# Decrease in Marginal Zone B-cells (CD27+IgD+CD38–) as Surrogate Marker of Rejection in Kidney Transplant Recipients

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## Abstract

***The biopsy of transplanted graft is the gold standard for the diagnosis of rejection in kidney transplantation; however, the procedure is invasive and may lead to a risk of graft lost.***
***The object of the present study was the assessment of surrogate markers that could complement or even replace the biopsy in kidney transplant recipients. Nineteen kidney transplant recipients with a median posttransplant follow-up of 216 days (range, 73-2,897) were recruited. A blood sample was drawn at the moment of impairment of graft function and just before diagnostic biopsy. The samples were stained with monoclonal antibodies to study different subpopulations of B lymphocytes and acquired on flow cytometry. Eight (42.1%) out of 19 were diagnosed with rejection. Five recipients were diagnosed with acute and three with chronic rejection. The median of circulating marginal zone B-cells in the recipient free of rejection was 7.0% (range, 4.2-9.5); nevertheless, the patients with rejection had a significant median decrease of 2.25% (range, 0.65-2.87; p = 0.0053). Receiver operating characteristic curve was performed to establish a cutoff value to differentiate patients diagnosed with rejection. This analysis set 3.55% marginal zone B-cells cells as the cutoff value to distinguish those patients with rejection at the moment prior to biopsy, with a sensitivity of 80.8% and specificity of 87.5% and an area under the curve of 91.5%.***
***Decreased frequencies of circulating marginal zone B-cells cells could be seen as a new surrogate marker of rejection in kidney transplantation.*** **(Trends in Transplant. 2013;7:51-5)**

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## Key words

***Kidney transplantation. Rejection. Surrogate marker. B-cell subpopulation. Marginal zone B-cell.***

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## Introduction

B-cells have gained interest in recent years in kidney transplantation, mostly due to the role of humoral response in allorejection and graft loss[1]. Besides the well-known function of antibody production, new aspects and paradoxical roles of B-cells are growing and acquiring new roles in transplantation[2]. Among these functions, B-cells may help T-cells as antigen presenting cells. Moreover, in a multicenter collaborative study of American (Immune Tolerance Network, ITN) and European (Reprogramming the Immune System for Establishment of Tolerance, RISET) networks to study tolerance in transplantation, operationally tolerant kidney transplant recipients (KTR), without immunosuppression and normal kidney graft function, showed a somewhat striking B-cell signature accompanied by increased circulating levels of B-cells and non-donor human leukocyte antigen (HLA) antibody production[3,4]. Recently, a B-cell subpopulation producing interleukin-10, with potential regulatory function supporting this hypothesis, has been demonstrated[5,6]. These findings led start several prospective studies to monitor B-cell subpopulations in KTR[7,8].

Nevertheless, the complexity of the B-cells increases because not only the acquired immune response interaction but also B-cells confined at marginal zone (MZB) are able to both induce T-cell-independent responses and participate in T-cell-dependent immune responses, capturing and importing blood-borne antigens to follicular areas of the spleen[9]. In addition, these MZB cells can participate in tolerogenic mechanisms. In summary, the different subsets of B-cells show paradoxical properties and are able to regulate alloresponses, with a potential role in graft tolerance, and to develop humoral rejection and induce cellular rejection.

In clinical practice, biopsy is the gold standard for diagnosis of clinical and subclinical rejection. However, despite the high utility and information given by biopsies, several cases of graft loss after biopsy have been reported[10]. Thus it is necessary to identify noninvasive surrogate markers of rejection for further validation in multicenter studies.

In the present study, different subpopulations of B-cells in KTR with rejection suspicion after graft function impairment were assessed as noninvasive surrogate biomarkers of rejection.

## Material and methods

The study was approved by our Institution's Ethics Committee and every patient recruited signed a written consent. Nineteen kidney transplant recipients were admitted for diagnostic biopsy due to impairment of kidney graft function. The demographic, clinical, and immunological parameters are summarized in table 1. Blood samples were taken prior to biopsy and processed within four hours after venipuncture.

