lek. Michał Gniewkiewicz

Ocena przydatności monitorowania przeciwciał przeciwko antygenom zgodności tkankowej dawcy u pacjentów po transplantacji nerki w stratyfikacji ryzyka immunologicznego utraty przeszczepu

Rozprawa na stopień doktora nauk medycznych i nauk o zdrowiu

w dyscyplinie nauki medyczne

Promotor: prof. dr hab. n. med. Magdalena Durlik

Klinika Medycyny Transplantacyjnej, Nefrologii i Chorób Wewnętrznych
Warszawskiego Uniwersytetu Medycznego

Obrona rozprawy doktorskiej przed Radą Dyscypliny Nauk Medycznych

Warszawskiego Uniwersytetu Medycznego

Warszawa 2023r.

Słowa kluczowe: anty-HLA DSA, wiązanie C1q, przeszczepienie nerki, utrata przeszczepu

Keywords: anti-HLA DSA, C1q-binding, kidney transplantation, graft loss

Nazwa i numer projektu badawczego

Praca naukowa finansowana ze środków budżetowych na naukę w latach 2018-2021, jako projekt badawczy w ramach programu **„Diamentowy Grant”**. Grant został przyznany przez Ministra Nauki i Szkolnictwa Wyższego (decyzja nr 0021/DIA/2018/47 z dnia 18.07.2018r.).

Numer projektu: DI2017 002147

Tytuł projektu: Ocena przydatności monitorowania przeciwciał przeciwko antygenom zgodności tkankowej dawcy u pacjentów po transplantacji nerki w stratyfikacji ryzyka immunologicznego utraty przeszczepu.

Kierownik projektu: lek. Michał Gniewkiewicz

Opiekun: prof. dr hab. n. med. Magdalena Durlik

Pragnę serdecznie podziękować wszystkim, dzięki którym powstanie niniejszej pracy było możliwe.

Dziękuję mojej Promotor Pani Prof. dr hab. n. med. Magdalenie Durlik za poświęcony czas, okazane wsparcie oraz zaufanie.

Dziękuję Pani Dr hab. n. med. Jolancie Gozdowskiej za zaangażowanie w działalność naukową, nieustanną motywację i cierpliwość.

Pracę dedykuję Rodzicom i Babci.

Wykaz publikacji stanowiących pracę doktorską

1. Gniewkiewicz M, Czerwińska K, Zielniok K, Durlik M. Association of Circulating Anti-HLA Donor-Specific Antibodies and Their Characteristics, including C1q-Binding Capacity, in Kidney Transplant Recipients with Long-Term Renal Graft Outcomes. *Journal of Clinical Medicine*. 2023; 12(4):1312. https://doi.org/10.3390/jcm12041312
   (Impact Factor: 3,900; Punkty MEiN: 140)

2. Gniewkiewicz M, Czerwińska K, Zielniok K, Durlik M. Impact of Resolved Preformed, Persistent Preformed, and De Novo Anti-HLA Donor-Specific Antibodies in Kidney Transplant Recipients on Long-Term Renal Graft Outcomes. *Journal of Clinical Medicine*. 2023; 12(10):3361. https://doi.org/10.3390/jcm12103361
   (Impact Factor: 3,900; Punkty MEiN: 140)

Spis treści

1.Wykaz stosowanych skrótów……...7

2. Streszczenie w języku polskim……...8

3. Streszczenie w języku angielskim…….11

4. Wstęp……14

5. Założenia i cel pracy…….17

6. Kopie opublikowanych prac…….18

7. Podsumowanie i wnioski……..44

8. Piśmiennictwo……...45

9. Opinia Komisji Bioetycznej……..50

10. Oświadczenia współautorów……...51

1. Wykaz stosowanych skrótów

ABMR – odrzucanie zależne od przeciwciał (ang. antibody-mediated rejection)

DSA – przeciwciała swoiste dla dawcy (ang. donor-specific antibodies)

HLA – ludzkie antygeny leukocytarne (ang. human leukocyte antigens)

KTx – przeszczepienie nerki (ang. kidney transplantation)

MFI – średnia intensywność fluorescencji (ang. mean fluorescence intensity)

