

# Раздел III

## СОВРЕМЕННЫЕ АСПЕКТЫ МЕДИЦИНСКОГО ОБРАЗОВАНИЯ

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UCD 616-092.19

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### DIAGNOSTICS OF RENAL ALLOGRAFT DYSFUNCTION: OUR EXPERIENCE IN APPLICATION OF BANFF-07 CLASSIFICATION

#### SUMMARY

Aim of research is practical application of the given classification of a pathology closely related kidneys allotransplantate and revealing the value of a complex of morphological researches in dynamics. Materials and Methods: was performed retrospective descriptive study, the depth of 5 years from January 2012 till February 2016. Sample – solid. Exclusion criteria – cadaver kidney transplantation. The work was conducted in the archive of pathomorphologic laboratory of National Scientific Surgery Center named after A.N.Syzganov. Results and discussion: it was about 48 cases of kidney transplantation. 64,58 % (n-31) cases is related transplantations. According to Banff-07 classification they have been divided into groups by investigated signs. Diagnostic categories of Banff-07 classification renal allograft biopsias was following: the norm – 51,61 % (n-16), antibody associated rejection – 3,22 % (n-1), boundary changes – 9,67 % (n-3), acute rejection – 9,67 % (n-3), chronic nephropathy of transplantate – 25,80 % (n-8), with inherent morphological signs.

Conclusion: future advances in analysis of renal allograft biopsies can already be predicted, and the classification and grading of acute/active rejection will continue to evolve. The significance of specific morphologic findings-including glomerulitis, arteriolitis, and infiltrates with unusual cellular features-for acute and chronic allograft dysfunction and outcome will continue to be investigated. Emphasis in biopsy assessment will shift from diagnosis to prediction of later allograft function and outcome, potentially enabling early intervention. Results of research have shown that lifetime morphological research of kidney transplant biopsies allows to give full representation about dynamics of a current, a stage of pathological process, a qualitative and quantitative estimation.

**Key words:** renal allograft biopsy, related transplantations, renal allograft rejection, lifetime morphological research.

According to the statement of Arthur Cohen, which is one of famous morphologist in the field of nephrology: the kidneys is mysterious and frightening organ for many medical students and young doctors, it is impossible so to probe or see kidneys simply. The anatomy and physiology, seems so difficult and difficult, clinical features of nephritic frustration are indirect and are not easily distinguished, and there are a secret language is necessary, used by nephritic pathologists. The anatomy of kidneys is a difficult and have structures with special type of blood supply by arteries and arterioles by the type

of an ended vessels, If occurs occlusion, the kidney tissue, blood supplied them is exposed to an ischemia, and result of which is necrosis. Almost all grafts have these displays in various degree [1].

One of prominent aspects is clinical diagnostics and timeliness of carrying out puncture biopsy for recognition and probably, prevention of development of certain symptomatics. At the elementary level, pathologists should see in kidneys biopates, at least one of these infringements connected with nephritic insufficiency, proteinuria and hematuria. Biopsy of kidney allograft should become ordinary

as only it works properly, in an equivalent to nephritic insufficiency [1].

Kidney transplant is the most common type of organ transplant and one of the most effective treatments for patients with end-stage renal disease. Management of patients with kidney transplant is a difficult clinical challenge and is often associated with a number of problems. Renal biopsy remains the gold standard by which essential diagnostic and prognostic information is obtained after kidney transplantation [1]. Biopsy methodologies have been devised to assess the acceptability of an organ before transplantation and to assess and predict renal allograft performance after implantation [2, 3].

Optimal method of treatment of patients with a chronic renal failure is heterotopic allotransplantation of a donor kidney and the result from such treatment directly depends on quality of receiving and the quality of the received donor kidney [1, 2]. Living and cadaveric donors can be a source of donor kidneys for transplantation to recipients. In case of an organ donorship from the living donor quality of the received donor kidneys and respectively result of transplantation are more optimal in comparison with transplantation when receiving a donor kidney from the cadaver, such difference of results is explained by various duration and intensity of influence of the damaging factors which are followed throughout time as at their receiving, preservation, and at their transplantation. Intensity of ischemic damage of a parenchyma of donor kidneys can have various expression, and its expression depends on aggression of a vasoconstriction of the dosage of cardiotoxic support depending on level, which is directly dictated by an initial condition of a hemodynamics of a donor's organism [3].