Whole blood was stained with monoclonal antibodies to define different B-cell subpopulations: CD27-FITC (clone M-T271, BD Pharmingen, San Diego, CA); CD19-PerCP (clone SJ25C1, BD Biosciences, San Diego, CA), CD38-PE-Cy7 (clone HIT2, Biolegend, San Diego, CA); IgD-PE (clone IA6-2, BD Pharmigen) as previously shown[11] and acquired in FACS-Canto-II flow cytometry (BD Biosciences). The results were analyzed with FACS-Diva Software (BD Biosciences). In the present study, the MZB cells were identified as CD19+CD27+IgD+CD38–.

The variables were non-parametrically distributed and a statistical analysis comparison of the median MZB cells of rejection and

**Table 1. Demographic, clinical and immunological variables of kidney transplant recipients**

| | Rejection-free group | Rejection group | p |
|---|---|---|---|
| (n) | 11 | 8 | |
| Cause of ESRD | | | |
| – Congenital | 2 | 1 | |
| – Glomerular | 2 | 4 | |
| – Interstitial | 1 | 0 | |
| – Systemic | 3 | 2 | |
| – Vascular | 3 | 1 | |
| Mismatches (mean) | | | |
| – A | 1.3 | 1.1 | NS |
| – B | 0.9 | 0.8 | NS |
| – DR | 0.9 | 1.1 | NS |
| Recipient age (years, median and interquartile range) | 43 (38-59) | 52 (23-58) | NS |
| Donor age (years, median and interquartile range) | 52 (44-60) | 42 (31-61) | NS |
| HLA antibodies | 2 | 0 | |
| (n, PRA peak) | 51%, 71% | 0 | |
| HLA antibodies | 6 | 0 | |
| (n, PRA maximum) | 23, 51, 65, 71, 78, 89 | 0 | |
| Induction therapy | 0 | 0 | |
| Acute tubular necrosis | 0/11 | 1/8 | |
| Treatment on biopsy | | | |
| – MMF | 11/11 | 6/8 | |
| – Tac/Rapa/CNI withdrawal | 11/0/0 | 6/1/1 | |
| Diagnosis | – | 5 acute rejection (3 cellular, 1 humoral, 1 BL) 3 chronic rejection | |

ESRD: end-stage renal disease; HLA: human leukocyte antigen; PRA: panel reactive antibody; MMF: mycophenolate mofetil; Tac: tacrolimus; Rapa: rapamycin; CNI: calcineurin inhibitor; NS: Non significant.

rejection-free groups was performed using U-Mann Whitney test. Receiver operating characteristic (ROC) curve was performed to assess the percentage of MZB cells that differentiate the rejection and rejection-free groups. To determine the risk of rejection in patients with high or low percentages of MZB cells, Chi-square with Fisher correction was applied. A p value $< 0.05$ was considered significant.

## Results

### *Marginal zone B-cells in kidney transplant recipients with rejection suspicion*

Upon impairment of graft function, the KTR were subsequently classified as rejection or rejection-free after biopsy diagnosis. No differences in both percentage and absolute numbers of B-cells between rejection and rejection-free groups were observed. The patients diagnosed with rejection had decreased percentage of MZB cells compared with the rejection-free group: median (interquartile range) 2.25% (range, 0.65-2.87) vs. 7.0% (range, 4.2-9.5), respectively; $p < 0.01$ (Fig. 1).

