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Evaluation of the level of alloantibodies and selected cytokines in kidney recipients

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Abstract

Introduction and Objective. Anti-HLA antibodies, especially anti-DSA, are believed to be potentially involved in acute and chronic rejection. New techniques, e.g. flow fluorimetry, can be an excellent supplement to the lymphocytotoxic tests currently used in pre-transplantation practice, and become a tool for monitoring post-transplant immunisation. The aim of the study was to assess the level of cytokines and alloimmunisation in kidney recipients, with the histopathological evaluation of the transplanted kidney biopsy.

Materials and method. The study included 62 graft recipients six months after transplant. The level of anti-HLA antibodies was assessed using the x-Map Luminex technique, and the level of cytokines (IFN-γ, IL-4, IL-10, IL-17) was determined using the ELISA test.

Results. Histopathological analysis showed that more than 45.2% of patients had changes in the biopsy material. Analysis of the level of alloimmunisation showed that in over 75.8%, the presence was detected of anti-HLA IgM class I antibodies, while anti-HLA IgM class II antibodies were found less often –17.8%. The level of anti-HLA IgG antibodies, depending on the type of the assessed class, respectively, was: class I – 43.5% and class II – 50%. More than half of the subjects also had anti-MICA antibodies. The level of analysed cytokines was low.

Conclusions. The results indicate significant alloimmunisation of kidney recipients, although they do not answer the question whether these are antibodies that appeared *de novo* after transplantation, and whether anti-DSA antibodies were present among them. For this purpose, the diagnostics should be expanded to include anti-HLA monitoring in the pre- and post-transplant period, using screening tests and tests to identify their specificity. The protocol biopsy and examination of level cytokine can also be a helpful tool in post-transplant diagnosis.

Key words

cytokines, graft, anti-HLA, anti-MICA, bioptats

INTRODUCTION

Currently, CRF (Chronic Renal Failure) is included on the list of civilisation diseases. It requires renal replacement therapy, which takes the form of dialysis or kidney transplantation. A successful and optimised transplant restores several excretory and endocrine functions performed by own, healthy kidneys. Therefore, when selecting a kidney donor-recipient system, it is crucial to evaluate both immunological and non-immunological parameters. One of the most significant factors, apart from determining the HLA (Human Leukocyte Antigen) compatibility, affecting the optimisation of the transplant, is the assessment and monitoring of the level of alloimmunisation in the recipient of the organ [1–3]. The chances of optimising the survival of the received graft are much lower in the group of immunised patients, compared with patients in whom the presence of alloantibodies is not observed. Kidney transplantation in an immunised recipient is associated with a higher risk of AMR (Antibody-Mediated Rejection), and even lacking AMR, graft survival in this group is significantly worse. Previously, it was believed that chronic rejection was primarily related to toxic organ damage from immunosuppressive drugs. Recent multicentre studies, however, indicate that humoral reaction is the leading cause of chronic rejection [4–6]. Even small amounts of donor-specific antibodies (anti-DSA) indicate the presence of memory cells, the clonal proliferation of which can quickly damage the graft. It should be borne in mind that the appearance of *de novo* anti-HLA antibodies, among which anti-DSA antibodies may be present, is also conducive to the activation of the rejection process [5–8]. Therefore, more sensitive methods should enable precise organ selection based on detecting harmful antibodies. Their quick identification would make it possible to spee- up the decision to modify treatment, especially in the case of subclinical processes [9,10].

OBJECTIVE

The aim of the study was to assess the function of the graft in kidney recipients, based on assessment of the protocol biopsy and parallel assessment of the level of alloantibodies and selected cytokines.

