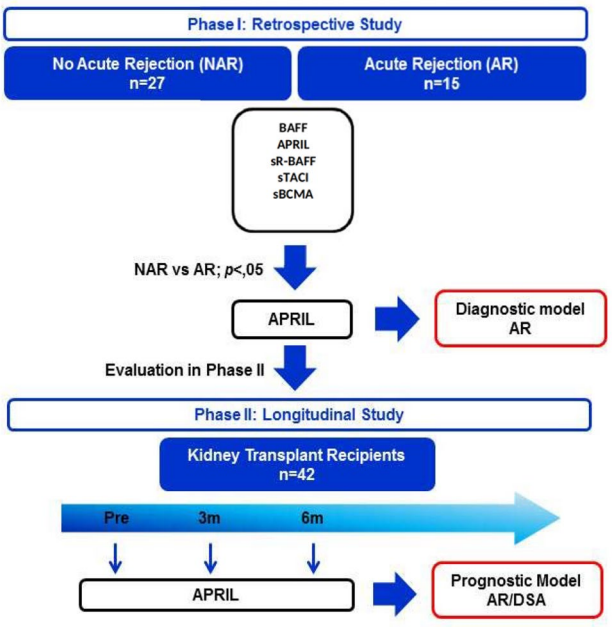

Fig. 1 Design of the study of soluble forms of the BAFF molecular system. In phase I, serum levels of BAFF, APRIL, and the soluble forms of their receptors (sR-BAFF, sTACI, and sBCMA) were analyzed in 42 samples of kidney transplant recipients (KTRs) without acute rejection ($n = 27$) and in 15 samples of KTRs at the moment of acute rejection (AR). Next, statistical models were developed to diagnose AR with those molecules in which statistical significance was obtained. In Phase II, those molecules with statistical significance in phase I was evaluated longitudinally in a cohort of 42 KTRs to verify their diagnostic capacity for AR

Table 1 Demographic data and clinical characteristics of the transplanted patients were included in the study of the soluble forms of the BAFF system

	Phase 1 cohort			Phase 2 cohort		
	NAR ($n=27$)	AR ($n=15$)	p	NAR ($n=36$)	AR ($n=6$)	p
Age (years)	53.47 ± 12.4	47.31 ± 10.1	0.217	55.6 ± 9.0	53.2 ± 18.8	0.751
Gender (male/female)	17/10	10/5	0.750	19/17	4/2	0.673
HLA[a] mismatches	4.14 ± 0.95	4.54 ± 1.12	0.325	4.03 ± 0.94	4.80 ± 1.30	0.192
Cold ischemia time (h)	10.7 ± 7.2	8.0 ± 4.2	0.488	9.1 ± 5.2	8.0 ± 2.9	0.645
Type of rejection (C/H)	–	5/10	–	–	4/2	–

Data expressed on mean ± SEM

Comparisons were made using Fisher's exact test or X^2 as appropriate

Values of $p < 0.05$ were considered significant

C cellular, *H* humoral, *NAR* non-acute rejection, *AR* acute rejection, *h* hours

[a]Total mismatches HLA-A, HLA-B, and HLA-DR

the Ethics Committee approved the protocol of HCUVA (PI15/01370 and PI19/01194).

Immunosuppressive Treatment

Oral tacrolimus (Prograf, Astellas, Ireland), mycophenolate-mofetil (MMF; CellCept, Roche, Switzerland), and prednisolone were given to all of the recipients (Dacortin, Merck, Spain). The tacrolimus (FK)-based protocol was started at a dose of (0.10–0.15 mg/kg/day) and was adjusted to maintain a trough level of FK in whole blood between 8 and 12 ng/ml during the first-month post-transplant, between 7 and 10 ng/ml during the second–third months after transplant, and between 5 and 8 ng/ml later. MMF was begun at a dose of 2000 mg/day, then gradually reduced to 1000–1500 mg/day during the first month after surgery, depending on white blood cell level. On the day of transplantation, days 12 and 34 after the procedure, methylprednisolone was given intravenously at 500, 250, and 125 mg/day, respectively. Oral prednisolone was started on day 5 after the operation at the dose of 20 mg and was then tapered to 5–10 mg/day within 2–3 months after transplantation.