2. Streszczenie w języku polskim

Monitorowanie odpowiedzi immunologicznej jest obecnie jednym z ważniejszych aspektów opieki nad pacjentem po przeszczepieniu nerki. Swoiste dla dawcy przeciwciała (DSA), skierowane przeciwko ludzkim antygenom leukocytarnym (HLA) są udowodnionym czynnikiem ryzyka rozwoju procesu odrzucania humoralnego oraz utraty przeszczepu. Przeciwciała mogą uszkadzać przeszczep w różnych mechanizmach, takich jak aktywacja układu dopełniacza, bezpośrednie oddziaływanie na komórki śródbłonka czy też cytotoksyczność zależna od przeciwciał. Na patogenność anty-HLA DSA mają wpływ ich dodatkowe właściwości: klasa HLA, specyficzność, średnia intensywność fluoroscencji (MFI), zdolność wiązania dopełniacza C1q, subklasa IgG. Zidentyfikowano trzy wzorce kliniczne ewolucji anty-HLA DSA po przeszczepieniu: zanikłe preformowane DSA, przetrwałe preformowane DSA i *de novo* DSA.

Niniejszą rozprawę doktorską tworzy cykl dwóch publikacji będących wynikiem projektu badawczego dotyczącego oceny przydatności monitorowania anty-HLA DSA u pacjentów po przeszczepieniu nerki, który został zrealizowany w ramach programu „Diamentowy Grant". Przeprowadzone badania miały na celu: 1) ocenę wpływu krążących anty-HLA DSA z uwzględnieniem ich właściwości (klasa HLA, specyficzność, średnia intensywność fluoroscencji (MFI), zdolność wiązania dopełniacza C1q, subklasa IgG) na odległe wyniki przeszczepienia nerki od dawcy zmarłego; 2) określenie znaczenia ewolucji anty-HLA DSA (zanikłe preformowane, przetrwałe preformowane, *de novo*) dla odległych wyników przeszczepienia nerki od dawcy zmarłego oraz identyfikacja czynników mających wpływ na tę ewolucję.

Do badania zostało włączonych 108 pacjentów, u których wykonano zabieg przeszczepienia nerki od dawcy zmarłego. Od wszystkich pacjentów pobrano surowice w trakcie hospitalizacji celem biopsji przeszczepu w okresie 3 do 24 miesięcy po zabiegu przeszczepienia nerki. W surowicach analizowano obecność przeciwciał przeciwko anty–HLA DSA oraz w przypadku ich wykrycia, określono ich charakterystykę. W badaniu przyjęto złożony punkt końcowy: utrzymujący się spadek szacunkowego wskaźnika filtracji kłębuszkowej powyżej 30% w odniesieniu do wartości w czasie wykonania biopsji lub niewydolność nerki przeszczepionej. Mediana czasu obserwacji pacjentów od momentu biopsji wynosiła 39 miesięcy.

U 19 pacjentów (17,6%) wykryto anty-HLA DSA. Ośmioro pacjentów miało immunodominujące anty-HLA DSA (przeciwciało z największą wartością MFI) w klasie I (42,1%), natomiast 11 pacjentów w klasie II (57,9%). U 10 pacjentów (52,6%) immunodominujące anty-HLA DSA miało zdolność wiązania dopełniacza C1q. Najpowszechniej występowała subklasa IgG1 (73,7%), następnie IgG3 (36,8%), IgG4 (21,1%) i IgG2 (10,5%). W analizie wieloczynnikowej predyktorem punktu końcowego było wykrycie anty-HLA DSA podczas biopsji (HR=5,133; 95% CI 2,150 – 12,253; p=0,0002) oraz zdolność anty-HLA DSA do wiązania dopełniacza C1q (HR=14,639; 95% CI 5,320 – 40,283; p=<0,0001).

Ponadto stwierdzono, że u 9 pacjentów (8,3%) po przeszczepieniu zaniknęły preformowane przeciwciała. Dziewięciu pacjentów (8,3%) miało przetrwałe preformowane anty-HLA DSA, natomiast u 10 pacjentów (9,3%) występowały anty-HLA DSA *de novo*. Obecność przetrwałych preformowanych DSA była predyktorem złożonego punktu końcowego badania (HR = 5,96; 95% CI 2,041–17,431; p = 0,0011), podobnie jak występowanie anty-HLA DSA *de novo* (HR = 4,48; 95% CI 1,483–13,520; p = 0,0079). Nie zaobserwowano zwiększonego ryzyka u pacjentów, u których zaniknęły preformowane DSA (HR = 1,10; 95% CI 0,139–8,676; p = 0,9305). Zaniknięcie preformowanych anty-HLA DSA było związane z użyciem globuliny antytymocytarnej jako indukcji immunosupresji. Przetrwanie preformowanych anty-HLA DSA było związane z wcześniejszą transplantacją i starszym wiekiem dawcy. Ponadto stwierdzono, że wcześniejszy przeszczep był czynnikiem ryzyka rozwoju anty-HLA DSA *de novo.*