Standardization of renal allograft biopsy interpretation and reporting is necessary to guide therapy in transplant patients and to establish an objective end point for clinical trials of new antirejection agents [4]. The Banff-07 Classification of Renal Allograft Pathology is an international scheme recently developed to fill this needs. The classification, which originated in a meeting held in Banff, Canada on August 2 to 4, 1991, was published in 1993, has been clinically validated in numerous studies, and is now widely used by center pathologists and in large international trials of immunosuppressive agents. Subsequent meetings have been held in Banff every two years to refine the classification. For National Institutes of Health clinical trials, a modification of the Banff grading system, the

Collaborative Clinical Trials in Transplantation classification was developed; this classification and a clinical validation study were published in late 2013 [5, 6].

There are two theories of the rejection development: –cellular theory (author is Peter Medawar): the process of graft rejection is immunologically dependent. The cells (easy to see on histological slides) were recognized and identified as a cause of tissue damage; humoral theory (author is Paul Terasaki): evidence that also in later periods after transplantation antibodies must participate in graft damage. Renal transplant biopsies uses for diagnosis of acute changes in renal function (acute rejection, drug toxic effects, infections), diagnosis of transplant-related changes in pancreas transplant, diagnosis of declining renal function over prolonged time (chronic rejection, recurrent diseases) and etc.

Approaches to renal transplant biopsy interpretation includes next positions: evaluation of all tissue components (glomeruli, interstitium, tubules, arteries and arterioles) and determination if changes are acute and/or chronic. There are standards for adequate renal biopsy: the cortex mandatory must present, at least 7 glomeruli, at least 2 arterial cross sections, not exclusively subcapsular, two cores are better than one for greater sensitivity. Tissue processing of renal transplant biopsies: light microscopy, immunofluorescence, immunoperoxidase C4d (may be limited) and electron microscopy if it is indicated.

The Banff classification characterizes five categories of renal allograft pathology: antibody-mediated rejection; suspicious of acute rejection; acute rejection; chronic sclerosing allograft nephropathy; and other – changes not considered due to rejection [7].

**Aim of research** is practical application of the given classification of a pathology closely related kidneys allotransplant and revealing the value of a complex of morphological researches in dynamics.

**Materials and Methods:** was performed retrospective descriptive study, the depth of 5 years from January 2012 till February 2016. Sample – solid. Exclusion criteria – cadaver kidney transplantation. The work was conducted in the archive of pathomorphologic laboratory of National Scientific Surgery Center named after AN Syzganov. The age of the donor ranged from 17 till 57 years.

Antiischemic actions provided by pharmacological protection of an organ which will be extract-

ed according to the general demands of the maintaining the potential donor. At conditioning the hemodynamics at the potential organ donor was corrected by the cardiotoxic medicine «Dofaminum» and monitoring the blood pressure level demanded various dosage of cardiotoxic. It is known that Dofaminum in a dose to 10 mkg/kg/min stimulates post-synaptic beta1-adrenoreceptors that causes positive inotropic effect and augmentation of minute volume of a blood, and also provides intensifying of a circulation in kidneys, the glomerular filtration and is stimulates removing of sodium ions and a diuresis. In high doses – more than 10 mkg/kg/min. or more prevail stimulation of alfa1-adrenoreceptors, there is a narrowing of renal vessels that in turn reduces earlier enlarged renal blood flow and a diuresis. For definition of a target dosage of Dofaminum counted intensity of its introduction to an organism of the donor, thus calculation was made on substance of the corresponding 1 kg of body weight of the donor and on rate of his introduction.

Extraction of donor kidneys at potential donors was made according to the standard scheme J.R.Ackermann. However the sequence of composition of solutions for primary perfusion of a kidney after their extraction was various. Options of sequences of solutions were such: either Custodiol solution, or a normal saline solution the incorporating Heparin divorced in the ratio 1:100 that is 1,0 ml of Solution of the Heparin on 100,0 ml of NaCl of 0,9 %, temperature of solution 14-16 C and after that further washing of kidneys with Custodiol.

Hypothermal pharmacocooling conservation of a donor kidney after its extraction from the potential organ donor, provides its initial washing from a blood the preserving solutions of relatives on ionic structure to intracellular liquid – Collins's solutions, Saks, SIUT and AO-2, Ross and Marshall, Bredshayder. Despite the above-mentioned actions, measures referred on conservation of a donor organ have the specific features as the task of an organ washing from a blood lays down on the conserving solutions having excess viscosity. As solution with the smallest viscosity in the studied work to conservation of a donor kidney and washing of a lumen of vessels of the finest order from a blood and bloody clots was applied the sequence of solutions – the Heparin divorced in a normal saline solution in the ratio of 1:100 that is 1,0 ml of Solution of the Heparin on 100,0 ml of NaCl of 0,9% in the temperature range 14-16 C and after that further washing of kidneys Custodiol solution.