Some cases were treated with induction therapy based on thymoglobulin or basiliximab based on the immune risk prior to transplantation.

Kidney Rejection Diagnosis

Acute cellular rejection (ACR) was defined as a 20% increase in serum creatinine over baseline, as well as biopsy-proven rejection (specimens were evaluated by light microscopy and immunofluorescence staining with a marker of classical complement activation (C4d) and classified according to the Banff classification (Cohen et al. 2012). The presence of identifiable histological abnormalities, positive C4d staining in peritubular capillaries, and the concomitant presence of DSA are all generally required to diagnose AMR (Legaz et al. 2021). Mild acute cellular rejection (Banff grade I) was treated with steroids pulse (500 mg methylprednisolone boluses) and increased maintenance immunosuppression. All other ACR were treated with anti-thymocyte globulin.

AR episodes were further classified as steroid-sensitive rejections (ACR Banff grade I) or steroid-insensitive rejections (ACR Banff grade II and II, and AMR). AMR was also treated with steroids pulse and intravenous immunoglobulin (0.25 g/kg and the last session 1 g/kg, (maximum 140 g) divided into two doses associated with plasmapheresis (three sessions a day, every 5 days). Later, we administered 500 mg anti-CD20 (Rituximab, Roche pharmaceuticals) intravenously. Anti-AMR treatment was also administered in two patients receiving anti-proteasome inhibitor Bortezomic (Velcade®, formerly PS-341).

Soluble Protein Analysis of the BAFF System by ELISA Technique

Serum samples were collected in dry tubes (BD Vacutainer, USA). The tubes were then centrifuged at 2000 rpm for 10 min, after which the supernatants were collected and stored at –60 °C until use.

The serum levels of BAFF and APRIL and the soluble forms of the receptors of the BAFF system (sR-BAFF, sTACI, and sBCMA) were measured in sera previously stored at –60 °C using commercial ELISA kits. The characteristics of the enzyme-linked immunosorbent assay (ELISA) kits used are summarized in Table 2. All kits were used in accordance with the manufacturer's specifications and recommendations. The analysis of the samples was carried out in duplicate, and the reading of the plates was carried out in Quanta Lyser 2 equipment (Inova Diagnostics, San Diego, CA, USA) at a wavelength of 450 nm and a reference wavelength 620 nm. The results were expressed in ng/ml.

Table 2 Characteristics of ELISA kits for serum quantification of BAFF system molecules

Molecules	Kit	Manufacturer	Sensitivity rango[a]
BAFF	Human BAFF/BLyS/TNFSF13B quantikine ELISA kit	R&D system	0.0625–4
APRIL	APRIL human ELISA kit	Thermo Fisher	0.78–50
sR-BAFF	TNFRSF13C/BAFFR ELISA kit (human)	Aviva systems biology	0.78–5
sTACI	TACI ELISA kit (human)	Aviva systems biology	0.016–2
sBCMA	TNFRSF17/BCMA ELISA kit (human)	Aviva systems biology	0.0312–2

[a]Data expressed on ng/ml

Statistical Analysis

The results were expressed as the mean ± standard error of the mean (SEM) for quantitative data or as percentages for categorical data. The X^2 test or Fisher's exact test compared categorical variables. The Kolmogorov–Smirnov test was used to ensure that the data were normal. The Mann–Whitney *U* test compared two groups with variables that did not account for normality. The Kruskal Wallis test and Dunn's post hoc test with Bonferroni correction for multiple comparisons compared three or more groups. As previously published, correlation analyses were carried out using the Spearman index (Cohen et al. 2012).

The Wilcoxon non-parametric test for related samples compared two related groups. The Friedman test with Wilcoxon post hoc compared three or more related groups.

The construction of ROC curves was used to assess the sensitivity and specificity of a biomarker, and area under curve (AUC) assessed the discriminating capability. The optimal cutoff value that maximizes sensitivity and specificity, the Youden index, the Benjamini–Hochberg, or the Bonferroni method, were used to correct the *p* value in multiple comparisons. For all statistical tests, $p = 0.05$, or p corrected = 0.05 in the event of multiple comparisons, were considered significant. The graphs and statistical analyses were created using the software packages Statistical Package for the Social Sciences (SPSS, v 22, Chicago, IL, USA) and GraphPad Prism (version 6, San Diego, CA, USA), as well as the R programming language, which was used in the Integrated Development R Studio version 3.4 environment.