Podsumowując, u pacjentów po przeszczepieniu nerki od dawcy zmarłego wykrycie przeciwciał przeciwko antygenom zgodności tkankowej dawcy oraz ich zdolność wiązania dopełniacza C1q są predyktorami gorszej czynności narządu przeszczepionego oraz utraty przeszczepu. Obecnie analiza zdolności wiązania C1q jest badaniem kosztownym, ale nieinwazyjnym, dostępnym i może być brana pod uwagę w praktyce klinicznej w opiece nad pacjentem po przeszczepieniu nerki. Stwierdzenie u biorcy nerki przeszczepionej anty-HLA DSA, a w szczególności tych wiążących C1q powinno skutkować zwiększeniem czujności lekarza transplantologa, dokładną analizą leczenia immunosupresyjnego czy też zorganizowaniem częstszych kontroli pacjenta.

Pacjenci z zanikłymi preformowanymi anty-HLA DSA po przeszczepieniu nerki mają podobne wyniki odległe jak pacjenci bez nich. Stwierdzenie przetrwałych preformowanych anty-HLA DSA oraz pojawienie się anty-HLA DSA *de novo* po przeszczepieniu nerki są predyktorami gorszej czynności i niewydolności nerki przeszczepionej. Na ewolucję

anty-HLA DSA po przeszczepieniu nerki ma wpływ leczenie indukcyjne za pomocą globuliny antytymocytarnej, poprzednie przeszczepienie czy też wiek dawcy. Kandydatów do przeszczepienia nerki, a zwłaszcza do retransplantacji należy poddać starannej ocenie ryzyka immunologicznego. Powyższe badania mogą być szczególnie przydatne w świetle rozwoju nowych protokołów odczulania przed przeszczepieniem.

Immunologia transplantacyjna rozwija się bardzo dynamicznie, ale istnieje wiele wątpliwości wymagających kolejnych badań.

3. Streszczenie w języku angielskim

Value of donor-specific anti-human leukocyte antigen antibodies monitoring in kidney transplant recipients for immunological risk stratification of graft loss

Immune monitoring is currently one of the important aspects of post-transplant care of kidney transplant recipients. The presence of donor-specific antibodies (DSAs) directed against human leukocyte antigen (HLA), is a proven risk factor for the development of antibody-mediated rejection (ABMR) and kidney allograft loss. Antibodies can damage renal graft through different mechanisms: activation of the complement cascade, direct interactions with the vascular endothelium, and antibody-dependent cellular cytotoxicity. The pathogenicity of anti-HLA DSAs is determined by antibody classes, specificity, mean fluorescent intensity (MFI), C1q-binding capacity, and IgG subclasses. There are three identified clinical patterns of anti-HLA DSAs post-transplant evolution: persistent preformed DSAs, resolved preformed DSAs, and *de novo* DSAs.

The doctoral dissertation consists of two articles that are the result of the "Diamond Grant" program research project regarding the value of anti-HLA DSAs monitoring in kidney transplant recipients. The aims of the conducted studies were: 1) to investigate the association of circulating DSAs and their characteristics, including antibody class, specificity, mean fluorescent intensity (MFI), C1q-binding capacity, and IgG subclasses, with renal allograft function and long-term outcomes; 2) to analyze the impact of resolved preformed, persistent preformed, and *de novo* anti-HLA DSAs on long-term renal allograft outcomes and to identify factors affecting the post-transplant evolution of anti-HLA DSAs.

The study included 108 consecutive patients from our transplant center who underwent kidney allograft biopsy 3 to 24 months after kidney transplantation from brain-dead deceased donors. From all the patients at the time of the biopsy, sera were collected for analysis of circulating anti-HLA DSAs and their characteristics. The primary endpoint was the composite of sustained 30% reduction from the estimated glomerular filtration rate at biopsy or death-censored graft failure. Patients were followed for a median time of 39 months.

Nineteen patients were identified with circulating anti-HLA DSAs at the time of the biopsy (17.6%). The immunodominant anti-HLA DSAs (antibody with the highest MFI) were class I in 8 patients (42.1%) and class II in 11 patients (57.9%). Ten patients (52.6%) had immunodominant anti-HLA DSAs with C1q-banding capacity. The most frequent

IgG subclass was IgG1 (73.7%), followed by IgG3 (36.8%), IgG4 (21.1%), and IgG2 (10.5%). The detection of anti-HLA DSAs at the time of biopsy (HR = 5.133, 95% CI 2.150–12.253, $p = 0.0002$) and their C1q-binding capacity (HR = 14.639, 95% CI 5.320–40.283, $p \leq 0.0001$) were independent predictors of the primary endpoint.

