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B-Cell Activating Factor (BAFF) As A Novel Alert Marker for The Immunological Risk Stratification After Kidney Transplantation: A Prospective Controlled Study

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ABSTRACT

Background: Kidney allograft failure is an essential issue in renal diseases and participates in the increasing number of subjects with end-stage kidney disease (ESKD). Rejection episodes remain impactful occasions after kidney transplant, in spite of the use of potent immunosuppressive agents. B-cell activating factor (BAFF) is essential for appropriate immune response mediated by B cells.

Patients and Methods: This concurrent prospective controlled cohort study included 120 patients classified into 2 groups, study group who experienced graft rejection during the study duration (n=20) and control group who did not develop graft rejection during the same study duration (n=100). Human Baf/BlyS/TNFSF13 B immunoassay was used to assess BAFF level in the serum. Patients were monitored for graft function, graft survival, episodes of graft rejection and other possible complications following kidney transplantation during the study duration and the follow-up period.

Results: Males were over-represented among recipients with rejection (p=0.0345), the distribution of HLA-A and B mismatches (0–4) did not differ significantly (p=0.1). For HLA-DR, 0 vs 1 mismatch showed insignificant difference (p=0.118), though the direction favored more 0-mismatch among controls. BAFF concentrations were higher in patients with rejection (3.79±1.58) compared with controls (2.61±0.78), with a statistically significant difference (p=0.003).

Conclusion: BAFF shows promise as a practical, non-invasive biomarker to enhance immunologic risk stratification and personalize post-transplant surveillance; rigorous validation and integration with molecular diagnostics are warranted before routine adoption.

Keyword: BAFF, Immunological Risk Stratification, Kidney Transplantation.

INTRODUCTION

Kidney allograft failure is an essential issue in renal diseases and participates in the increasing number of subjects with ESKD, now exceeding seven million globally as of 2020. Several health organizations have emphasized the requirement for clinically adjusted kidney allograft survival prediction model that could improve decision making as well as patient treatment [1].

Rejection episodes are still impactful occasions following kidney transplant, in spite of the usage of potent immunosuppressive agents. Nowadays, histologic diagnosis based on allograft specimens has been considered the best diagnostic tool for rejection. This method is invasive and is liable to sampling mistakes [2].

B cells have an important role in the biologic process, which mediates renal graft rejection and tolerance across different effector mechanisms, such as the formation of alloantibodies, antigen (Ag) presentation to T cells, and cytokine synthesis. Throughout B cell proliferation, the ability of cytokines, which offer survival signals, which include the IL-7 or the BAFF, is essential for the proper immune response mediated by B cells [3,4]. One study displayed that BAFF-activated B cells considerably encouraged the activity of co-cultured T cells and raised the percentages of CD4+, CD154+ T cells[5].

BAFF and a proliferation-inducing ligand (APRIL) are TNF family cytokines with a homotrimeric type II transmembrane structure. B-cells are the main source of R-BAFF and (APRIL receptor) R-APRIL. R-BAFF and R-APRIL could be detached from the cellular membrane and serve as a negative feedback mechanism, inhibiting B-cell-mediated immune responses by blockade the serious BAFF and APRIL effects [6].

The soluble form of the BAFF receptors remains poorly evaluated. Regarding multiple myeloma, the increased serum level of BCMA is correlated with plasma cell number as well as the clinical state, while in lupus erythematosus, BCMA and TACI are correlated with the disease activity [7].

AIM OF WORK

To analyse the changes in BAFF system expression in pre- and post-kidney recipients in presence or absence of rejection, for the purpose of enhancing prediction of rejection and prediction of graft survival using molecular analysis.

PATIENTS AND METHODS

This concurrent prospective controlled cohort study comprised 146 cases with end stage renal failure underwent kidney transplant in the period between January 2023 to December 2024 and completed at least six months of follow-up. Patients less than 15 years (n=16), re-transplant patients (n=8) and those who lost for follow up (n=2) were ruled out. The remaining patients (n=120) were classified into 2 groups, study group who experienced graft rejection during the study duration (n=20) and control group who did not develop graft rejection during the same study duration (n=100).