Results

Correlation Between the Serum Forms of the BAFF Molecular System

The correlations between the soluble forms of the BAFF system are shown in Fig. 2. Soluble TACI showed a significant correlation with BAFF levels ($r = 0.458$, $p = 0.011$) and soluble R-BAFF (sR-BAFF) ($r = 0.649$, $p < 0.001$). Soluble BCMA levels also positively correlated with APRIL levels ($r = 0.548$, $p = 0.002$). On the other hand, BAFF levels strongly correlated with sR-BAFF levels, although the differences were not significant ($r = 0.356$; $p = 0.053$). Similarly, APRIL levels positively correlate with sTACI levels, although they were not significant ($r = 0.361$; $p = 0.051$). In general, the results showed a close relationship between the different soluble molecules of this system.

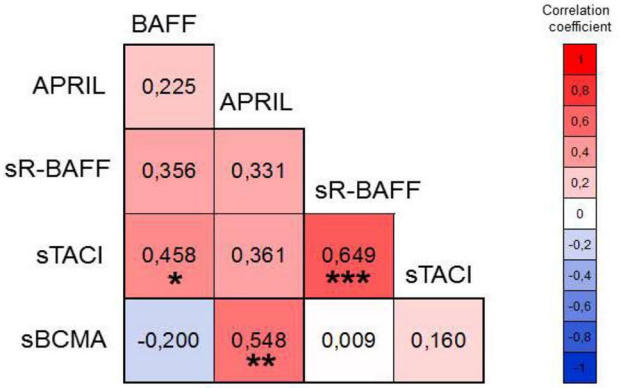

Fig. 2 Correlation of the soluble forms of the BAFF system. The figure shows a correlation matrix based on a color grading from red (correlation index: + 1) to blue (correlation index: –1). Within each cell of the figure, the Spearman correlation index is indicated. Data were analyzed using Spearman's correlation index. Values $p < 0.05$ were considered significant. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$

Serum Forms of the BAFF Molecular System in AR

The serum levels of the soluble forms of the BAFF system molecules were evaluated in 27 KTRs with good post-transplant renal function (NAR) and in 15 KTRs with AR. Figure 3 shows a statistically significant increase in APRIL levels in the AR group (11.4 ± 2.7 vs. 5.2 ± 0.8 ng/ml, $p = 0.006$). Regarding serum BAFF levels, a slight tendency is observed for the KTRs of the NAR group to have higher levels than those of the AR group, although the differences were not significant ($p = 0.086$). On the other hand, the serum levels of BAFF receptors in their soluble forms (sR-BAFF, sTACI, and sBCMA) did not show any significant differences between the different study groups (Fig. 3).

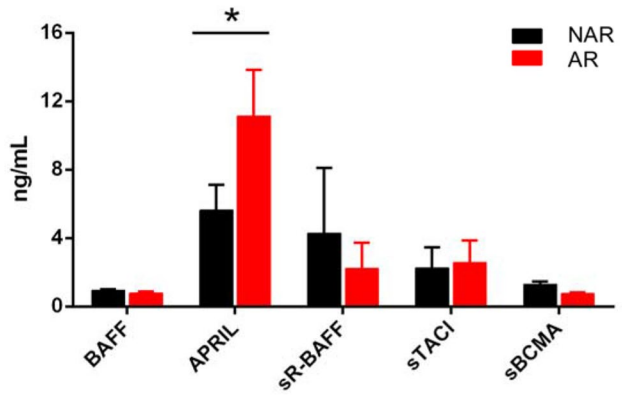

Fig. 3 Serum levels of the molecules of the BAFF system in their soluble forms. Comparison of BAFF, APRIL, sR-BAFF, sTACI, and sBCMA gene expression levels in peripheral blood samples among kidney recipients with good graft function during the first year after transplantation (NAR) and at the time of acute rejection (AR). Data are represented as the mean ± SEM. Statistical analyzes were performed using the Mann–Whitney *U* test. Values $p < 0.05$ were considered significant. *$p < 0.05$