Based on the DSA status at the time of the biopsy, there were identified 9 patients (8.3%) with resolved preformed anti-HLA DSAs, 9 patients (8.3%) with persistent preformed anti-HLA DSAs and 10 patients (9.3%) with *de novo* anti-HLA DSAs. The identification of persistent preformed DSAs at the time of biopsy was the most significant predictor of the primary endpoint of the study (HR = 5.96, 95% CI 2.041–17.431, $p = 0.0011$), followed by the occurrence of *de novo* DSAs (HR = 4.48, 95% CI 1.483–13.520, $p = 0.0079$). No increased risk was observed in patients with resolved preformed DSAs (HR = 1.10, 95% CI 0.139–8.676, $p = 0.9305$). The use of anti-thymocyte globulin as an induction of immunosuppression was a risk factor for resolved preformed anti-HLA DSAs. The persistence of preformed anti-HLA DSAs was associated with previous transplantation and the increased age of the donor. Moreover, a prior transplant was identified to be a risk factor for the development of *de novo* anti-HLA DSAs.

To summarize, identification of circulating anti-HLA DSAs and their C1q-binding capacity are independent predictors for inferior renal allograft function and graft failure after kidney transplantation from a brain-dead deceased donor. Currently, analysis of C1q is limited by its cost, but it is noninvasive, easily accessible, and might be considered in routine clinical practice in post-transplant management. Detection of circulating DSA (in particular those binding C1q) should result in a thorough analysis of immunosuppressive treatment and careful patient monitoring.

Patients with resolved preformed anti-HLA DSAs have similar graft prognoses as patients without DSAs. The persistence of preformed anti-HLA DSAs after kidney transplantation and the occurrence of *de novo* anti-HLA DSAs are independent predictors of inferior kidney graft function and graft failure. Post-transplant evolution of anti-HLA DSAs is associated with anti-thymocyte globulin therapy, prior transplantation, and the donor's age. Candidates for renal transplantation (particularly for retransplantation) should undergo a cautious immunological risk assessment. These findings are valuable in light of the development of new desensitization protocols.

Transplant immunology evolves rapidly, but there are many gaps in the current knowledge, and therefore further studies are needed to answer clinical questions.

4. Wstęp

Przeszczepienie nerki (KTx) jest preferowaną metodą leczenia pacjentów ze schyłkową niewydolnością nerek [1]. W ostatnich latach, dzięki wprowadzeniu nowoczesnych leków immunosupresyjnych, rozwoju technik chirurgicznych oraz lepszej opiece pooperacyjnej krótkoterminowe wyniki u biorców przeszczepu nerki uległy znaczącej poprawie, natomiast długoterminowe wyniki nie poprawiły się w podobnym stopniu [2]. Odrzucanie zależne od przeciwciał (ABMR) jest główną przyczyną dysfunkcji przeszczepionej nerki, a następnie jej utraty [3]. Obecność u biorcy swoistych dla dawcy przeciwciał (DSA), zwłaszcza skierowanych przeciwko ludzkim antygenom leukocytarnym (HLA), jest udowodnionym czynnikiem ryzyka rozwoju ABMR [4]. Występują również DSA nie-anty-HLA, takie jak przeciwciała przeciwko receptorowi angiotensyny II typu 1 lub przeciw receptorowi endoteliny-1 typu A, których rola jest obecnie analizowana [5,6].

W ciągu ostatniej dekady metody oznaczania anty-HLA DSA poprawiły się diametralnie dzięki zastosowaniu techniki fazy stałej z wykorzystaniem mikrokulek opłaszczonych antygenami HLA [7]. DSA uszkadzają przeszczep w różnych mechanizmach, takich jak aktywacja układu dopełniacza, bezpośrednie oddziaływanie na komórki śródbłonka czy też cytotoksyczność zależna od przeciwciał [8]. O patogenności DSA decyduje szereg ich dodatkowych właściwości: klasa HLA, specyficzność, średnia intensywność fluoroscencji (MFI), zdolność wiązania dopełniacza C1q, subklasa IgG [9-11] Przeciwciała przeciwko HLA klasy II występują częściej niż przeciwko HLA klasy I [12,13]. Stwierdzono związek między wysokimi wartościami MFI anty-HLA DSA, a częstszym występowaniem ABMR i zmniejszoną przeżywalnością przeszczepu [14,15]. Wykazano, że zdolność wiązania dopełniacza C1q jest predyktorem ABMR i koreluje z przeżyciem przeszczepu [16,17]. Jednakże nie jest jasne czy zwiększone ryzyko jest związane ze zdolnością wiązania dopełniacza lub wysokimi wartościami MFI, ponieważ istnieje silna korelacja między zdolnością DSA do wiązania C1q, a MFI [18,19]. Podklasy IgG1 i IgG3 są silnymi przeciwciałami wiążącymi dopełniacz, podczas gdy podklasy IgG2 i IgG4 są uważane za przeciwciała niewiążące dopełniacza [20]. Udowodniono, że IgG3 i IgG4 są silnie związane z ABMR i skorelowane z jego fenotypami (IgG3 z ostrym ABMR, IgG4 z subklinicznym ABMR). Ponadto immunodominujące DSA subklasy IgG3 są związane z niewydolnością przeszczepu [21].

