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Analysis of selected diagnostic factors of organic kidney damage revealed in protocol biopsy among renal transplant recipients

Short title: Lukasz Jankowski et al. Protocol biopsy

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ABSTRACT

Introduction: Kidney transplantation is the most effective treatment for end-stage renal disease, but long-term graft survival remains a significant challenge. Subclinical graft rejection and other pathological changes often go undetected by standard clinical tests, leading to graft dysfunction and failure. The goal of the study was to determine the usefulness of protocol biopsies in clinical practice by examining the frequency and severity of histological problems, the effectiveness of the transplanted organ, and the potential beneficiaries of protocol biopsies following a kidney transplant.

Material and methods: A retrospective analysis was conducted on 72 kidney transplant recipients from the Department of Transplantation Medicine, Nephrology, and Internal Diseases at the Medical University of Warsaw. A statistical analysis was performed to identify significant differences between patients with and without organic kidney changes.

Results: Among the 72 kidney transplant recipients, 40% of the biopsies revealed abnormal histological findings. There were big differences in the lipid profiles of patients with and without organic renal damage. The group with renal damage had lower levels of total cholesterol, HDL, and LDL ($p < 0.05$).

Conclusions: The results showed significant differences in the lipid profile between patients with organic renal damage and those without organic renal changes, highlighting the value of biopsies in identifying patients at risk of graft failure.

Keywords: kidney transplantation; protocol biopsy; graft loss; kidney allograft dysfunction; diagnostic utility

Introduction

The epidemiology of kidney diseases shows a growing number of chronic kidney disease (CKD) cases, which in the end stage often necessitate kidney transplantation (KTx) [1]. CKD affects approximately 10–15% of the global population, and its prevalence is higher in older adults, women, and those with underlying conditions like diabetes mellitus and hypertension [2]. In particular, diabetes and hypertension are the two leading causes of kidney failure, contributing together to around 40–60% of all CKD cases globally. Furthermore, a significant burden of kidney diseases falls on racial minorities, further complicating access to care and outcomes [2]. In Poland, the incidence of kidney transplants has diminished in recent years owing to a scarcity of suitable organs and an older demographic of patients. This decrease aligns with global patterns in organ shortages when the demand for transplants exceeds the availability of viable kidneys. Recent data reveal that preemptive kidney transplantation, which can markedly enhance patient outcomes, constitutes about 9–11% of deceased donor kidney transplants [2]. The durability of graft survival for transplanted kidneys has significantly improved over the past thirty years, primarily due to advancements in immunosuppressive treatments and post-transplant management, resulting in a 20–30% rise in graft survival rates compared to previous decades [2].

Prolonging the long-term viability of renal transplants is a primary challenge in contemporary transplantation. Despite substantial advancements in recent decades, the long-term survival rates for kidney transplants continue to pose concerns. Data indicate that the survival rates for kidney transplants at 1, 3, 5, 10, and 15 years are 99%, 97%, 93%, and 70%, respectively, demonstrating a consistent decrease in graft viability over time [3]. The primary reason for graft loss in long-term follow-up is the buildup of permanent chronic alterations, frequently resulting from untreated or treatment-resistant rejection processes. Chronic allograft dysfunction is a primary factor in graft failure, responsible for 35–50% of all kidney transplant losses [4]. The decline in transplanted kidney function, typically indicated by elevated blood creatinine levels and reduced eGFR, frequently represents a late-stage manifestation of underlying conditions. At this point, irreversible harm may have already transpired. Histopathological analysis of kidney biopsy specimens facilitates the identification of early alterations, including inflammation or fibrosis, before the abnormality of clinical indicators such as creatinine levels [4]. Early detection via biopsy provides an opportunity for prompt management and can enhance transplant survival by mitigating problems before the onset of chronic disease. This is why many clinics, including the authors' Clinic of Transplantation Medicine, have implemented a clinical program of protocol biopsies. These

biopsies are performed immediately after transplantation and at pre-determined intervals (typically 3 and 12 months after KTx) in clinically asymptomatic patients.