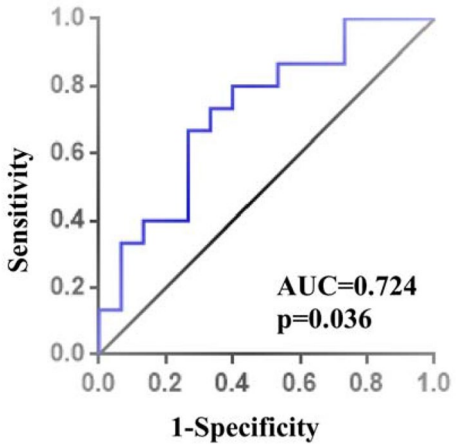

Fig. 4 ROC curve for predicting AR using serum APRIL levels. The non-discrimination diagonal (black line) is used as a reference. The area under the curve (AUC) and statistical significance are shown on the graph

The above results showed that APRIL levels were elevated at the time of rejection. Analysis using ROC curves showed an AUC = 0.724 [0.541–0.908] for APRIL with a significant result of $p = 0.036$. The Youden index indicates that the best cutoff point for predicting rejection was 6.05 ng/ml (Fig. 4), with a sensitivity of 66.7% and a specificity of 73.3% for this cutoff level.

Post-transplant Evolution of Serum APRIL Levels

In the study of the post-transplant dynamics of serum APRIL levels in the longitudinal cohort, we observed a significant decrease, both at 3 months (19.7 ± 18.7 vs. 10.4 ± 10.2 ng/ml; $p = 0.002$) as at 6 month post-transplantation (19.7 ± 18.7 vs. 5.5 ± 4.6 ng/ml; $p < 0.001$) compared with pretransplantation (Fig. 5A). However, no significant differences were obtained when comparing sAPRIL levels over time (pre-transplantation, 3 and 6 month post-transplantation) between KTRs of the NAR and AR groups (Fig. 5B).

Levels of Soluble APRIL in Recipients with AR

The cutoff level obtained in the previous section by ROC curve analysis (6.05 ng/ml) was analyzed to verify its prognostic utility of sAPRIL for AR. In Fig. 6, we observed that when applying this cutoff level to the post-transplant samples of the longitudinal cohort of phase 2, 64.9% (37/57) of the patient samples of the NAR group were correctly classified as samples without risk of acute rejection. In contrast, in the samples of patients in the AR group, only 50% (4/8) were correctly classified as samples with AR risk.

Levels of Soluble APRIL as a Risk Biomarker for the Production of DSAs

Finally, we evaluate the usefulness of sAPRIL levels as a biomarker for the appearance and development of post-transplant de novo DSA. When comparing APRIL levels at pre- and 3, and 6 month post-transplantation between KTRs without DSA ($n = 38$) and recipients with DSA ($n = 4$), we did not observe significant differences (Fig. 7A). However, when performing a survival analysis to see the link between APRIL levels and the time of appearance of DSA antibodies, we observed that recipients with high pre-transplant levels of APRIL (Tertil 3, APRIL ≥ 22.98 ng/ml) generate DSAs earlier than patients with lower APRIL levels (Fig. 7B; log-rank $p = 0.027$). At 36 month post-transplantation, 76.9% (10/13) of KTRs with elevated pre-transplant APRIL levels (Tertil 3) did not develop DSA antibodies, compared to 96.1% (25/26) of recipients with pre-transplant medium and low APRIL levels (Tertiles 1 and 2).

Discussion

This study aimed to analyze the soluble forms of the BAFF system in patients undergoing kidney transplantation, analyzing their role in the de novo DSA production and AR development. All this is to help discover expression profiles that allow stratifying the risks in RRs.