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MATERIALS AND METHOD

Test Group. The study included 62 kidney transplant recipients under the constant care of the Department of Nephrology, Transplantology and Internal Diseases of the Independent Public Clinical Hospital No. 2 in Szczecin, Poland. The organ transplants were performed in the period 2009–2018. To assess the function of the graft, a protocol biopsy was performed in the sixth month after transplant. The biopsy assessment was based on the 2009 Banff classification (Banff scale) [11,12], which includes categories of renal transplant lesions and types of rejection. The category of changes of varying severity includes, e.g. borderline lesions (BC), acute T-cell rejection (AR-T), acute humoral rejection (AHR), stromal fibrosis and tubular atrophy (TA/IF), nephrotoxicity of calcineurin inhibitors (CNI) and features of chronic nephropathy (CAN). Simultaneously with the biopsy assessment, an analysis of immunological parameters was performed, based on determination of the serum level of anti-HLA class I and II antibodies and anti-endothelial antibodies (anti-MICA) in two classes (IgM and IgG), and the level of selected cytokines (IFN-γ, IL-4, IL-10, IL-17).

The study was conducted following the guidelines of the Declaration of Helsinki and approved by the Ethics Committee of the Pomeranian Medical University in Szczecin (Consent No. KB-0012/114/12)

LAB Screen Mixed Class I and II. Quantifying alloantibodies in the examined patients was carried out using the LABScreen Mixed Class I and II test (One Lambda, CA, USA) in two classes – IgM and IgG, according to the manufacturer's recommendations. Antibody values are given in MFI (Mean Fluorescent Intensity) units.

Enzyme-Linked Immunosorbent Assay (ELISA). Quantifying IFN-γ, IL-4, IL-10, and IL-17 levels in patients enrolled in the study was performed using the Quantikine ELISA (R&D System, Minneapolis, MN, USA), according to the manufacturer's instructions. Serum samples were tested in native form. Before starting the assays, all samples were centrifuged (4 min, 11,000 rpm) to remove the cellular fraction. IL-4, IL-10, and IL-17 concentrations were calculated based on a standard 8-point curve, and in the case of IFN-γ, a 7-point curve. The plates were read using a BioTek ELx800 analyser (Boston Industries, Walpole, MA, USA) at a wavelength of 450/620 nm.

Statistical Analysis. The statistical significance of the results was determined using a one-way ANOVA test. The level of statistical significance was $p<0.05$. Statistical analysis was performed using GraphPad Prism 5.02 (GraphPad Software Inc., San Diego, CA, USA).

RESULTS

In the case of 62 biopsies performed in the study group, 34 gave a negative result – during their assessment, no histopathological changes were found, e.g. for features of acute rejection (AR-0). On the other hand, in 28 biopsies, according to the Banff scale, the appearance of histopathological changes was observed: in 18 biopsies, changes were found in favour of chronic nephropathy of mild or severe severity (CAN; TA/IF), including 12 cases related to nephrotoxic effects calcineurin inhibitors (CINs); borderline lesions (BC) were found in 4 biopsies; in 3 changes indicating acute T-cell rejection of the graft (AR-T), and in 3 features of acute humoral rejection (AHR).

The assessment of the level of anti-HLA IgM alloantibodies showed the presence of anti-HLA class I antibodies in the serum of 47 subjects, whose MFI ranged from 120.0 – 1820.0 (mean = 568.72); anti-HLA class II antibodies in 7 subjects in the MFI range of 100.0 – 2070.0 MFI (mean = 957.14), and anti-MICA in 22 subjects in the range of MFI 180.0 – 3510.0 (mean = 1526.36) (Fig. 1).

The level of alloimmunisation regarding anti-HLA IgG antibodies showed the presence of anti-HLA class I antibodies in 27 subjects, MFI range: 100.0 – 11740.0 (mean = 2924.44); anti-HLA class II antibodies in 31 subjects, MFI range: 100.0 – 10450.0 (mean = 2266.45) and anti-MICA in 24 subjects, MFI range: 100.0 – 1570.0 (mean = 368.33) (Fig. 1).

In the tested sera, in only 12 patients, IL-4 was detected in the concentration range of 27.12 – 807.95 pg/ml (average concentration = 308.30 pg/ml); in a few patients, IL-10 (patients 7 and 35) and IFN-γ (patients 7 and 15). IL-17 was not detected in any plasma samples isolated from patients (Tab. 1).