The main clinical criteria of quality of the carried-out actions at stages of extraction the donor kidneys referred on their «protection» namely at a stage of conditioning and a stage of their conservation were the beginning of excretory function after immediate transplantation and further conservation of these parameters with long-term adequate functioning. The condition of a nephrosites, convoluted tubules, vascular glomulus, degree of a dystrophia and a condition of a canaliculus of a kidney, existence of contents and fullness of a vascular glomulus of a kidney were the main morphological data of quality of extraction of donor kidneys, these data were regarded at 400 and 1000 multiple augmentation when coloring by a hematoxylin and eosine and the basal fuchsine of an azur 2 methylene blue terms of opportunity to trace such data were at the time of conservation of a donor kidney, for the 5th and 21st days after transplantation of this kidney.

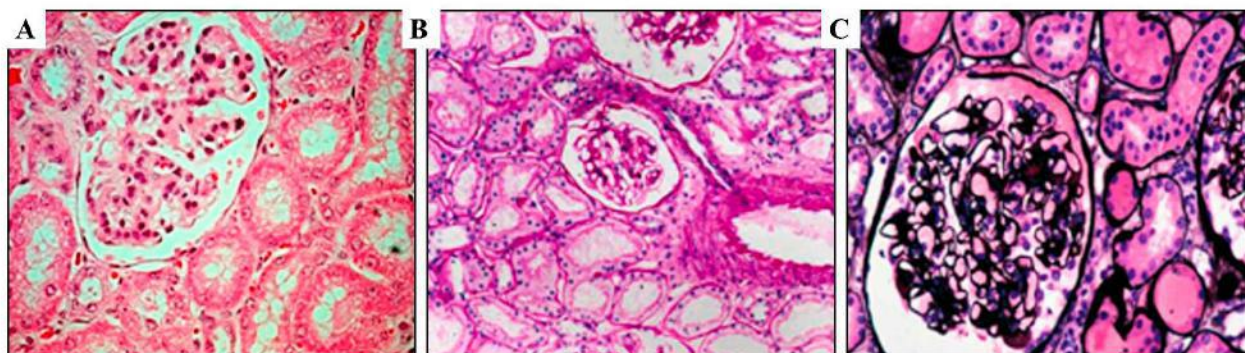
The pathogenesis of acute antibody-mediated rejection includes: subsequent complement activation and adhesion of cells in result of necrosis of endothelial-cells, produced by platelet deposition and coagulation. It is denotes polymorphonuclear cells. Usually mononuclear cells adherence to the endothelium of the glomeruli is produced, which process is accompanied by C4d deposition in the glomeruli and peritubular capillaries, in the peritubular capillaries between ghost outlines of the tubules.

Chronic allograft rejection – is immune injury of the graft, developed due to a failure to maintain sufficient immunosuppression which controlled by residual antigraft antibodies and lymphocytes. Which is include a progressive decline in renal function, infiltration and invasion of the renal parenchyma by T-cells, and persistent infiltration of the interstitial space by T-cells and macrophages, proliferation of smooth-muscles and hyperplasia vessel walls, with formation a neointima; focal destruction and necrosis of internal elastic lamina, result of which is total vascular occlusion.

Acute antibody-mediated rejection is divided on: acute antibody-mediated rejection (type I: expression of positive C4d, minimal signs of inflammation, type II: expression of positive C4d, leukocytic infiltration, type III: expression of positive C4d, transmural arteritis), chronic active antibody-mediated rejection.

The pathogenesis of second theory mainly includes processing of endogenous and exogenous antigens by class I and class II of major histocompatibility complex pathways. The first one is endog-





**Figure 1.** **A.** The glomerulus is presented by the afferent and efferent arterioles forming «Malpighie's» body. Capsule's walls are thin. In canaliculi nephrothelium is high cylindrical, in cytoplasm are visible small vacuole (H&E stain). **B.** Low magnification of cortex with portions of two glomeruli, tubules, and interstitium and interlobular artery with arteriolar branch (periodic acid-Schiff (PAS) stain). **C.** Normal glomerulus with surrounding normal tubules and interstitium (Jones silver stain)