Recent studies have shown that increased levels of sBAFF are associated with an increased risk of AMR (Clark et al. 2011; Min et al. 2016; Pongpirul et al. 2018; Xu et al. 2018), but there are few studies on the cytokine APRIL and the soluble forms of R-BAFF receptors, TACI and BCMA

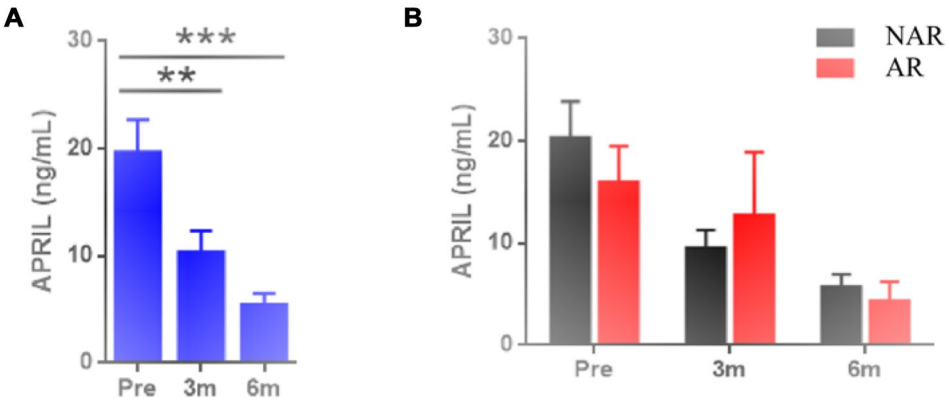

Fig. 5 Dynamics of serum APRIL levels. **A** Serum APRIL levels in kidney recipients at pre-, 3, and 6 month post-transplantation. The Wilcoxon test for related samples was compared between post- and pretransplantation times. **B** Comparison of APRIL levels at different times between patients without acute rejection (NAR) and acute rejection (AR). Comparisons between the groups at each time were made using the Mann–Whitney *U* test. Values are expressed as the mean ± SEM. Values of $p < 0.05$ were considered significant. $^{**}p < 0.01$; $^{***}p < 0.001$

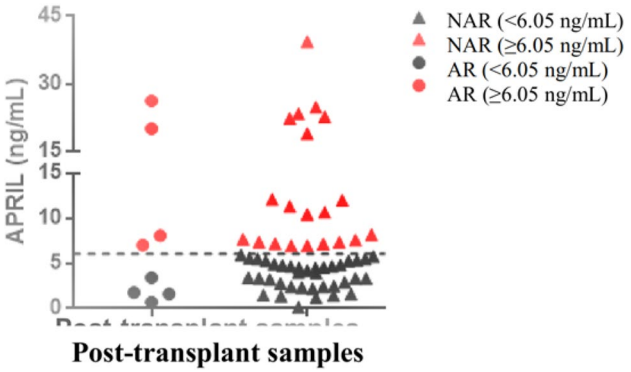

Fig. 6 Post-transplant concentrations of sAPRIL and its relationship with the outcome and kidney transplantation outcome. The graph shows the sAPRIL levels after transplantation of renal recipients without acute rejection (NAR, triangles) and with acute rejection (AR, circles). The cutoff level to discriminate between the NAR and AR groups (6.05 ng/ml), calculated using the ROC curve in the previous section, is represented as a horizontal dashed line. Samples that exceed the cutoff level are represented in red, others in black

(Thibault-Espitia et al. 2012). Our study evaluated soluble forms' diagnostic and prognostic utility throughout the BAFF system. The study was divided into two phases; In the first phase, we carried out a retrospective study in which we analyzed the levels of soluble forms in sera from KTRs at the time of AR and NAR. In the second phase, a longitudinal study was carried out. Those molecules that showed significant differences in the first phase were analyzed, measuring the concentrations at different times after transplantation, studying their prognostic utility for AR, and triggering DSA antibody production.

The phase I results of our study show that soluble levels of APRIL are elevated in KTRs at the time of AR. However, no studies link increased APRIL levels with AR development in kidney transplantation. APRIL pre-transplant levels have been associated with a greater recurrence of IgA nephropathy in KTRs with a living donor graft (Jo et al. 2019). Thus, APRIL is crucial for the survival of plasma cells (Benson et al. 2008), so its elevation in rejection processes can promote the differentiation and survival of allospecific plasma cells. On the other hand, it has been shown that APRIL could also act as a costimulator of T cell proliferation, for which it can potentially also act to enhance cell-type rejections (Yu et al. 2000). This part would explain why we have obtained differences between both groups despite our cohort having a mixture of cell-type and antibody-mediated rejections. However, a recent study showed that the APRIL/TACI pathway also has essential immunosuppressive functions through the maintenance and survival of Tregs (Tai et al. 2019).