No statistical significance was shown between the assessed immunological parameters, i.e. measurable cytokines and anti-HLA class I and II IgM/IgG and anti-MICA IgM/IgG alloantibodies obtained in the group of patients with a negative biopsy result, and patients with a positive result.

Detailed results of the protocol biopsy and the analysed immunological parameters are presented in Tab. 1.

DISCUSSION

Despite the enormous progress that has been made in terms of selection criteria for transplantation in the donor-recipient system and the use of immunosuppressive drugs, rejection is still one of the causes of kidney loss. Particularly at risk of this complication are immunised recipients who, to a greater extent than non-immunised recipients, are exposed to the risk of rejection associated with the activation of humoral immunological factors, i.e. specific anti-HLA antibodies and complement activation [5, 6, 13, 14]. Therefore, significant factors associated with graft rejection include HLA antigen mismatch in the donor-recipient system, and prior sensitisation of potential recipients. Alloimmunisation of recipients most often occurs during organ transfusion, transplantation, and re-transplantation. Furthermore, in the case of female recipients, past miscarriages and pregnancies increase the chance of this process. Other factors include infections preceding the transplant, during which molecular mimicry occurs, i.e. the similarity of the pathogen's antigens and the transplanted organ's antigens. Re-infection or reactivation of the pathogen causes a parallel host response against the infecting agent and the graft antigens [15].

Kidney transplantation in an immunised recipient is therefore at higher risk of AMR, and even in its absence, graft survival in this group is significantly worse, which may be associated with chronic rejection of the organ dependent on antibodies. Even small amounts of anti-DSA antibodies indicate the presence of memory cells, whose clonal proliferation can quickly damage the graft [5–8].