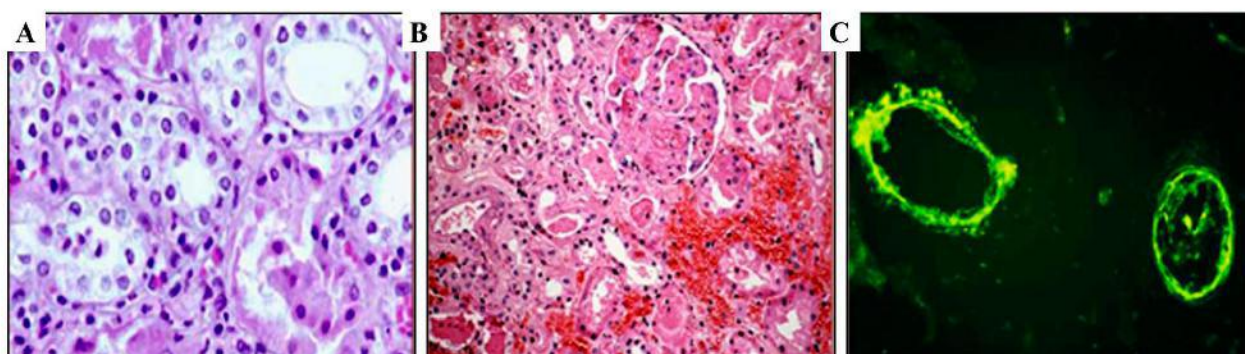
enous antigens are digested by proteosomes into peptides and are leads into class I major histocompatibility complex. Exogenous antigens are degraded by endosomes and are leads into class II major histocompatibility complex. Assembly of the major histocompatibility complex within the cell's endoplasmic reticulum leads for its transport through the Golgi apparatus and provides its expression on the cell surface in connection with peptide, where the major histocompatibility complex – peptide complex connects with CD8<sup>+</sup> CD4<sup>+</sup> T lymphocytes. Next stage is activation and differentiation of T-lymphocytes from dendritic-cell maturation till activation and proliferation of effector T-cells with its active products.

T-cell rejection is divided on: acute T-cell rejection (Type IA: moderate intimal arteriitis; severe intimal arteriitis and moderate tubulitis; type IB: severe tubulitis. Type IIA: mild-moderate intimal ar-

teritis. Type IIB: severe intimal arteritis. Type III: transmural arteritis) Chronic active T-cell rejection.

**Results and discussion:** it was about 48 cases. 64,58 % (n-31) cases is related transplantations of a kidney. According to Banff-07 classification they have been divided into groups by investigated signs. Diagnostic categories of Banff-07 classification renal allograft biopsias was following: the norm- 51,61 % (n-16), antibody associated rejection – 3,22 % (n-1), boundary changes – 9,67 % (n-3), acute rejection – 9,67 % (n-3), chronic nephropathy of transplantate – 25,80 % (n-8), with inherent morphological signs.

The antibody-associated rejection, presumably mediated by antibodies is typically characterized by the accumulation of polymorphonuclear leukocytes in glomerular and peritubular capillaries in the early period after transplantation, followed by damage to the endothelium and capillary thrombosis (fig. 2).



**Figure 2.** **A.** Humoral rejection, type II. Neutrophils are in peritubular capillaries and glomeruli; interstitial hemorrhage is present. Anti-donor class II antibodies were demonstrable (H&E stain). **B.** Hyperacute rejection. The cortex shows diffuse hemorrhage and neutrophils in peritubular capillaries with prominent glomerular thrombi 1 day after transplantation (H&E stain). **C.** Hyperacute rejection due to ABO incompatibility (Immunofluorescence stain shows immunoglobulin M (IgM) in arterial walls)



However, the histological diagnosis of acute ABMR is still problematic, especially in cases with few outward symptoms [8, 9]. The studies using immunofluorescence to detect immunoglobulins (IgG, IgM and IgA), complement factors (C3, C4 and C1q) and fibrin are not very accurate [10].

Boundary changes – «suspicion`» on acute rejection [11]. This category is used when there is no intimal arteriitis, but the presence of foci of weak tubulitis.