Views on the diagnostic and prognostic utility of the biopsy protocol for the transplanted kidney have varied over the past 15 years. After the introduction of drugs with potent immunosuppressive effects and the associated decline in the incidence of episodes of acute T-cell rejection, the belief among many investigators was that performing protocol biopsies was unwarranted, as they did not contribute information that would lead to modifications in therapeutic management [5]. Studies have demonstrated the diagnosis of subclinical rejection in a significant number of patients, reporting instances of silent acute rejection in as many as 13% of protocol biopsies [6]. These findings underscore the significance of protocol biopsies in identifying asymptomatic rejection processes that, if addressed, may result in chronic allograft malfunction and eventual graft loss. Protocol biopsies are increasingly considered a crucial instrument in transplant surveillance, particularly in identifying early rejection and histological alterations in high-risk patients. [7].

Despite its probable benefits, protocol biopsy is a rarely used tool for monitoring a transplanted kidney, both in Poland and internationally. This appears to be due to the paucity of studies dedicated to evaluating its usefulness in the management of renal transplant recipients. Although the procedure is invasive and can cause complications, it's crucial to identify the patient who would benefit most.

The goal of the study was to determine the usefulness of protocol biopsies in clinical practice by examining the frequency and severity of histological problems, the effectiveness of the transplanted organ, and the potential beneficiaries of protocol biopsies following a kidney transplant.

Material and methods

The authors retrospectively analyzed the clinical and laboratory data of 72 consecutive kidney transplant recipients operated on in 2014–2018, under the care of the Department of Transplantation Medicine, Nephrology, and Internal Diseases of the Medical University of Warsaw, who performed protocol biopsies of the kidney transplant. The evaluation included 144 protocol biopsy results, routinely performed 3 and 12 months after the date of transplantation. Every procedure involved taking and analyzing two fragments of renal tissue, and all biopsies used the 16G needle. The biopsy lesions were classified qualitatively and quantitatively using the uniform criteria of the Banff classification. Routinely determined was the presence of complement C4d deposits, polyomavirus SV40 antigen, immunoglobulins,

fibrinogen light chains, and complement components C3 and C4 in transplanted kidney biopsies. The Pathology Laboratory of the Clinic assessed all biopsies with the same experienced pathologist. Histopathological data were then correlated with clinical and laboratory data of the renal transplant recipient, including: the degree of immunization before transplantation, assessed by serological method (PRA: panel reactive antibodies) or solid phase method (DSA: donor specific antibodies), comorbidities, cause of end-stage renal disease, duration and method of dialysis therapy; data related to the donor: donor characteristics (age, sex, BMI, smoking status, standard donor, extended criteria donor, living donor), immunological selection of the donor and recipient in terms of HLA A-B- and DR antigens, immunological selection of the donor and recipient in terms of groups major blood; data characterizing the organ transplantation procedure: cold ischemia time, occurrence of delayed graft function (DGF, defined as the need to perform hemodialysis at least once in the patient in the first week after kidney transplantation); data on the course of the post-transplantation period before the protocol biopsy, including: episodes of acute rejection in the period before the biopsy, immunosuppressive treatment regimen used, ultrasound imaging of the abdominal cavity with Doppler assessment of renal flow. The frequency and type of renal graft pathology were assessed and searched for risk factors that might lead to clinically silent changes in protocol biopsies of the transplanted kidney. Histological findings were, for the sake of statistical analysis, classified into one of the subgroups: 1) normal biopsy; 2) borderline changes; 3) subclinical cell-mediated rejection; 4) subclinical antibody-mediated rejection; 5) glomerulitis; 6) thrombotic microangiopathy; 7) polyomavirus BKV; 8) proliferative arteriopathy.

Statistical analysis

This study utilized both descriptive and inferential statistical approaches to examine the data, to compare different clinical parameters between individuals who have normal kidney function and those who have kidney damage. For continuous variables, the authors computed the mean and standard deviation (SD), as well as the count and percentage for categorical variables. To ascertain the statistical significance of disparities between the two groups (normal kidney vs. kidney injury), the authors employed the independent t-test to compare the means of the two groups for continuous variables, taking into consideration the uneven variances using Welch’s t-test. The authors employed the Chi-square test of independence to evaluate the correlation between each categorical variable and kidney function status. In the absence of any link between the variables, the authors compared the

observed frequencies in each category with the predicted frequencies. These tests yielded p-values that reveal the statistical significance of the group disparities; a p-value below 0.05 is considered statistically significant. The statistical studies were performed using Python and the appropriate statistical libraries. The table presents the relationship between different parameters and kidney injury, including the sensitivity, specificity, and AUC (area under the curve) values for each variable. The statistical techniques used in this study include correlation analysis, which involves using the Pearson correlation coefficient to measure the strength and direction of relationships between variables. Additionally, predictive analysis was conducted using logistic regression to evaluate the impact of individual factors on the risk of kidney injury. Logistic regression yields regression coefficients that serve as indicators of the magnitude and direction of the association between independent variables (such as sex, age, BMI, etc.) and the dependent variable (kidney damage).