Ethical Considerations

Throughout its implementation, the study complied with the Helsinki Declaration. Approval

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was taken from Mansoura University Institutional ethics committee to conduct this study. An informed and written consent was taken from each patient. All cases were informed about the study design.

METHODS

Before kidney transplantation, all donor–recipient pairs were ABO blood group compatible. Donor and recipient HLA-A, B and DR typing was conducted by the routine molecular by sequence-based typing (SBT) INNO-LiPA method [8]. Full clinical data were comprised in this investigation. Creatinine and estimated glomerular filtration rate (eGFR) were measured in entire recipients. Other data were collected and included patient viral profile. Ultrasonographic examinations of kidney allograft were carried out.

After kidney transplantation, for all patients, blood specimens were obtained pre-transplant and at six weeks post-transplant. Elective time zero renal allograft biopsy at the time of donor nephrectomy was done and ultrasound-guided percutaneous biopsy was done at the time of unexplained rise of serum creatinine (Ser Cr). Biopsy was processed for light microscopy (LM) and immunofluorescence staining for C4d. Acute rejection was diagnosed according to Banff Schema 2017 [9].

Acute rejection was further categorized into acute AMR, acute cellular rejection, or mixed type of rejection. Additionally, biopsies were scored by utilizing a graded scale as next: no humoral rejection; C4d deposition only (C4d+ peritubular capillaries (PTC) by immunofluorescence with no histologic alterations by LM), moderate AHR (minimal tubular damage, neutrophil infiltration), and severe AHR (glomerular thrombosis, necrosis, mesangiolysis. C4d accumulation without histological affection on LM wasn't considered AMR.

Serial traditional CDC cross-match and Luminex lab screen at time of transplantation and post-transplantation in the 3rd month and on the time of eventful graft biopsy were done. After determination of lymphocytotoxic cross match against donor T and B cells, patients' sera were assessed for the existence of HLA Abs using the Lab screen mixed assay with Luminex microbeads, each coated with purified HLA Class I molecules and HLA Class II molecules. Incubation of the sera were done with the beads for half an hour, following washing, they were incubated with an anti-human IgG phycoerythrin (PE) conjugated monoclonal Ab for half an hour. Results were reported based on median fluorescence intensity of PE staining. Lab. screen software was utilized to detect positivity [10].

Immunosuppressive Treatment

Following kidney transplant, the recipients mainly received immunosuppressive agents based on the standard protocols of our Centre. Immunosuppressive therapy following transplantation can be stratified based on recipient risk factors. Category I included first transplants with zero panel-reactive antibodies (PRA), where induction therapy with basiliximab was used, and maintenance was steroid-free, typically combining tacrolimus (Tac) and mycophenolate mofetil (MMF). Category II encompassed first transplants from unrelated donors, first transplants with PRA between 1–20%, and those with PRA 20–40% but less than a 3/6 HLA mismatch. In this group, induction was also with basiliximab, but maintenance required a triple protocol of steroids, tacrolimus, and MMF. Category III included higher-risk patients such as first transplants with PRA 21–40%, repeat transplants with PRA 11–40%, repeat transplants with prior aggressive rejection leading to graft loss within five years, and those with 5/6 HLA mismatch (requiring individualized assessment). For those patients, induction was performed with antithymocyte globulin (ATG), and maintenance had the full triple immunosuppressive protocol of steroids, tacrolimus, and MMF.

Regarding frequency of sampling, first sample was before transplantation, second sample was two weeks after kidney transplantation, then monthly for three months, then every 3 months for 9 months.

BAFF-ELISA analysis

Measurement of BAFF level in the serum was conducted by utilizing the human Baf/BlyS/TNFSF13 B immunoassay (R&D Systems, Minneapolis, Unites States of America) based on the manufacturer's recommendations. The minimal detectable level was 62.5 pg/ml and OD-measurement was conducted with a Tecan reader (Männedorf, Switzerland).

Histological Analyses

The specimens obtained from biopsy underwent pathologic diagnosis after processes of embedding, sectioning, and staining. The diagnosis was based on Banff 2015 criteria. The streptavidin-perosidase (SP) linkage approach was utilized to assess the expression levels of BAFF, C4d, and CD20 in transplanted renal tissues.