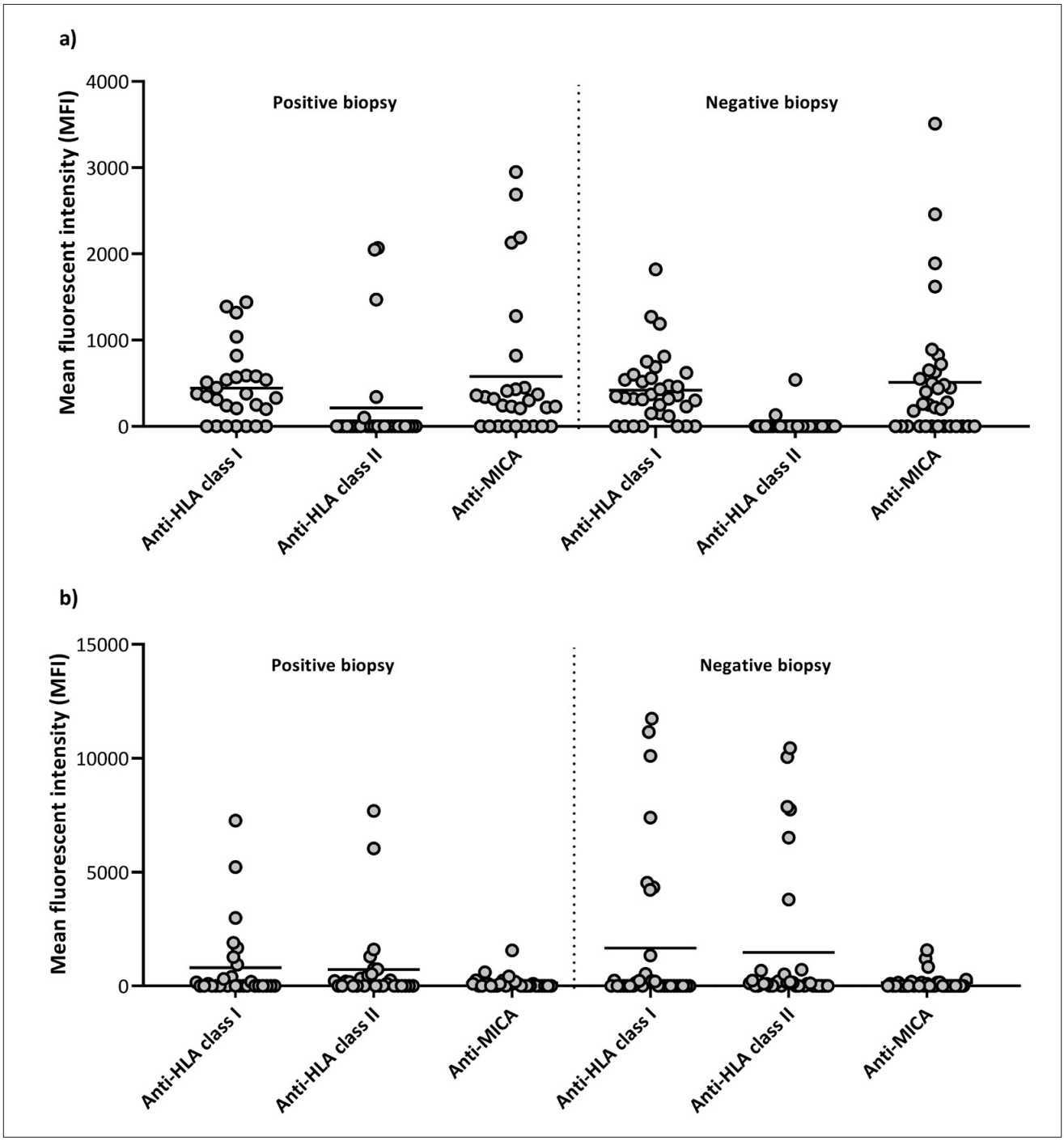

Figure 1. Determination of the level of anti-HLA class I and II antibodies and anti-MICA IgM (a) and IgG (b) antibodies in the serum of biopsy-positive kidney recipients compared to negative recipients

In connection with the above, it is crucial to determine the immunological status of the kidney recipient, which is effortlessly alloimmunised, both in the peri- and post-transplantation period. However, despite the generally used common name for immunisation assessment, which is abbreviated to PRA, it hides various diagnostic methods with significantly different sensitivity and specificity, which create difficulties in interpreting the results. Moreover, the guidelines for performing them in waiting patients vary [9, 10].

Depending on the recommendations in individual countries, various methods are used, starting from the biological method PRA-CDC (Panel Reactive Antibodies-Complement Dependent Cytotoxicity) performed in the potential recipient from the moment of entering the transplant waiting list until the procedure is performed, through tests with higher resolution, such as SPA (Solid Phase Assay) using a flow fluorimeter (Luminex), flow cytometer or ELISA technique, allowing to identify the specificity of anti-HLA antibodies and differentiate their classes [2, 8, 16, 17]. These tests should include assessing antibodies in the group of potential recipients and monitoring the humoral response in transplant patients. Determination of anti-HLA specificity before transplantation allows for calculating the virtual PRA, enabling precise donor selection, even for highly

Table 1. Detailed results of the protocol biopsy and the analysed immunological parameters in kidney recipients

