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**INTEGRATION OF MORPHOMETRIC AND MORPHOLOGICAL METHODS IN THE COMPREHENSIVE ASSESSMENT OF KIDNEY TRANSPLANT FUNCTION****Muminov Javohir Asliddinovich**

Independent Researcher, Abu Ali ibn Sino Bukhara State Medical Institute

ORCID: 0009-0006-5199-0392

**Abstract.** Objective: To evaluate the informativeness of morphometric, ultrasound, and morphological methods for diagnosing the functional status of kidney grafts in the early postoperative period. The study included 72 recipients divided into two groups according to sensitization level (MFK-1, n = 41; MFK-2, n = 31). Serial ultrasound measurements of renal size, parenchymal thickness, and resistive index (RI) were performed, together with morphometric analysis of biopsy specimens according to Banff (2017) and assessment of correlations between functional, hemodynamic, and histological parameters. A significant correlation was found between elevated RI and the severity of interstitial-tubular changes ( $r = 0.68$ ;  $p = 0.01$ ). The combination of instrumental and morphological methods increases the diagnostic accuracy of early graft function assessment.

**Keywords:** kidney transplantation, ultrasound morphometry, morphology, Banff classification, resistive index, correlation analysis.

**Background**

Kidney transplantation is the optimal treatment for end-stage chronic kidney disease (CKD), providing patients with a higher quality and longer duration of life compared with maintenance dialysis therapy [1,2,9]. However, despite contemporary advances in surgical technique and immunosuppressive therapy, functional graft complications in the early postoperative period remain one of the main causes of graft loss. According to international registries, the incidence of acute rejection and ischemia-reperfusion injury during the first 3 months after transplantation ranges from 10% to 25% [3,4,5,17].

Traditionally, the main tools for evaluating graft function are clinical and biochemical parameters (creatinine, urea, and glomerular filtration rate), but these change only after pronounced morphological disturbances have developed. Puncture biopsy remains the gold standard for diagnosing the causes of graft dysfunction, yet its use is associated with several limitations, including invasiveness, the risk of bleeding and infectious complications, and subjectivity in the interpretation of histological data [6,7,8,20].

In this context, noninvasive imaging methods, especially ultrasound and Doppler ultrasonography, are of particular importance because they allow real-time assessment of the structural and functional parameters of the graft. Measurement of morphometric parameters, including organ size, parenchymal thickness, echostructure, and resistive index (RI), makes it possible to identify early disturbances in intrarenal hemodynamics that may indicate rejection or acute tubular necrosis [9,10,11,21].

According to Halloran et al. (2020), an RI above 0.70 is associated with interstitial-tubular inflammation and vascular changes corresponding to Banff t2i2v1. Studies by Belavina et al. (2023) confirmed that the correlation between RI and the morphological indices t, i, and v may reach  $r = 0.65-0.72$ , making ultrasound one of the most important noninvasive tools for early diagnosis [12,13,18].

The integration of morphometric and morphological methods has particular scientific and practical value. Combining ultrasound data with biopsy findings assessed according to the Banff

criteria (2017–2022) makes it possible not only to objectify the interpretation of morphological findings, but also to predict the probability of immune injury without the need for repeated biopsy. Contemporary studies by Loupy et al. (2018) and Lefaucheur et al. (2017) show that multiparametric approaches increase the diagnostic accuracy of rejection to 90–92%, significantly reducing the number of invasive procedures and improving transplantation outcomes [14,15,16,19].

Thus, the development of a comprehensive system for assessing kidney graft status based on a combination of morphometric and morphological methods is a relevant direction in modern transplantology. Such an approach makes it possible to objectively evaluate the dynamics of graft functional parameters, identify early signs of rejection, and individualize therapeutic strategy in patients with different levels of immunological risk.

The aim of this study was to evaluate the diagnostic significance of integrating morphometric and morphological methods in the comprehensive follow-up of kidney recipients during the early postoperative period.

#### Materials and Methods

The study was conducted at the Department of Angiosurgery and Transplantation of the Samarkand Regional Multidisciplinary Medical Center (SRMMC) from 2021 to 2025.

#### Study design and sample characteristics

A single-center prospective-retrospective study included 72 patients (49 men and 23 women) with end-stage chronic kidney disease (stage V CKD) who underwent living-related kidney transplantation. The mean age of the patients was  $41.2 \pm 11.3$  years (range, 18 to 63 years), and the mean duration of maintenance hemodialysis before transplantation was  $28 \pm 9$  months.