Follow up

After transplantation patients were kept inpatient for 10-13 days, follow up after discharge was twice weekly for one month, then, weekly for two months, then, every 2 weeks till the 6th month, then, monthly till the 1st year post transplantation and lastly every 3 months.

Outcome Measurements

Patients were monitored for graft function, graft survival, episodes of graft rejection and other possible complications following kidney transplantation during the study duration and the follow- up period. Allograft ACR was described as a twenty percent increase in Ser Cr beyond basal level and biopsy-conformed rejection (samples were assessed by LM and immunofluorescence stain with a marker of complement stimulation (C4d) and classified based on the Banff classification [11]. The diagnosis of acute Ab mediated rejection needs unique histopathological results, positive C4d staining in PTC, and the concurrent existence of de novo donor-specific Abs (DSA).

Statistical Analysis

Data entry and analysis was conducted using SPSS version 25 (Inc., Chicago, IL, USA). Continuous data were presented as mean±SD when normally distributed and as median and range when non-normally distributed. Categorical data were presented as frequency and percentage and were compared by Fisher's exact test. The Kolmogorov–Smirnov test was utilized to confirm that the data were normal. The Mann-Whitney U test was utilized to compare two groups with variables that weren't normalized. A significance level ≤ 0.05 was applied for all *P* values.

RESULTS

Table **(1)** shows that mean recipient age was not significantly different between the 2 studied groups. Donor age did not also differ. Males were over-represented among recipients with rejection (90% vs 66%), whereas donor sex distribution was similar. Consanguinity showed a non-significant trend toward higher "related" status in controls.

Table (1): Baseline pre-transplantation personal factors

variable	**Rejection (n = 20)**	**No Rejection (n = 100)**	**p-value**
Recipient's age (years), mean (SD)	**26.3 (11.4)**	**21.7 (8.7)**	**0.1 ***
Donor's age (years), mean (SD)	**41 (8.9)**	**41.3 (9.9)**	**0.89 ***
Recipient's sex, number of patients (%)			**0.04 *****
Male	18 (90)	66 (66)	
Female	2 (10)	34 (34)	
Donor's sex, number of patients (%)			**0.6 ****
Male	8 (40)	32 (32)	
Female	12 (60)	68 (68)	
Consanguinity, number of patients (%)			**0.09 *****
Related	16 (80)	93 (93)	
Non-related	4 (20)	7 (7)	

*****Independent t-test**, **** Chi-square test**, ***** Fisher's exact test

Table **(2)** shows that ABO identical vs different did not differ between the 2 studied groups. The distribution of recipient blood groups (A/B/AB/O) and donor blood groups was similar across groups. Prior transfusion status and the number of transfusions also showed no significant differences.

Table (2): Baseline pre-transplantation hematological factors:

variable	Rejection (n = 20)	No Rejection (n = 100)	p-value
ABO Compatibility, number of patients (%)			**1.00 ***
identical	19 (95)	93 (93)	
different	1 (5)	7 (7)	
Recipient blood group, number of patients (%)			**0.35 ****
A	7 (35)	53 (53)	
B	6 (30)	16 (16)	
AB	3 (15)	11 (11)	
O	4 (20)	20 (20)	
Donor blood group, number of patients (%)			**0.59 ***
A	7 (35)	39 (39)	
B	4 (20)	21 (21)	
AB	1 (5)	1 (1)	
O	8 (40)	39 (39)	
Prior Blood Transfusion, number of patients (%)			**0.61 ****
No	12 (60)	66 (66)	
Yes	8 (40)	34 (34)	
Number of blood transfusion, number of patients (%)			**0.78 ***
1-3 times	6 (75)	27 (79.4)	
4-5 times	1 (12.5)	5 (14.7)	
≥ 6 times	1 (12.5)	2 (5.9)	

* Fisher's exact test**,** ** Chi-square test

Table **(3)** displays that the distribution of HLA-A and B mismatches (0–4) did not differ significantly between the 2 groups. For HLA-DR, 0 vs 1 mismatch showed no significant difference, though the direction favored more 0-mismatch among controls. BAFF concentrations were higher in patients with rejection (mean 3.79 ± 1.58) compared with controls (2.61 ± 0.78), with a significant difference.