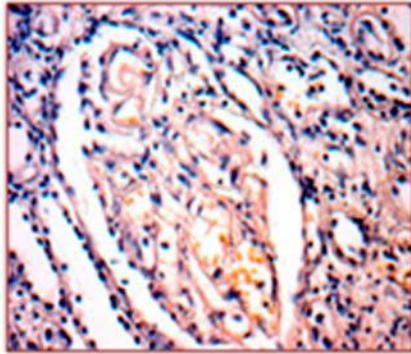


Figure 3. There is an edema in a glomerulus, full-blooded vessels. Canalliculi lumens are stretched, are marked by mononuclear cells: 1-3 in cross-section (weak tubulitis) (H&E stain)

In grafts undergoing acute rejection, T lymphocytes infiltrate and proliferate within the interstitial space, whence they invade renal tubules, causing tubulitis. Invading CD8 T lymphocytes, which have immunologic specificity for the allograft, cross the basement membrane of the tubule, where they proliferate and induce apoptosis of tubular cells [12]. Sublethally injured tubular cells can also transform from their native epithelial phenotype into primitive

mesenchymal myofibroblasts, promoting interstitial fibrosis [13, 14]. Necrosis of tubular epithelial cells and basement membrane rupture cause urinary leakage, graft dysfunction, and progressive tubular atrophy [15, 16]. The histologic characteristics of vascular rejection (also termed arteritis or endarteritis) include the infiltration of vessels by mononuclear cells, endothelial-cell apoptosis, and the synthesis of matrix proteins and collagens by intimal myofibroblasts.

Chronic allograft rejection – ongoing immune injury to the graft – is due to a failure to maintain sufficient immunosuppression to control residual antigraft lymphocytes or antibodies [13]. Its features include a progressive decline in renal function, invasion of the renal parenchyma by T cells, and persistent infiltration of the interstitium by T cells and macrophages [17]. Occasionally, one also sees smooth-muscle proliferation and hyperplasia in vessels, forming a neointima; focal destruction of internal elastic lamina; and finally, vascular occlusion.

**Conclusion:** future advances in analysis of renal allograft biopsies can already be predicted, and the classification and grading of acute/active rejection will continue to evolve. The significance of specific morphologic findings-including glomerulitis, arteriolitis, and infiltrates with unusual cellular features-for acute and chronic allograft function and outcome will continue to be investigated. Emphasis in biopsy assessment will shift from diagnosis to prediction of later allograft function and outcome, potentially enabling early intervention. Results of research have shown that lifetime morphological research of kidney transplant biopsies allows to give full representation about dynamics of a current, a stage of pathological process, a qualitative and quantitative estimation.

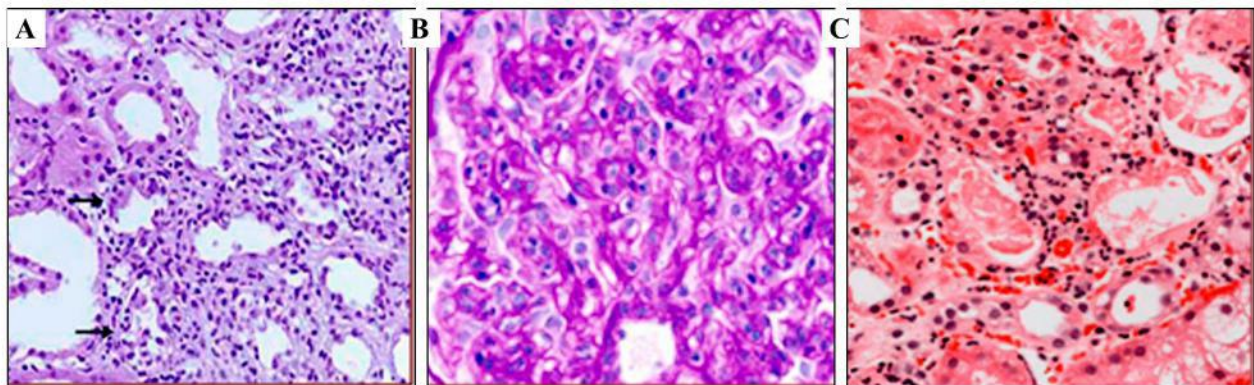


Figure 4. **A.** Acute cellular rejection (type I). A diffuse mononuclear infiltrate is present with edema and tubulitis (periodic acid-Schiff (PAS) stain). **B.** Acute allograft glomerulopathy. This pattern of glomerular injury can occur as a feature of cellular or humoral rejection (PAS stain). **C.** There was no graft function. Tubules show severe acute damage, with a heavy infiltrate of neutrophils in intertubular capillaries (H&E stain)

