# THE POSSIBLE ROLE OF EARLY POST-TRANSPLANT INFLAMMATION IN LATER ANEMIA IN KIDNEY TRANSPLANT RECIPIENTS

Goran Imamović[1]*, Enver Zerem[2], Safet Omerović[3], Enes Osmanović[4], Emir Hodžić[1]

[1] University Clinical Center Tuzla, Department of Nephrology and Dialysis, Trnovac bb, 75 000 Tuzla, Bosnia and Herzegovina
[2] University Clinical Center Tuzla, Department of Interventional Ultrasound, Trnovac bb, 75 000 Tuzla, Bosnia and Herzegovina
[3] Regional Medical Center "Dr Safet Mujić", Titova bb, 88 000 Mostar, Bosnia and Herzegovina
[4] BH Heart Center Tuzla, Izeta Sarajlića bb, 75 000 Tuzla, Bosnia and Herzegovina

* Corresponding author

## Abstract

Delayed kidney graft function and acute rejection in the early post-transplant period affect both short and long-term allograft survival. Allograft rejection, as an inflammatory state, results in increased erythropoietin resistance, which leads to decreased haemoglobin (Hb) level. We conducted this study to evaluate whether inflammation in the early post-transplant period could predict later anemia.

This is a retrospective cohort study based on the analysis of 64 existing clinical records. *Predictor*: White blood cells (WBC) count obtained by the end of the first week post-transplant (W1). *Covariates*: Donor's age, recipient's age and sex. *Outcome*: Anemia identified at 12 months (M12) post engraftment.

Median WBC count at W1 was 9,5 x$10^3$/μL (5th – 95th percentile 5,2 x$10^3$/μL -17,8 x $10^3$/μL). Mean Hb values at M12 were 129,9 ± 20,3 g/L, in males 136,2 ± 20,1 g/L and in females 119,4 ± 16,2 g/L. The significant correlation was found between WBC at W1 and Hb at M12. Pearson coefficient of correlation r was -0,26, and 95% confidence interval (CI) for r was -0,47 to -0,015 (p=0,03). Univariate logistic regression showed significant association between WBC at W1 and Hb at M12 (OR 1,20; 95% CI 1,04 to 1,39, p=0,01). After the adjustment for donor's and recipient's age by transplantation and recipient's sex, multiple regression showed that WBC count remained predictive of anemia at M12 (OR 1,17; 95% CI 1,01 to 1,36, p=0,03).

Early post-transplant inflammatory response predicts later anemia in kidney transplant recipients. An increase in WBC count in the first week post-transplant by $10^9$/L increases the risk for anemia after twelve months by 17%.

KEY WORDS: leukocytosis, graft rejection, erythropoietin, kidney transplantation, inflammation

## Introduction

The anemia of chronic kidney disease is, in most patients, normocytic and normochromic, and is due primarily to reduced production of erythropoietin (EPO) by the kidney (a presumed reflection of the reduction in functioning renal mass), and to shortened red cell survival (1). Post-transplant anemia attracts less attention than anemia in chronic kidney disease, it is common and still remains substantial problem. The prevalence of anemia at 10 years post-transplant was reported to be as high as 93,2% (2). Anemia can be observed both in early and in late post-transplant period (3). In the early post-transplant period anemia assessment that can be attributed to renal reasons is not reliable because the perioperative blood loss, frequent phlebotomies and fluid shifts interfere with its proper evaluation (4). Therefore, the studies that evaluate anemia usually start with anemia assessment performed at least 3 months after transplantation (5), although there have been recently the indicators that in modern era anemia could be relialably assessed even earlier (6). Inflammation is known to contribute to anemia in general population and in chronic kidney disease. Likewise, infection in kidney transplant recipients has been associated with an increased risk of anemia (7). It has been suggested that allograft rejection, as an ongoing inflammatory state, results in increased EPO resistance, which leads to decreased haemoglobin (Hb) level (8). Acute rejection in the early post-transplant period (1 to 12 weeks) is known to affect kidney function adversely (9) and kidney dysfunction is a predictor of anemia, due to decreased EPO production (10). The incidence of subclinical rejections may vary between 15-43 % in protocol biopsies taken at various time points between 1 and 6 months post-transplant (11). In the presence of acute allograft rejection 11 genes involved in haemoglobin transcription were found down-regulated (12). However, no reports have been published about the association between early inflammatory response and later post-transplant anemia. Therefore, knowing that delayed graft function within one week post-transplant has a major adverse impact upon both short and long-term allograft survival (13) and knowing that acute rejection in the early post-transplant period (1 to 12 weeks) is known to affect kidney function adversely (9), it could be postulated that the early inflammatory response could be associated with later post-transplant anemia. Therefore, we conducted this study to evaluate whether an inflammatory response in the early post-transplant period could predict later post-transplant anemia.