Table (3): Baseline pre-transplantation immunological factors and biomarkers correlation with recurrent rejection

variable	Rejection (n = 20)	No Rejection (n = 100)	p-value
HLA-A and B mismatch (MM), number of patients (%)			**0.1 ***
0 mismatches	2 (10)	4 (4)	
1 mismatch	5 (25)	31 (31)	
2 mismatches	8 (40)	57 (57)	
3 mismatches	3 (15)	6 (6)	
4 mismatches	2 (10)	2 (2)	
HLA-DR mismatch, number of patients (%)			**0.11 ***
0 mismatches	1 (5)	21 (21)	
1 mismatch	19 (95)	79 (79)	
BAFF, mean (SD)	3.79 (1.58)	2.61 (0.78)	**0.003***

* Fisher's exact test

DISCUSSION

In this prospective controlled cohort of 120 kidney-transplant recipients, we found that circulating BAFF measured early after transplantation was significantly higher among cases who developed biopsy-proven rejection than in contemporaneous controls without rejection (3.79 ± 1.58 vs 2.61 ± 0.78; p = 0.003). This signal emerged despite broadly comparable pre-transplant hematologic factors and HLA mismatch distributions, underscoring a potential role for BAFF as an early, non-invasive indicator of heightened immunologic risk. Our cohort demonstrated no significant between-group differences in ABO compatibility, recipient/donor blood-group distribution, prior transfusion exposure, or transfusion frequency strata. Likewise, the burden of HLA-A and B (0–4) and HLA-DR (0–1) mismatches didn't vary significantly between groups. These balanced baselines reduce confounding from classic alloimmunization surrogates and strengthen the association between elevated BAFF and rejection risk observed here.

Our results align with prior reports implicating BAFF biology in kidney allograft injury and antibody-mediated processes [12,13]. Studies have linked higher soluble BAFF to de novo DSA formation, chronic active ABMR, and poorer graft outcomes [14]; our data

add prospective, real-world evidence in a living-donor–dominant setting and support the feasibility of BAFF monitoring alongside standard surveillance.

If validated in larger cohorts, serum BAFF could complement functional markers (creatinine/eGFR) and histology by: (i) flagging patients for intensified surveillance (e.g., early DSA testing or protocol biopsy), (ii) informing individualized immunosuppression adjustments, and (iii) aiding risk stratification in trials targeting B-cell pathways. Because our study captured a clear difference using a commercially available ELISA with standard operating characteristics, translation into routine workflows appears practical.

Strengths include a prospective design, standardized immunosuppression protocols, predefined sampling windows, and histology-based rejection adjudication, which together mitigate misclassification. Baseline balance in ABO, transfusion history, and HLA mismatches reduces confounding for the BAFF–rejection association. Limitations include a modest number of rejection events (n = 20) limiting multivariable adjustment power; absence of systematic DSA quantification at all time-points; potential center-specific practice effects; and a follow-up horizon focused on early outcomes, precluding definitive statements about long-term graft survival or chronic ABMR trajectories. These caveats mirror gaps highlighted in prior BAFF literature and should guide next-step studies.

Validate BAFF thresholds in larger, multi-center cohorts and compute ROC-optimized cut-offs with external calibration; 2) integrate BAFF with emerging molecular assays (e.g., dd-cfDNA, gene-expression panels) to test composite risk scores; 3) perform longitudinal modeling to determine whether dynamic BAFF trajectories precede histologic or functional injury; and 4) explore whether BAFF-targeted or B-cell–directed therapies (e.g., belimumab, anti-CD20, BAFF/APRIL blockade) modify outcomes in BAFF-high phenotypes within controlled trials.

CONCLUSION

In summary, elevated serum BAFF early after kidney transplantation was accompanied by the occurrence of biopsy-proven rejection in our cohort, independent of conventional pre-transplant immunologic and hematologic factors. BAFF shows promise as a practical, non-invasive biomarker to enhance immunologic risk stratification and personalize post-transplant surveillance; rigorous validation and integration with molecular diagnostics are warranted before routine adoption.

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