Patient number	Biopsy date	Bioptats		Cytokines (pg/ml)				Anti-HLA IgM (MFI)			Anti-HLA IgG (MFI)		
		Interpretation	Results	IL-17	IL-4	IL-10	IFN-γ	Class I	Class II	MICA	Class I	Class II	MICA
1.	13-09-2009	POS	CAN-D	0	0	0	0	590.0	0.0	370.0	0.0	0.0	0.0
2.	26-10-2009	NEG	AR-0, CAN-0	0	296.46	0	0	0.0	0.0	1890.0	0.0	120.0	100.0
3.	30-11-2009	POS	BC	0	0	0	0	540.0	340.0	0.0	7260.0	7690.0	0.0
4.	12-09-2009	POS	CAN-M, CNI-M	0	0	0	0	1390.0	0.0	0.0	0.0	220.0	0.0
5.	25-03-2010	POS	CAN-M, CNI-M	0	328.83	0	0	0.0	0.0	2130.0	930.0	1290.0	240.0
6.	07-04-2010	POS	BC	0	0	0	0	450.0	0.0	0.0	400.0	250.0	0.0
7.	11-05-2010	NEG	AR-0, CAN-0	0	65.59	41.95	15.26	470.0	0.0	1620.0	0.0	100.0	150.0
8.	08-07-2010	POS	AHR-IIA	0	805.95	0	0	580.0	0.0	220.0	1670.0	740.0	250.0
9.	29-09-2010	POS	TA/IF-M, CNI-S	0	0	0	0	1320.0	0.0	450.0	0.0	0.0	100.0
10.	08-12-2010	POS	TA/IF-M	0	0	0	0	540.0	0.0	430.0	100.0	0.0	0.0
11.	12-01-2011	POS	AR-IA, CNI-M/S, TA/IF-S	0	0	0	0	820.0	0.0	820.0	0.0	330.0	1560.0
12.	24-02-2011	POS	BC, CAN-M. ATN-D, CNI-S	0	0	0	0	0.0	1470.0	2950.0	0.0	100.0	100.0
13.	25-02-2011	POS	CAN-M	0	0	0	0	250.0	2070.0	240.0	1900.0	460.0	0.0
14.	04-03-2011	POS	CAN-S, CNI-S	0	0	0	0	0.0	0.0	410.0	0.0	0.0	100.0
15.	01-04-2011	POS	AR –II, CAN- M	0	0	0	67.41	1040.0	0.0	0.0	0.0	0.0	100.0
16.	13-05-2011	POS	AHR-IIA	0	209.99	0	0	570.0	0.0	0.0	0.0	730.0	600.0
17.	29-08-2011	NEG	AR-0	0	0	0	0	690.0	0.0	0.0	190.0	0.0	0.0
18.	09-13-2011	NEG	AR-0	0	0	0	0	370.0	0.0	250.0	0.0	0.0	1200.0
19.	14-09-2011	POS	TA/IF-M	0	0	0	0	330.0	0.0	0.0	0.0	0.0	0.0
20.	27-09-2011	POS	CNI-M, IF-D	0	0	0	0	0.0	0.0	300.0	190.0	0.0	0.0
21.	28-10-2011	POS	CNI-M, TA/IF-M	0	0	0	0	510.0	0.0	1280.0	0.0	0.0	0.0
22.	07-12-2011	NEG	AR-0	0	0	0	0	540.0	0.0	2460.0	10100.0	710.0	0.0
23.	13-12-2011	POS	CNI-M	0	0	0	0	0.0	0.0	340.0	5220.0	200.0	0.0
24.	05-03-2012	NEG	AR-0	0	402.12	0	0	150.0	130.0	500.0	1340.0	0.0	0.0
25.	07-03-2012	NEG	AR-0	0	0	0	0	1820.0	0.0	450.0	0.0	0.0	150.0
26.	23-03-2012	POS	TA/IF-M	0	0	0	0	200.0	0.0	360.0	160.0	0.0	0.0
27.	30-03-2012	NEG	AR-0	0	0	0	0	320.0	540.0	480.0	0.0	0.0	0.0
28.	13-06-2012	POS	CNI-S	0	0	0	0	380.0	0.0	230.0	2990.0	1600.0	0.0
29.	16-08-2012	POS	BC	0	0	0	0	310.0	0.0	210.0	0.0	0.0	0.0
30.	24-08-2012	NEG	AR-0	0	0	0	0	360.0	0.0	440.0	0.0	0.0	830.0
31.	16-05-2013	POS	AR-T-IB	0	0	0	0	240.0	100.0	0.0	0.0	0.0	100.0
32.	16-10-2009	NEG	AR-0, CAN-0	0	0	0	0	460.0	0.0	630.0	0.0	0.0	0.0
33.	03-08-2010	NEG	AR-0	0	0	0	0	1190.0	0.0	220.0	0.0	0.0	150.0
34.	22-09-2010	NEG	AR-0	0	0	0	0	1270.0	0.0	650.0	0.0	0.0	100.0
35.	22-01-2010	NEG	AR-0, CAN-0	0	807.95	25.06	0	750.0	0.0	550.0	150.0	120.0	280.0
36.	23-11-2010	NEG	AR-0	0	0	0	0	150.0	0.0	3510.0	4330.0	0.0	1570.0
37.	30-09-2011	NEG	AR-0	0	0	0	0	0.0	0.0	180.0	0.0	0.0	0.0
38.	07-03-2011	NEG	AR-0	0	0	0	0	0.0	0.0	830.0	240.0	210.0	160.0
39.	07-03-2011	NEG	AR-0	0	0	0	0	600.0	0.0	400.0	0.0	150.0	0.0
40.	18-11-2011	NEG	AR-0	0	0	0	0	250.0	0.0	260.0	0.0	150.0	0.0
41.	09-01-2012	NEG	AR-0	0	0	0	0	520.0	0.0	0.0	0.0	0.0	0.0
42.	29-03-2012	NEG	AR-0	0	0	0	0	120.0	0.0	0.0	0.0	0.0	0.0
43.	16-04-2012	NEG	AR-0	0	0	0	0	330.0	0.0	280.0	530.0	3800.0	0.0
44.	17-12-2015	NEG	AR-0	0	0	0	0	810.0	0.0	0.0	220.0	7750.0	100.0
45.	15-02-2016	NEG	AR-0	0	0	0	0	230.0	0.0	0.0	0.0	670.0	0.0
46.	27-05-2016	NEG	AR-0	0	0	0	0	560.0	0.0	0.0	230.0	7870.0	0.0
47.	06-10-2016	NEG	AR-0	0	0	0	0	310.0	0.0	720.0	0.0	510.0	190.0
48.	10-05-2016	NEG	AR-0	0	0	0	0	0.0	0.0	200.0	4530.0	240.0	0.0
49.	17-07-2016	NEG	AR-0	0	0	0	0	0.0	0.0	0.0	11740.0	10050.0	0.0