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## ТҮЙІН

Жақын туыстық донор бүйрегінің аллотрансплантатының патологиясы кезінде берілген классификацияны тәжірибелік тұрғыда қолдану және клиникалық процесс динамикасын даморфологиялық зерттеулердің жиынтығының мағынасын анықтау. 5 жылауқымындағы, 2012 ж. қаңтарынан 2016 ж. ақпан айын қоса алғандағы, ретроспективті сипаттамалық зерттеулер. Сұрыптау – тұтас. Ерекше критеріі – бүйректің кадаверлік трансплантациясы. Жұмыс А.Н.Сызғанов атындағы ұлттық ғылыми хирургия орталығының патоморфология зертханасының мұрағатында жүргізілді. Бүйрек трансплантациясының 48 жағдайы қарастырылды. 64,58 % (n-31) жағдай туыстық трансплантация. Banff-07 классификациясына байланысты олар зерттеу белгілеріне байланысты топтарға бөлінді. Banff-07 классификациясына байланысты бүйрек аллотрансплантациясының биопаттары келесі морфологиялық ерекшеліктерімен көрінді: қалыпты – 51,61 % (n-16), антидене-ассоциирленген қабылдамаушылық – 3,22 % (n-1), шеткері өзгерістер – 9,67 % (n-3), жедел қабылдамаушылық – 9,67 % (n-3), транспланттың созылмалы нефропатиясы – 25,80 % (n-8).

Бүйрек аллотранспланттарының биопсияларын талдау аймағында келешектегі жетістіктері алдын ала болжамдалып, жедел/ белсенді қабылдамаушылықтың жіктемесі мен градириленуі жақсарады. Нақты морфологиялық көріністерінің маңыздылығы, жедел және созылмалы аллотранспланттарының бұзылы-



сы, соның ішінде, гломерулонефрит, артериолит және әдеттен тыс жасушалық көрінісі бар инфильтраттары, олардың нәтижесі зерттелетін болады. Ерте қол сұғу мақсатында, аллотрансплантант қызметін анықтау үшін диагноз қоюдан бастап, болжамы мен нәтижесін бағалауға ерекше назар аударылады. Зерттеу нәтижесі бойынша, бүйрек трансплантантының биопсиясын тіршілік жағдайында морфологиялық зерттеу, оның динамикалық ағымын, патологиялық үрдіс дәрежесін, өзгерістердің сандық және сапалық қасиетін толық көрсетеді.

**Түйінді сөздер:** бүйрек трансплантантының биопсиясы, туыстық трансплантация, бүйрек трансплантантының криздік қабылдамаушылығы, тіршілік жағдайындағы морфологиялық диагностика.

#### АННОТАЦИЯ

Практическое применение данной классификации при патологии аллотрансплантатапочек близкородственного донора и выявление значения комплекса морфологических исследований в динамике клинического процесса. Ретроспективное описательное исследование, глубиной 5 лет в период с января 2012 г. по февраль 2016 г. включительно. Выборка – сплошная. Критерии исключения – кадаверная трансплантация почки. Работа проводилась в архиве лаборатории патоморфологии национального научного центра хирургии им. А.Н.Сызганова. 48 случаев трансплантации почки. 64,58 % (n-31) случаев родственной трансплантации. Согласно Banff-07 классификации они были разделены на группы по исследуемым признакам. Диагностические категории Banff-07 классификации биоптатоваллотрансплантации почки с присущими морфологическими признаками были следующими: норма – 51,61 % (n-16), антитело-ассоциированное отторжение – 3,22 % (n-1), пограничные изменения – 9,67 % (n-3), острое отторжение – 9,67 % (n-3), хроническая нефропатия трансплантата – 25,80 % (n-8).

Будущие достижения в области анализа биопсий почечных аллотрансплантатов может быть предсказаны, а также классификация и градирование острого / активного отторжения будет продолжать улучшаться. Значимость конкретных морфологических признаков, в том числе гломерулит, артериолит и инфильтраты с необычными клеточные признаками, при острых и хронических дисфункциях аллотрансплантата, а также исход будут исследованы. Особое внимание при оценке биопсии будет сдвигаться от постановки диагноза до прогнозирования поздней функции аллотрансплантата и исход, потенциально позволяя раннее вмешательство. Результаты исследования показали, что прижизненное морфологическое исследование биопсий почечного трансплантата позволяет дать полное представление о динамике течения, стадии патологического процесса, качественную и количественную оценку изменений.

**Ключевые слова:** биопсия почечного трансплантата, родственная трансплантация, кризы отторжения почечного трансплантата, прижизненная морфологическая диагностика.