Patients were divided into two groups according to the degree of immunological sensitization:

- MFK-1 group (control): 41 patients with a low level of panel-reactive antibodies (PRA < 15%) and no donor-specific antibodies (DSA).
- MFK-2 group (highly sensitized): 31 patients with an increased level of sensitization (PRA > 50%) and/or DSA > 3000 MFI according to the Luminex test.

The main clinical and demographic parameters (age, sex, dialysis duration, comorbidities, and anamnestic sensitization factors such as blood transfusions and pregnancy) were comparable between the groups ( $p > 0.05$ ).

#### Immunological typing

HLA typing at loci A, B, and DR was performed by PCR-SSP using commercial One Lambda kits (USA). The levels of PRA and DSA were determined by the Luminex Single Antigen Bead Assay on a LabScan 3D analyzer (One Lambda, Thermo Fisher Scientific, USA).

The results were expressed as percentages (PRA) and Mean Fluorescence Intensity (MFI) units. MFI values  $\geq 3000$  were considered clinically significant.

Crossmatch testing was performed by two methods:

- CDC-XM (complement-dependent cytotoxicity crossmatch): positive if more than 10% of cells died.
- Flow-XM (flow cytometry crossmatch): positive if fluorescence shifted by more than 50 channels compared with the negative control.

### Ultrasound examination (morphometry)

Ultrasound examination was performed using a GE LOGIQ P7 unit (GE Healthcare, USA) with a 3.5–5.0 MHz convex transducer in B-mode, color Doppler, and pulsed-wave Doppler modes. Examinations were carried out at standard time points: before transplantation and on postoperative days 1, 7, and 30.

The following parameters were measured:

- longitudinal and transverse dimensions of the graft (mm);
- parenchymal thickness in the middle segment (mm);
- cortical echogenicity relative to the liver;
- prominence of the pyramids, capsule status, and corticomedullary differentiation;
- resistive index (RI) in the segmental arteries of the upper, middle, and lower poles with an insonation angle  $\leq 60^\circ$ ;
- peak systolic velocity (PSV) and end-diastolic velocity (EDV).

RI was calculated using the formula:

$$RI = (PSV - EDV) / PSV$$

Each measurement was performed three times, and the mean value was calculated. An RI increase  $\geq 0.70$  was regarded as a sign of impaired intrarenal hemodynamics.

### Morphological examination and morphometric analysis

Percutaneous graft biopsies were performed under ultrasound guidance (5 MHz probe, 16–18G guiding needle). The biopsy specimens were fixed in 10% neutral formalin, dehydrated, and embedded in paraffin according to the standard protocol. The following staining methods were used:

- hematoxylin and eosin (H&E): general assessment of cellular composition and structure;
- PAS reaction: assessment of basement membranes and tubules;
- Masson's trichrome: visualization of fibrosis;
- Jones stain when required.

Morphological assessment was performed according to the Banff (2017) criteria with determination of the indices t, i, v, g, ptc, ci, ct, cg, ah, and C4d. Morphometric analysis of biopsy specimens was performed in conjunction with ultrasonography. The following parameters were determined:

- cortical layer thickness ( $\mu\text{m}$ );
- diameter of the renal tubules;
- relative interstitial area;
- vascular bed density (per 1  $\text{mm}^2$  of field of view).

### Comprehensive assessment algorithm

1. Immunological verification of compatibility (PRA, DSA, crossmatch).
2. Ultrasound morphometric monitoring (RI, parenchymal thickness, echogenicity).
3. Biopsy in cases of persistent  $RI \geq 0.70$  or a creatinine increase  $> 20\%$ .
4. Comparison of ultrasound and morphological data with calculation of correlation coefficients.

The study structure is presented schematically below:

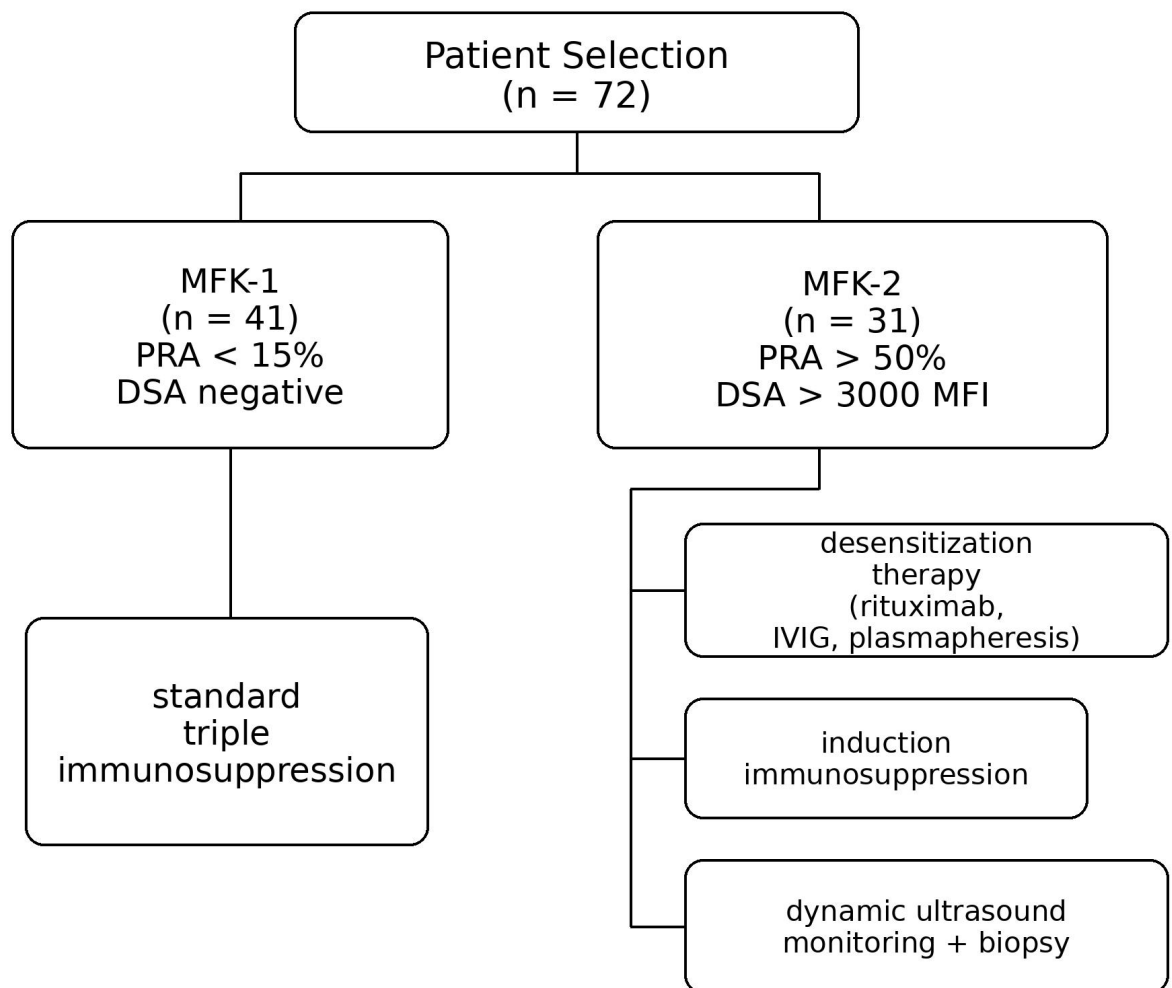


Figure A. Study design and distribution of patients by immunological class

## Results and Discussion

A comprehensive analysis of ultrasound, morphometric, and morphological parameters was carried out in 72 kidney transplant recipients. Comparison of the MFK-1 group (low sensitization) and the MFK-2 group (high sensitization) made it possible to identify pronounced differences in the dynamics of morphometric parameters, vascular indices, and histological signs of graft injury.

### 1. Dynamics of ultrasound parameters

Ultrasound monitoring was performed at standard time points: before transplantation and on postoperative days 1, 7, and 30. The mean resistive index (RI) in MFK-1 patients was  $0.69 \pm 0.05$  on day 1 and decreased to  $0.64 \pm 0.04$  by day 30. In MFK-2 patients, RI was significantly higher at all stages of observation ( $0.74 \pm 0.06 \rightarrow 0.71 \pm 0.05$ ;  $p < 0.05$ ). A gradual decrease in parenchymal thickness was also observed in MFK-2 patients, from  $18.2 \pm 2.1$  mm to  $17.0 \pm 1.7$  mm ( $p < 0.05$ ), which was interpreted as a manifestation of interstitial edema and incipient tubular atrophy.