## Materials and Methods

*Design*: Retrospective cohort study based on the analysis of 64 existing clinical records.

*Setting*: University-based tertiary internal medicine hospital in the town of Tuzla, Bosnia and Herzegovina.

*Participants*

Inclusion criteria: Patients transplanted and followed up at Tuzla University Medical Center from 1999 – 2007. There were in total 70 of them.

Exclusion criteria: Patients who did not reach the first year post-engraftment, patients with a renal allograft other than the first allograft and patients with missing data. Three patients were excluded because they died before reaching the first year post-engraftment, 1 has lost his graft in the early post-transplant course, 1 was excluded because of a renal allograft other than the first allograft and 1 because of the missing datum on white blood cell count (WBC) in the chart, leaving a total of 64 patients in the study, 29 males and 35 females.

*Anamnesis*

All patients fulfilled standard criteria to be eligible to receive their transplants, that is they were free from any untreated current infection, active malignancy with short life expectancy, chronic illness with life expectancy of less than one year, poorly controlled psychosis and active substance abuse. Two patients transplanted in 2004 were receiving EPO treatment by 1 year post-transplant and both were still anemic by that time. Other anemic patients started receiving EPO later than 1 year post-transplant because EPO treatment was introduced in our center only in 2005.

*Diagnosis*

Biopsy-proven acute rejection was diagnosed in 10 patients, 2 exhibited signs of delayed graft function, 6 suffered from postoperative surgical complications, 39 were hypertensive and 22 hyperlipemic. There were 5 diabetics, 3 with coronary heart disease and 4 with other cardio-vascular diseases. The study was approved by the local medical ethics committee.

*Main predictor*: WBC count obtained by the end of the first week post-transplant (W1).

*Covariates*: Donor's age, recipient's age and sex.

*Main outcome measure*: Anemia identified at 12 months (M12) post-transplant.

*Measurements*

Anemia was expressed as decreased qualitative variable of Hb. As recommended by the American Society of Transplantation, it was considered present if the Hb concentration was ≤ 13 g/dl in men or ≤ 12 g/dl in women. Donor and recipient age at transplantation were represented by quantitative variables in years. Induction immunosuppressive protocol included steroids, cyclosporine, antithymocyte globulin (up to 2002) and humanized anti-IL-2 receptor antibodies (later). Maintenance immunosuppressive regimen consisted of cyclosporine, azathioprine (up to 2002), mycophenolate mofetil (later) and steroids.

*Statistical analysis*

Statistical analyses were performed with MedCalc software (version 8.1.0.0 for Windows, MedCalc). The degree of association between WBC and Hb was assessed with correlation analysis using Pearson coefficient of correlation after a log-transformation to correct for a lack of normality, where appropriate. Univariate logistic regression analysis was applied to test the relationship between the main predictor and the main outcome, followed by multivariate model adjusted for other covariates.

## Results

There were 66 patients initially enrolled. Mean age was 32,2 ± 9,9 years, males 34,5 ± 9,2 and females 28,4 ± 10,2 (p=0,02). Median WBC count at W1 was 9,5 x103/μL ($5^{th}$ – $95^{th}$ percentile 5,2 $x10^3$/μL -17,8 $x10^3$/μL). Mean Hb values at M12 were 129,9 ± 20,3 g/L, in males 136,2 ± 20,1 g/L and in females 119,4 ± 16,2 g/L. Anemia prevalence at M12 was 45,3%. There were 51,7% anemic males and 71,4% anemic females (p=0,17).