Similarly, APRIL and not BAFF can stimulate IL-10 secretion in B cells (Hua et al. 2016). Although these results show the dual role of APRIL in regulating the immune system, our data suggest a pro-inflammatory role in transplantation. In allogeneic hematopoietic stem cell transplantation, elevated APRIL levels, rather than BAFF, have been associated with chronic graft-versus-host disease (Chasset et al. 2016). Contrary to what was expected in our study, the BAFF levels were not significant, but we also observed a particular trend in which these patients who did not develop rejection tended to have higher levels. The clinical significance of BAFF in predicting AR is unclear. Some research shows that BAFF levels can be a risk factor for the development of AR and allosensitization (Friebus-Kardash et al. 2018; Wang et al. 2019). However, another study (Slavcev et al. 2016) found that transplants with AMR had lower levels than KTRs without rejection, similar to our present study, and found no correlation between BAFF levels and DSA

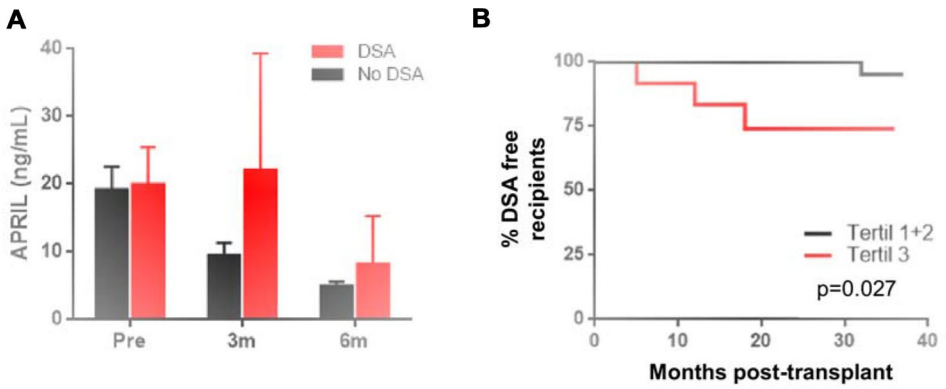

Fig. 7 Association of serum APRIL levels and incidence of DSA antibodies. **A** Comparison of APRIL levels at different times between kidney recipients without and with DSA antibodies. Comparisons between the groups at each time were made using the Mann–Whitney *U* test. Values are expressed as the mean ± SEM. **B** Percentage of DSA antibody-free recipients based on pre-transplant APRIL levels. The comparisons of the Kaplan–Meier curves were made using the log-rank test. Values of $p < 0.05$ were considered significant

production. Therefore, the use of BAFF as a biomarker in kidney transplantation remains in doubt, given the controversy between different studies (Afzali et al. 2021; Alfaro et al. 2021a; Irure-Ventura et al. 2020).

Recently, it has also been shown that the BAFF and APRIL receptors exist in soluble forms and can be detected in body fluids (Hoffmann et al. 2015; Laurent et al. 2015; Smulski et al. 2017). The circulating levels of these molecules reflect the state of activation and accumulation of B cells. Since then, the soluble forms of these receptors have been postulated as potential biomarkers in hematopoietic cancers or autoimmune diseases, although they were not studied in kidney transplantation (Ghermezi et al. 2017; Gutierrez et al. 2019; Thaler et al. 2017). Our results did not show significant differences between KTRs with and without AR (Hoffmann et al. 2015) showed that sTACI and sBCMA are released from activated B cells and plasma cells, respectively, and that their presence in cerebrospinal fluid from multiple sclerosis patients correlated with intrathecal IgG production.

On the other hand, other authors observed that in a chronic kidney transplant, blocking APRIL/BLyS resulted in a significant drop in autoantibody production and disturbed splenic germinal center formation. However, no difference in kidney transplant pathology was seen, which could be due to the lack of T cell-centric immunosuppression. This study data suggest that inhibiting APRIL/BLyS could help to reduce antibody development in kidney recipients over time (Bijkerk et al. 2017).