Table 1. Dynamics of morphometric and hemodynamic graft parameters

Parameter	Time point (days)	MFK-1 (n = 41)	MFK-2 (n = 31)	p
Parenchymal thickness, mm	1	17.8 ± 1.9	18.2 ± 2.1	0.47
	7	18.4 ± 1.8	17.1 ± 1.6	0.03
	30	18.9 ± 2.0	17.0 ± 1.7	0.01
RI	1	0.69 ± 0.05	0.74 ± 0.06	0.04
	7	0.67 ± 0.05	0.72 ± 0.05	0.03
	30	0.64 ± 0.04	0.71 ± 0.05	0.02

An RI  $\geq$  0.70 during the first 7 days after transplantation was observed in 28 patients (38.9%), predominantly in the MFK-2 group (61.3% vs. 22.0% in MFK-1;  $p < 0.01$ ). These patients more often demonstrated a delayed decline in creatinine and ultrasound signs of graft edema.

## 2. Morphological and morphometric biopsy results

Graft biopsy was performed in 27 patients (15 from MFK-1 and 12 from MFK-2). According to the Banff (2017–2022) criteria, minimal changes (t1i1v0) corresponding to ischemic injury predominated in MFK-1. In MFK-2, signs of tubulointerstitial inflammation (t2i2v1) and vascular endotheliitis (v1–v2) predominated. Positive C4d staining ( $> 10\%$  of peritubular capillaries) was found in 9 patients (29.2%), all of whom belonged to the MFK-2 group.

**Table 2. Comparative morphological characteristics of graft biopsy specimens**

Banff index	MFK-1 (n = 15)	MFK-2 (n = 12)	p
t (tubulitis)	1.1 ± 0.5	1.9 ± 0.6	0.02
i (interstitial inflammation)	1.0 ± 0.4	2.0 ± 0.5	< 0.01
v (vasculitis)	0.4 ± 0.2	0.9 ± 0.3	0.03
C4d (positive), %	0	29.2	< 0.01
ci (interstitial fibrosis)	0.8 ± 0.3	1.4 ± 0.5	0.04
ct (tubular atrophy)	0.7 ± 0.4	1.3 ± 0.5	0.03

Morphometric analysis of the biopsy specimens showed that patients in MFK-2 had a larger interstitial area ( $26.3 \pm 4.1\%$  vs.  $18.9 \pm 3.7\%$ ;  $p = 0.01$ ), while the mean parenchymal thickness and vascular density were lower ( $r = -0.56$ ;  $p = 0.02$ ).

### 3. Correlation between ultrasound and morphological parameters

The analysis demonstrated significant relationships between hemodynamic and histological parameters:

**Table 3. Correlation between ultrasound and morphological parameters**

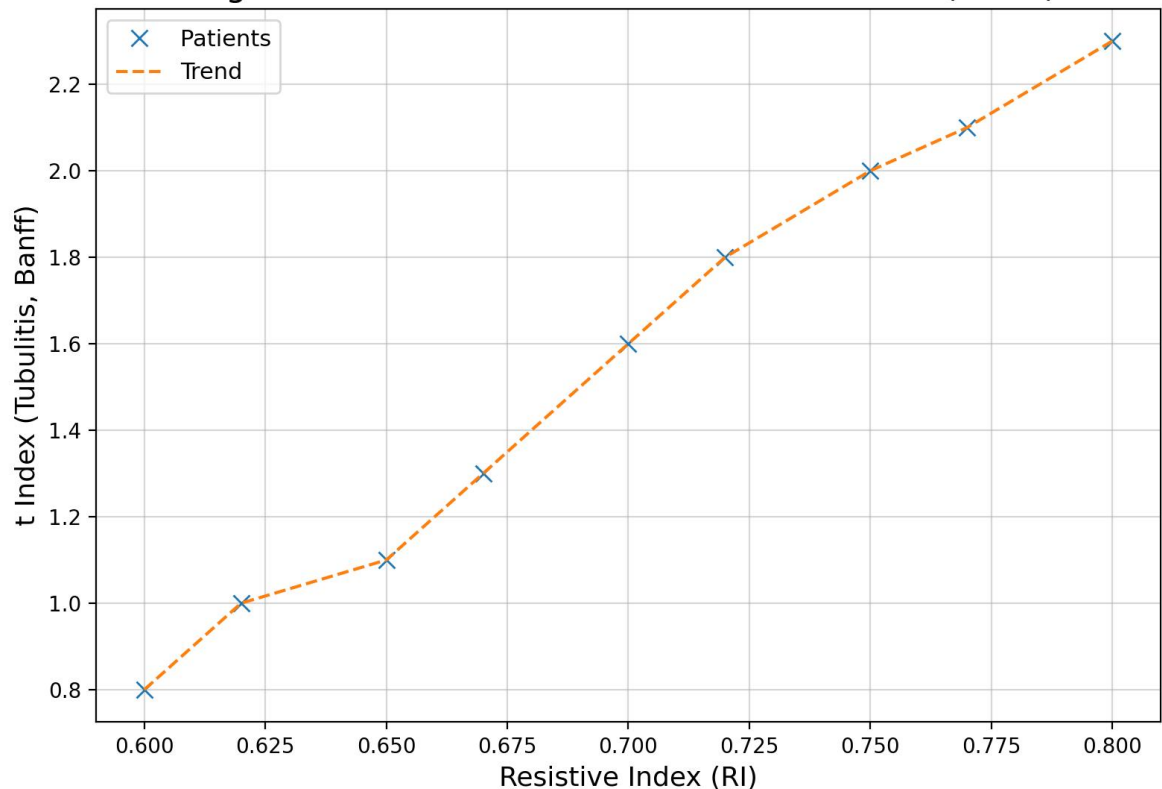
Parameters	r	p
RI ↔ t index	0.68	0.01
RI ↔ i index	0.63	0.02
RI ↔ parenchymal thickness	-0.53	0.02
Parenchymal thickness ↔ ci	-0.49	0.03
Positive C4d ↔ RI ≥ 0.70	0.71	0.01