### *Cutoff level of marginal zone B-cells to detect rejection in patients with impaired kidney graft function*

The ROC curve analysis gave 3.55% of MZB cells in peripheral blood as a cutoff value that differentiated patients suffering an episode of rejection from the patients with

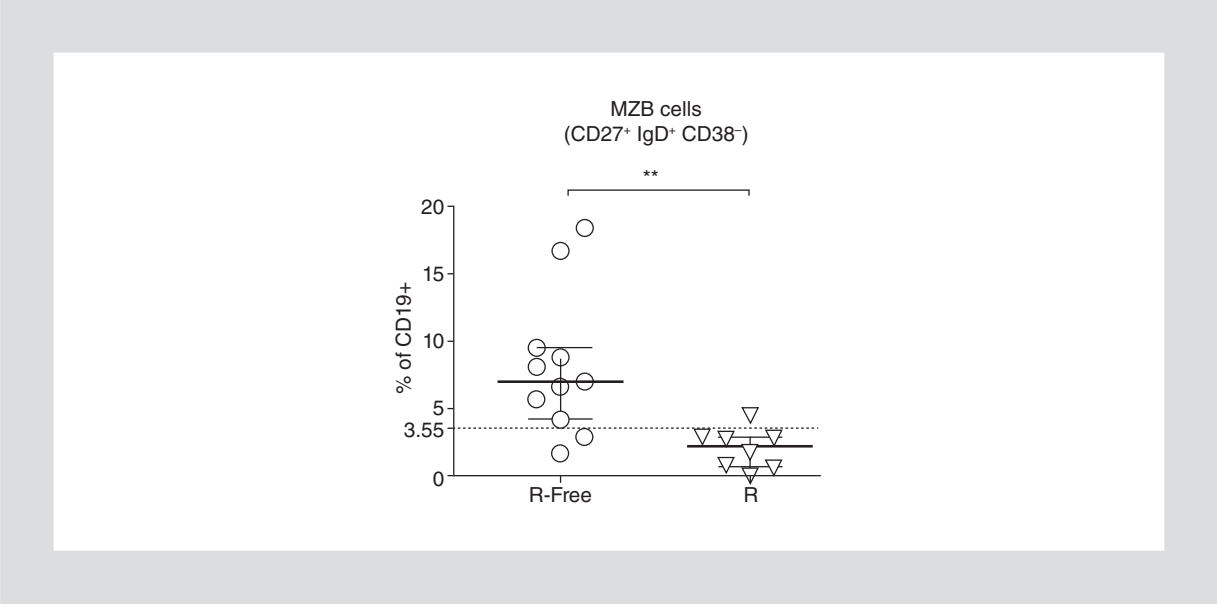


**Figure 1.** *Comparison of the percentage of marginal zone B-cells in kidney transplant recipients with impaired graft function. The kidney transplant recipients (KTR) were divided according to biopsy results: group of patients with rejection (R), white triangles and R-free, white circles. The medians and interquartile range of the percentage of marginal zone B-cells (MZB) (CD27+IgD+CD38–) in each group are depicted. The dotted line represents the cutoff value that best differentiates both groups with MZB cell percentage levels. The median comparison was performed using U-Mann Whitney test (**p < 0.01).*

impaired graft function included in rejection-free group, with 81.8% sensitivity and 87.5% specificity. The area under the curve was 91.5%. The relative risk of rejection in a patient with impaired graft function, with MZB cells below 3.55%, was 7.5 (Chi-square with Fisher´s correction, p = 0.009).

## Discussion

The rate of acute rejection in KTR has decreased during recent years due to the improvements in immunosuppressive drugs[12]. However, still around 5% of KTR suffers an acute rejection episode within the first year posttransplantation. The diagnostic gold standard is biopsy, but potential side effects could still appear, even graft loss[10]. It is of great interest to develop noninvasive surrogate markers to complement biopsy in the diagnosis of graft rejection[13,14]. The present study shows that a decrease in MZB cells in peripheral blood below 3.55% (of CD19+ cells) is associated with increased risk of rejection in patients with impaired kidney graft function.

The decline in MZB cells could be explained by their recruitment in the graft or lymph organs, as previously shown in rejection of xenotransplants[15], although the reason for the decrease in MZB cells in acute rejection remains to be elucidated. Moreover, the main limitation of the study is its single center nature. To increase the interest in MZB cell as surrogate markers of rejection, these results should be validated in multicenter studies with a larger sample size.

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