Hoffmann et al. (2015) hypothesize that soluble levels of TACI and BCMA are associated with the production of anti-HLA antibodies, although we have not performed this analysis, and future studies will be necessary at this point. In our study, we have also analyzed the correlation between the soluble forms of the BAFF system, and our results show that, as expected, BAFF levels are positively correlated with the soluble levels of R-BAFF and TACI, although statistical significance was not reached in the case of R-BAFF. BAFF can bind to R-BAFF and TACI but not to BCMA; the binding of BAFF to these receptors causes their cleavage by the proteases ADAM10 and ADAM17 (Hoffmann et al. 2015; Smulski et al. 2017). Similarly, sAPRIL levels positively correlate with the two receptors to which it can bind, TACI and BCMA, although the correlation does not reach statistical significance in the case of TACI. Like the other receptors, the stimulation of BCMA by APRIL produces cleavage by γ-secretase, and it is released into the medium (Laurent et al. 2015).

In phase 1 of the study, we observed that APRIL levels were increased in KTRs with AR. The analysis using ROC curves showed the cutoff point at 6.05 ng/ml for the diagnosis of AR. In phase 2 of the study, we evaluated its prognostic utility in another cohort by measuring levels at pre- and 3, and 6 month post-transplantation. The longitudinal study results did not show significant differences between KTRs that develop AR and present DSA antibodies and those that do not; however, survival analyses show that the KTRs who present high APRIL levels pretransplantation generate, with a shorter period post-implantation time, DSA antibodies.

On the other hand, several studies observed the importance of sCD30 serum levels before transplantation in predicting renal AR and allograft failure (Nafar et al. 2009; Solgi et al. 2012; Wang et al. 2007). Renal transplant/graft candidates have higher pre-transplant serum sCD30 levels than healthy people, predictive of early renal graft loss. The post-transplant sCD30 levels were also investigated as an early predictor of impending graft rejection (Pelzl et al. 2003; Süsal et al. 2002). Besides, a study found no apparent relationship between serum sCD30 levels on day 28 after kidney transplantation and the incidence of AR,

suggesting that the level of sCD30 on day 7 post-transplantation provides valuable data to predict AR (Shimizu et al. 2016).

On the other hand, AR after kidney transplantation was associated with circulating microRNAs and vascular injury (Bijkerk et al. 2017). miR-192 was involved in developing renal fibrosis, suggesting a pathogenic link of these miRNAs to AR and vascular injury (Krupa et al. 2010). The reduced clearance effect observed in these patients could be caused by increased levels of these circulating miRNAs (Alfaro et al. 2021c; Grenzi et al. 2015).

Our results in the previous gene expression study showed that the KTRs that generate DSA antibodies have a greater increase in TACI expression in the first months after transplantation (data own not shown and submitted). This observation, together with the results of the study of the soluble forms of the BAFF system, suggests that high levels of APRIL at pretransplantation can promote a greater expression of its receptors at post-transplantation and may promote the breakdown of tolerance toward antibodies development. However, the low incidence of AR and the presence of DSA antibodies included in the second phase cohort make it necessary for the obtained results to be validated in larger cohorts. In addition to designing studies that simultaneously analyze transcripts and soluble proteins to establish a correlation between expression levels and secretion levels, their relationships could be significant with types of B cells implicated in different transplant status (Alfaro et al. 2021b).

The study's main limitations are the limited cohort and the non-inclusion of groups separated by type of rejection. Furthermore, we have not analyzed the association of these molecules with the incidence of DSA antibodies, as in other types of transplant papers (Legaz et al. 2020).

In conclusion, this is the first study carried out on kidney transplant patients that evaluates the association of the entire molecular system of BAFF in its soluble forms with the incidence of AR. Our results show that elevated serum levels of APRIL may be helpful as a biomarker for the diagnosis of AR, although the longitudinal study shows that it is not helpful as a prognostic biomarker.

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Declarations

Conflict of Interest The authors declare that this research was conducted without any commercial or financial relationships construed as a potential conflict of interest.

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