Results

In 72 patients, in whom the protocol biopsy was performed in the Clinic either 3 or 12 months after KTx, in 29 (40%) abnormal histological results were found either in the course of observation (Table 1).

Patients with organic kidney changes had a significantly lower mean total cholesterol level (36.59 mg/dL, SD = 66.92) compared to those without changes (94.4 mg/dL, SD = 93.66, $p = 0.003$). Similarly, the average HDL level was lower in the group with renal organic changes (12.06 mg/dL, standard deviation = 23.64) compared to the group without organic changes (26.76 mg/dL, standard deviation = 25.69, $p = 0.015$). In addition, patients with organic kidney injury had a considerably lower mean LDL level (22.52 mg/dL, SD = 37.54) compared to those without organic kidney changes (63.96 mg/dL, SD = 57.71, $p < 0.001$). The average triglyceride level was significantly lower in the group with organic renal changes (31.02 mg/dL, standard deviation = 72.6) compared to the group without kidney changes (72.58 mg/dL, standard deviation = 97.81, $p = 0.044$). The results indicate a noticeable change in lipid profile parameters in individuals with organic renal impairment (Fig. 1).

The ischemic variable (0.22) showed the strongest positive correlation, indicating that patients with ischemic problems are more likely to experience organic kidney damage (Table 2). Further significant positive correlations include BMI (0.21) and hypercholesterolemia (0.20), indicating that higher body mass index and elevated cholesterol may be risk factors for kidney damage. The variables: age (0.18) and diabetes mellitus (0.16) also have a moderate

positive correlation, suggesting that older age and diabetes may be associated with a higher risk of organic kidney damage.

The variable sex has a minimal correlation with organic kidney damage (0.10), indicating that gender is not a significant factor in this analysis. In contrast, cholesterol-related variables such as HDL, total cholesterol, and LDL have a negative correlation with organic kidney damage. The authors recorded the strongest negative correlation for LDL (–0.38) and total cholesterol (–0.32), suggesting that higher levels of total cholesterol and LDL may have an inverse relationship with organic kidney damage. The variable HDL (–0.28) also shows a moderate negative correlation.

Discussion

Significant lipid profile differences were found one year after KTx between patients without organic kidney abnormalities and those with organic renal impairment. Statistically, organic renal damage patients had lower total cholesterol than those without kidney abnormalities. HDL and LDL values were lower in the renal injury group, with LDL considerably lower. These findings support prior research on dyslipidemia and chronic kidney disease (CKD) development. Poorer outcomes in CKD patients often result from low HDL and LDL levels, leading to aberrant lipid metabolism. This study's negative connection between LDL and renal damage supports past research linking hypocholesterolemia to inflammation and lower kidney function [8]. Protocol biopsies detect a high prevalence of subclinical rejection; therefore, future studies should examine their long-term cost-effectiveness in transplant patient treatment, especially in high-risk groups. This could help determine the biopsies’ ideal frequency and time. The study shows substantial links between IHD, BMI, and renal impairment. The positive correlation between BMI and cholesterol levels bolsters the notion that obesity and metabolic syndrome serve as risk factors for chronic renal disease [9–11]. Due to several factors, creatinine levels before biopsy did not differ significantly between those with and without organic changes. Organic changes in the transplanted kidney may not immediately raise creatinine. Biopsy-detected subclinical rejection or early fibrosis may not affect creatinine levels. The study found that routine biopsies detect these changes before creatinine levels rise, explaining the same results in both groups. Although damaged, the transplanted kidney can maintain normal creatinine levels and correct initial irregularities. Minor or localized injuries often show this. Kidney transplant recipients utilize immunosuppressive medicine to treat early inflammation or rejection, which maintains elevated creatinine levels even when lesions are present on biopsy. Creatinine is not

a sensitive early indication of renal failure, especially in transplant recipients. Interstitial fibrosis or inflammation may precede creatinine elevation [12–14].