Patient number	Biopsy date	Bioptats		Cytokines (pg/ml)				Anti-HLA IgM (MFI)			Anti-HLA IgG (MFI)		
		Interpretation	Results	IL-17	IL-4	IL-10	IFN-γ	Class I	Class II	MICA	Class I	Class II	MICA
50.	15-02-2016	NEG	AR-0	0	0	0	0	350.0	0.0	0.0	11150.0	10450.0	0.0
51.	08-11-2016	NEG	AR-0	0	0	0	0	0.0	0.0	0.0	200.0	100.0	0.0
52.	29-12-2016	POS	CNI-S	0	307.18	0	0	380.0	0.0	0.0	310.0	180.0	100.0
53.	27-06-2018	POS	BC	0	0	0	0	350.0	0.0	230.0	0.0	0.0	0.0
54.	06-12-2018	NEG	AR-0, CAN-0	0	172.54	0	0	620.0	0.0	890.0	7390.0	6510.0	0.0
55.	26-06-2018	POS	AR-T-IB	0	27.12	0	0	0.0	0.0	0.0	0.0	520.0	190.0
56.	29-06-2018	NEG	AR-0, CAN-0	0	210.08	0	0	430.0	0.0	0.0	0.0	120.0	0.0
57.	26-07-2018	POS	AR-0, CNI-M	0	0	0	0	210.0	0.0	320.0	0.0	0.0	420.0
58.	17-07-2018	POS	BC	0	0	0	0	0.0	0.0	2690.0	0.0	0.0	0.0
59.	18-06-2018	NEG	AR-0	0	0	0	0	320.0	0.0	0.0	0.0	100.0	0.0
60.	27-09-2018	POS	AR-T-IB	0	0	0	0	1440.0	2050.0	2190.0	1270.0	6040.0	0.0
61.	06-11-2018	NEG	AR-0, CAN-0	0	67.84	0	0	0.0	0.0	0.0	4220.0	180.0	0.0
62.	06-11-2018	NEG	AR-0	0	0	0	0	300.0	0.0	0.0	0.0	0.0	0.0