The significant correlation was found between WBC at W1 and Hb at M12. Pearson coefficient of correlation r was -0,26, and 95% confidence interval (CI) for r was -0,47 to -0,015 (p=0,03) (Figure 1). Univariate logistic regression analysis showed significant association between WBC at W1 and Hb at M12 (OR 1,20; 95% CI 1,04 to 1,39, p=0,01). After the adjustment for donor's and recipient's age by transplantation and recipient's sex, multiple regression model showed that WBC count remained predictive of anemia at M12 (OR 1,17; 95% CI 1,01 to 1,36, p=0,03) (Table 1).

| Predictors | Units of increase | Adjusted OR | 95% CI |
|---|---|---|---|
| [1]WBC at [2]W1 | $10^9$ cells/L | 1,17<br>1,0 (ref.) | 1,011 – 1,362 |
| Recipient's age | 1 year | 0,94<br>1,0 (ref.) | 0,887 – 1,003 |
| Recipient's sex | male<br>female | 0,68<br>1,0 (ref.) | 0,209 – 2,239 |
| Donor's age | 1 year | 0,96<br>1,0 (ref.) | 0,924 - 1,015 |

TABLE 1. Association between WBC obtained by the end of the first week post-transplant and anemia after 12 months

**Legend: [1]WBC - white blood cells; [2]W1 - end of the first week post-transplant**

## Discussion

Our results showed significant correlation and association, between WBC count obtained by the end of the first week post-transplant and anemia identified at M12. An increase in WBC count at W1 by $10^9$ / L increased the risk for anemia at M12 by 17% (Table 1), which clearly demonstrates the impact of inflammation in the early post-transplant period on anemia at 12 months. The anemia of chronic disease (inflammation), was initially thought to be associated primarily with infectious, inflammatory, or neoplastic disease. However, other observations have shown that anemia of chronic disease can be seen in a variety of conditions, including severe trauma, heart failure, diabetes mellitus, and in those with acute or chronic immune activation (14-18). The anemia is typically normochromic, normocytic, and hypoproliferative. Recurrent or chronic inflammatory processes are common in individuals with chronic kidney disease, including those with chronic renal failure. This is due to many underlying factors, including the uremic milieu, elevated levels of circulating proinflammatory cytokines, oxidative stress, carbonyl stress, protein-energy wasting, enhanced incidence of infections and others. It has been suggested that the underlying inflammatory medical condition causes the release of cytokines such as the interleukins (eg, IL-1 and IL-6) (19) and tumor necrosis factor (TNF-alpha) by activated monocytes. These cytokines may then unleash a cascade including the secretion of interferon (IFN)-beta and IFN-gamma by T lymphocytes. As an example, IFN-gamma when given to experimental animals can produce the picture of anemia of chronic disease with most of the abnormalities noted above (20. Among patients with chronic kidney disease, the presence of an inflammatory state may also be closely related to accelerated atherogenesis, protein-energy malnutrition, and anemia (21-23)