Kidney transplantation is the best treatment for end-stage renal failure (ESRD), improving quality of life and longevity over dialysis [15]. The graft's long-term survival and efficacy are major concerns after transplantation. Many recipients face transplant malfunction and failure despite immunosuppressive drug advances. Researchers are using protocol biopsies to identify subclinical rejection and other pathological abnormalities that could jeopardize transplant survival. Understanding how lipid profile changes affect graft function may lead to targeted dyslipidemia treatments for transplant recipients. Regardless of clinical status, protocol biopsies were performed at preset intervals after transplantation. This study sought early histological abnormalities, including subclinical rejection, chronic rejection, and other detrimental changes before they appear clinically. These biopsies uncover asymptomatic, progressive illnesses that would normally go undiagnosed until they cause damage. "For-cause" biopsies, which only occur when clinical signs indicate a problem, contrast with this proactive approach [16, 17].

Numerous studies have examined how protocol biopsies improve graft outcomes. This discovery emphasizes the need for early detection post-transplant when management can significantly alter the disease trajectory. Garcia-Lopez's 2024 meta-analysis supports protocol biopsies' short- and long-term safety and efficacy [18]. Terrec et al. examined kidney transplantation biopsies three months later. They observed that the three-month protocol biopsy improved transplant survival regardless of donor age, kidney type (living or deceased), or immunosuppressive regimen [19]. Early detection of histological changes allowed timely intervention, improving long-term effects. Protocol biopsies are useful for detecting histological changes, especially those suggesting antibody-mediated rejection (ABMR) or chronic allograft nephropathy (CAN).

Protocol biopsies can detect subclinical rejection — histological signs of rejection without clinical symptoms — according to numerous studies. Subclinical rejection can lead to chronic allograft dysfunction if left untreated. According to Cieślik et al., routine biopsies one year after transplantation effectively detect early illnesses such as subclinical rejection, interstitial fibrosis, and tubular atrophy. Early treatment improved performance and slowed graft failure [20].

Protocol biopsies protect kidney transplant recipients by detecting antibody-mediated rejection. Their research revealed that ABMR is on the rise, often progressing

asymptomatically and going undetected by serum creatinine or eGFR tests [8]. Protocol biopsies provide intervention before permanent rejection in these cases.

Although useful, protocol biopsies have limits. People fear protocol biopsies due to their invasiveness [21, 22]. Biopsies are typically safe, but bleeding, infection, and transplant damage are rare. Schwarz et al. found that 16-gauge needles were more useful than 18-gauge needles for protocol biopsies while maintaining a similar risk profile [23]. This suggests that biopsy techniques may reduce procedural risks.

Non-invasive biopsies have garnered attention in recent years. Biomarkers like donor-derived cell-free DNA (dd-cfDNA) can detect graft deterioration non-invasively. Mehta et al. found that non-invasive biomarkers are promising but cannot yet replace biopsy [24]. Developing non-invasive tools may reduce the need for biopsies, especially if biomarkers' precision and predictive relevance improve [25–29]. Biopsies are still the best way to detect subclinical rejection and other early pathology.

Protocol biopsies have long-term benefits, as shown by numerous studies. Continuous biopsies have shown a decrease in chronic allograft dysfunction and late-stage graft failure at one-year post-transplantation. Moein et al. (2023) and Garcia-Lopez found that protocol biopsies improve outcomes and graft life, especially in high-risk patients [18, 30]. Protocol biopsies benefit patients at high risk of rejection, such as those with sensitization or marginal donor organs. This patient population has high rates of subclinical rejection, which can progress quickly without detection. Huang et al. recommended protocol biopsies for high-risk transplant patients [7].

Protocol biopsies can detect early pathological changes in kidney transplant recipients, but the domain is dynamic. Graft health monitoring may be safer and more patient-centered with non-invasive methods. Until these approaches match the diagnostic accuracy of biopsies, protocol biopsies are necessary in post-transplant treatments. The dispute over routine biopsies will continue, especially with new technology [21, 31, 32]. Protocol biopsies are necessary for screening and treating subclinical issues in high-risk individuals before they cause lasting graft damage. With careful patient selection and biopsy technology developments, protocol biopsies will remain essential to post-transplant therapy.