POS – positive result; NEG – negative result; MFI – mean fluorescence intensity; AR-0 – no acute rejection; AR-IA/IIA/IIIA – acute rejection type IA.II. IIIA according to Banff; AHR-IA/IIA/IIIA – acute humoral rejection type IA/II/IIIA according to Banff; CNI-M/D – nephrotoxic effects of low or high-intensity calcineurin inhibitors; ATN-M/D – signs of acute mild or severe urethral damage; CAN-O – no chronic nephropathy; CAN-M/D – mild or severe chronic nephropathy; CNI-S/M – weak or severe isomeric vacuolation in the renal tubular epithelium; BC – histopathological picture with borderline changes; TA/IF-M/D – stromal fibrosis and tubular atrophy of low or high degree (chronic nephropathy); AR-T-IB/IIB – acute T-cell rejection of graft type IB/IIB according to Banff

immunised recipients [17, 18]. In addition, the use of this test after transplantation may facilitate monitoring of the level of alloimmunisation, as well as allowing the detection and assessment of the level of DSA-specific antibodies synthesised *de novo* in the patient. It appears that monitoring alloantibodies may be an excellent prognostic indicator for graft function, and the titer increase may precede other subclinical symptoms, thus enabling prompt intervention by clinicians [7, 9, 10, 17].

In the case of own results obtained in kidney recipients 6 months after transplantation, it was found that in more than 45.2% (n = 28) patients, biopsy material showed changes indicating features of nephropathy of varying severity (n = 21), or changes behind acute humoral (n = 3), or T-cell (n = 3) rejection. Fu et al. proved that subclinical rejection changes occur in as many as 8.4% of patients, and subclinical BC borderline as 43.4% for the first 5 years after kidney transplantation [19]. Cosio et al. showed in post-transplant histology analyse at 1 year that 72.6% of grafts had minor abnormalities (favourable histology), 20.2% unfavourable histology – dominated BC and SCR and 7.2% glomerulonephritis [20].

However, according to the literature data, the problem of alloimmunisation concerns many recipients. Depending on the method of antibody assessment, the immunised constitute from 10% to over 40% of those reported for the first transplant [10]. A much higher level of alloimmunisation was observed in the group of patients qualified for re-transplantation: 59.4% [21], 73.91% [22], and 75% [15]. Analysis of alloimmunisation in the current study showed the presence of anti-HLA IgM class I antibodies in over 75.8% (n = 47), and class II antibodies in only 17.8% of the subjects (n = 5). The prevalence of anti-HLA IgG antibodies in the study group, respectively, was: for class I – 43.5% (n = 27) and class II – 50% (n = 31). In both classes, more than half of the subjects were also found to have anti-MICA antibodies. In own research, a higher level of anti-HLA antibodies was obtained for the IgG class (average MFI – 2266.45) than for IgM (average MFI – 957.14). However, it should be borne in mind that significant discrepancies in the number of immunised recipients may result from differences in the sensitivity of the methods used to assess them and the impact of the patient's immunising factors [10].

Studies by Sadeghi et al. indicate that in the pre- and early transplant period (up to the sixth month after surgery), the level of IFN-γ and IL-2 is low, while the level of IL-4 and IL-10 is elevated [23]. This study also showed that this situation changed between 12 – 24 months after transplantation, with a significant increase in IFN-γ. Similar conclusions were reached in the case of cytokine evaluation. An increase in IL-4 was observed in the study group, although in the case of 7 out of 12 patients with elevated levels of this interleukin, no changes were found in the biopsy material.

CONCLUSIONS

Post-transplant protocol biopsy seems to be a helpful tool in assessing graft function. Similarly, assessing the level of alloimmunisation is a vital exponent of humoral rejection. This assessment should be supplemented by identifying the specificity of anti-HLA antibodies and based on their systematic monitoring in the pre- and post-transplant period.

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