**Note.** r = Pearson correlation coefficient; p = level of statistical significance.

Pearson correlation analysis (r) with assessment of statistical significance (p) revealed a pronounced direct relationship between an increase in the resistive index (RI) and the severity of tubulitis (t) and interstitial inflammation (i) ( $r = 0.68$  and  $r = 0.63$ , respectively;  $p < 0.05$ ). A moderate inverse relationship was observed between RI and parenchymal thickness ( $r = -0.53$ ;  $p = 0.02$ ), as well as between parenchymal thickness and the severity of interstitial fibrosis (ci) ( $r = -0.49$ ;  $p = 0.03$ ). A positive C4d reaction was significantly more common when  $RI \geq 0.70$  ( $r = 0.71$ ;  $p = 0.01$ ), which indicates a relationship between increased vascular resistance and antibody-mediated graft injury.

The figure below shows a scatter plot demonstrating a direct linear relationship between the resistive index (RI) and the degree of tubulitis (t index) according to the Banff classification. As RI rises above 0.70, the severity of tubulitis progressively increases, indicating a direct association between impaired intrarenal hemodynamics and inflammatory changes in the graft ( $r = 0.68$ ;  $p = 0.01$ ).

Figure 1. Correlation Between RI and t Index (Banff)



*Figure 1. Correlation between RI and the Banff t index*

The obtained data confirm that the combined use of ultrasound and morphological methods makes it possible to detect early signs of immune-mediated and ischemic graft injury.

An  $RI \geq 0.70$  is associated with interstitial-tubular inflammation (t2i2v1) and is frequently accompanied by C4d positivity, indicating a possible antibody-mediated mechanism.

Similar results were reported by Halloran et al. (2020), who demonstrated a high correlation between the molecular profile of rejection and Doppler changes ( $RI > 0.70$ ) [6]. Loupy and Lefaucheur (2018) also noted that the combination of noninvasive (morphometry) and invasive (biopsy, C4d) methods increases diagnostic sensitivity to 90–92% [14–16].

Thus, the integration of ultrasound morphometry, morphological analysis, and immunohistochemical criteria is an optimal tool for evaluating graft function in the early post-transplant period and makes it possible to substantially reduce the need for repeated biopsies.

#### Conclusions

- The integration of morphometric, ultrasound, and morphological methods makes it possible to comprehensively assess the condition of the kidney graft and identify early signs of dysfunction with high diagnostic accuracy.

- An elevated resistive index ( $RI \geq 0.70$ ) is accompanied by increased severity of tubulitis and interstitial inflammation according to the Banff criteria (t2i2v1), indicating a direct relationship between impaired intrarenal hemodynamics and morphological changes in the graft.

- Significant correlations were established between morphometric and morphological parameters: an increased RI was associated with reduced parenchymal thickness ( $r = -0.53$ ;  $p = 0.02$ ) and greater severity of interstitial fibrosis ( $r = -0.49$ ;  $p = 0.03$ ).

• The combined use of ultrasound morphometry, Doppler ultrasonography, and morphological analysis increases the informativeness of kidney graft function monitoring and contributes to early prediction of possible graft dysfunction.

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