The study has flaws. The retrospective nature of this study increases selection bias and makes it harder to prove causality between histological alterations and clinical outcomes. The study included 72 kidney transplant recipients with 144 biopsy results, but the sample size may be too small to extrapolate the findings to other populations due to the complexity of kidney transplant patient profiles and immunosuppressive protocol heterogeneity (only 6

patients in the present group have a PRA > 0). The study lacked long-term clinical follow-up beyond biopsy intervals, which is necessary to establish histological findings' prognostic value. The lack of long-term graft survival data makes it difficult to analyze how protocol biopsies affect transplant durability. The present group, however, had a significant rate of aberrant findings (40%), allowing doctors to initiate treatment immediately despite subclinical changes.

Conclusions

The results showed significant differences in the lipid profile between patients with organic renal damage and those without organic kidney changes, highlighting the value of biopsies in identifying patients at risk of graft failure. The use of protocol biopsy may help improve long-term graft survival by early detection of lesions not seen on standard clinical tests. Further studies are recommended with larger patient populations and longer follow-up periods to better assess the long-term benefits of protocol biopsies and confirm their impact on graft survival. In the future, researchers should also look at comparing protocol biopsies with newer, less invasive ways to check on the health of grafts, like donor DNA-based biomarkers. This might cut down on the need for invasive procedures.

Article information

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Table 1. Baseline characteristics of patients

Parameter	Kidney without organic damage (n = 43)	Kidney with organic damage (n = 29)	p-value
Sex, male	19 (44.2%)	10 (35.7%)	0.644
Age, y	47.03 (13.7)	42.28 (12.66)	0.142
BMI	24.17 (3.78)	25.8 (3.9)	0.084
Comorbidities			
Smoker	4 (9.3)	1 (3.6)	0.654
Dyslipidemia	4 (9.3)	7 (25.0)	0.147
Hypertension	38 (88.4)	26 (92.9)	0.832
Diabetes mellitus	3 (6.9)	4 (14.3)	0.547
IHD	1 (2.3)	4 (14.3)	0.147
Laboratory findings			
Creatinine	1.462 (0.480)	1.455 (0.409)	0.949
Hemoglobin	13.28 (13.77)	11.27 (1.67)	0.348
Hematocrit	34.2 (3.63)	34.57 (3.91)	0.690
Total cholesterol	94.4 (93.66)	36.59 (66.92)	0.003
HDL	26.76 (25.69)	12.06 (23.64)	0.015
LDL	63.96 (57.71)	22.52 (37.54)	< 0.001
Total glicerides	72.58 (97.81)	31.02 (72.6)	0.044
HLA_A			0.658
0	3 (7.0)	3 (10.7)	
1	21 (48.8)	11 (39.3)	
2	14	**11 (??**	
HLA_B			0.508
0	3	1	
1	22	12	
2	13	12	
HLA_DR			0.334
0	9	6	
1	23	18	
2	6	1	
Kidney status			
No organic damage	43	–	
Borderline lesions	-	6	
Subclinical cellular changes	-	14	
Subclinical humoral changes	-	1	
Glomerular changes	-	2	
Thrombosis	-	2	
BK virus	-	4	

BMI — body mass index; HDL — high-density lipoprotein; IHD — ischemic heart disease; LDL — low-density lipoprotein

Table 2. Correlation of parameters in relation to the risk of kidney damage

	Correlation with Organic kidney changes	**Sensitivity**	**Specificity**	**AUC**
Sex, male	0.098	34.5%	55.8%	0.549
Age, y	0.178	62.1%	60.5%	0.615
BMI	0.207	51.7%	72.1%	0.622
Comorbidities				
Smoker	–0.042	0.0%	100%	0.488
Hypercholesterolemia	0.202	24.1%	90.7%	0.574
Hypertension	0.078	93.1%	11.6%	
Diabetes mellitus	0.160	17.2%	93.0%	0.551
IHD	0.221	13.8%	97.7%	0.557
Laboratory findings				
Hgb	–0.093	24.1%	90.7%	0.510
Hct	0.048	65.5%	48.8%	0.533
Total cholesterol	–0.324	89.7%	11.6%	0.390
HDL	–0.282	96.6%	11.6%	0.315
LDL	–0.377	0.0%	100%	0.325
TG	–0.226	92.2%	13.9%	0.400
HLA_A	0.072	11.5%	97.4%	0.529
HLA_B	0.134	46.2%	65.8%	0.568
HLA_DR	–0.097	76.9%	23.7%	0.457

Figure 1. Updated correlation matrix of parameters

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