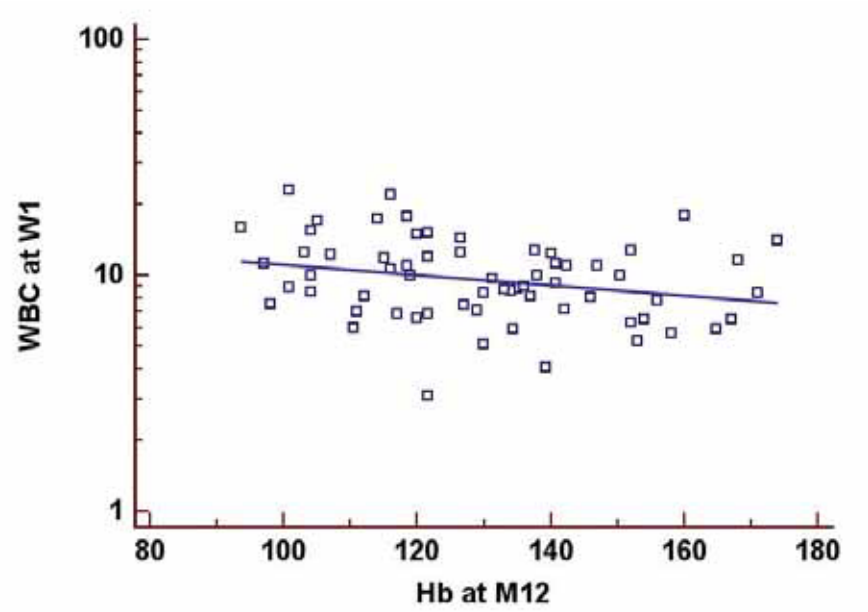


FIGURE 1. Correlation between white blood cells (WBC) obtained by the end of the first week post-transplant (W1) and hemoglobin (Hb) obtained by 12 months (M12) post-transplant.

Likewise, the relationship between inflammation and anemia in kidney transplant recipients has already been established (24). Winkelmayer et al. describe an inflammatory response in the form of rejection and conclude that the rejection could have caused in their study a decrease in Hb level through EPO resistance (8). We have taken into account all risk factors for anemia that could have confounded the association between WBC count at W1 and Hb at M12. Those factors were: impaired kidney function, acute rejection episodes, increased donor and decreased recipient age, female recipients and a renal allograft other than the first allograft (25, 26). Thus, one patient with a renal allograft other than the first allograft was excluded from the study and recipient's age and sex and donor's age were adjusted for in the multivariate analysis. However, we did not adjust for kidney function and acute rejection episodes in the multivariate analysis. Impairment of kidney function was not adjusted for because inflammation elevates serum creatinine. Winkelmayer et al. in their study of association between anemia and allograft loss did adjust their analysis for creatinine clearance, although they specified that "anemia would be a surrogate of chronic rejection and would reflect a state of chronic inflammation due to a failed graft" (8). Thus, creatinine clearance in this study should not have been a confounder to be used for the adjustment because chronic rejection and inflammation deteriorate kidney function, which renders creatinine clearance an intervening variable on a causal pathway to the allograft loss (27). Besides, the problem of collinearity between anemia as a surrogate for chronic rejection and creatinine clearance as a marker of deterioration of kidney function seems to be obvious in this analysis, too. We did not make an adjustment for acute rejection episodes in our study either, even though they are known risk factors for anemia (25, 26). However, acute rejection episodes did not meet the criteria to be a confounding variable in our analysis. The predictor was WBC count which was a surrogate for inflammation / rejection so that we have avoided the problem of collinearity in a statistical analysis by not adjusting for acute rejections (28). Furthermore, acute rejection is also an intervening variable on a causal pathway between the predictor and the outcome, since inflammation can trigger the rejection in kidney transplant recipients (29). That is also why WBC count should not have been adjusted for rejection (30).

*Limitations*

Unfortunately we do not have additional parameters of inflammation, such as cytokines, or CRP, but WBC itself is a valid marker for heightened inflammatory state. It could be argued that antirejection drugs including steroids used during this stage might have confounded WBC. This, however, is unlikely since all our patients received steroids at the same dose (7 mg/kg) in an induction immunosuppressive protocol and dose variation as a possible cause of varying WBC count can be ruled out. This raises the likelihood that the more brisk host-response to steroids is perhaps associated with later post-transplant anemia. However, sustained elevation in WBC is more likely to represent a heightened inflammatory state and less likely due to variable

host response to the same dose of steroid. Furthermore, all patients received induction treatment, either with antithymocyte globulin (up to 2002), or Basiliximab (later), which are both myelodepressive, so there was no confounding effect on WBC count. The same stands for Mycophenolate mofetil, or Azathioprine.

## Conclusion

Early post-transplant inflammatory response predicts later anemia in kidney transplant recipients.
An increase in WBC count in the first week post-transplant by $10^9$ / L increases the risk for anemia after twelve months by 17%.
Larger scale studies addressing the limitations discussed above are needed to establish possible association between early post-transplant inflammatory response and later anemia in kidney graft recipients.

*List of Abbreviations*

| | | |
|---|---|---|
| WBC | - | White blood cells |
| W1 | - | first week post-transplant |
| M12 | - | 12 months post-transplant |
| EPO | - | erythropoietin |

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