Anty-HLA DSA mogą powstać jeszcze przed przeszczepieniem (przeciwciała preformowane) lub mogą rozwinąć się *de novo* po przeszczepie [22]. Preformowane DSA mogą powstać

w wyniku ekspozycji na alloantygeny podczas ciąży, transfuzji krwi lub podczas wcześniejszego przeszczepu [23,24]. Obecnie jednym z elementów doboru biorcy do dawcy jest wirtualna próba krzyżowa, która ma na celu uniknięcie kojarzenia dawców z biorcami z obecnymi DSA [25,26]. Anty-HLA DSA *de novo* zwykle pojawiają się w ciągu roku od przeszczepu i są zazwyczaj skierowane przeciwko HLA klasy II [9]. Rozwój DSA *de novo* może być związany ze zmniejszeniem immunosupresji (z różnych przyczyn, np. działania niepożądane leków, nieprzestrzeganie zaleceń lekarskich), z ciążą, transfuzją krwi bądź wszczepieniem homograftu [27-29]. Istnieją trzy zidentyfikowane wzorce kliniczne ewolucji DSA po przeszczepieniu: zanikłe preformowane DSA, przetrwałe preformowane DSA i *de novo* DSA [30]. Pacjenci z przetrwałymi DSA są bardziej narażeni na ABMR lub utratę przeszczepionej nerki w porównaniu z pacjentami, u których DSA nie wykryto bądź ustąpiły po KTx. Czynnikami związanymi z przetrwaniem DSA po przeszczepieniu są klasa II, wysokie MFI i zdolność wiązania dopełniacza C1q [30,31]. Pokazano również, że pacjenci z wykrytymi *de novo* DSA wykazują istotnie zwiększone ryzyko przewlekłego ABMR i utraty przeszczepu nerki w porównaniu z pacjentami z wykrytymi DSA przed KTx [32,33]. Ukazały się prace dowodzące, że niezależnie od statusu przeciwciał (preformowane bądź *de novo*), ich obecność odpowiada większej częstości rozpoznania subklinicznego ABMR [34,35]. Ponadto stwierdzono, że obecność DSA po KTx wiąże się ze zwiększoną ekspresją genów związanych z ABMR, nawet w bioptatach, w których nie stwierdzono odrzucania [36].

W przypadku przeszczepów narządów miąższowych monitorowanie anty-HLA DSA jest obecnie przedmiotem intensywnych badań. W styczniu 2023 roku opublikowano rekomendacje dotyczące oceny anty-HLA DSA po przeszczepieniu [37]. W publikacji przedstawiono również wiele wątpliwości, które wiążą się z tym zagadnieniem. Nie jest wiadome, kiedy i jak często wykonywać badania przesiewowe anty-HLA DSA u biorców ze stabilną funkcją przeszczepu nerki oraz jakie dodatkowe właściwości przeciwciał brać pod uwagę [11]. Ponadto nie ma konsensusu co do postępowania z biorcami z krążącymi *de novo* DSA bez dysfunkcji przeszczepu [35]. Badany jest również związek między pojawieniem się anty-HLA DSA *de novo*, a rozwojem ABMR, ponieważ nie u wszystkich pacjentów DSA-dodatnich rozwija się ABMR [38,39]. Obecnie nie ma skutecznej metody leczenia ABMR, dlatego wykrycie DSA stanowi dla lekarza transplantologa ważną informację umożliwiającą podjęcie wczesnej interwencji, takiej jak optymalizacja leczenia immunosupresyjnego, częstsze kontrole pacjenta czy wykonanie biopsji nerki przeszczepionej, która pozwala zdiagnozować ABMR na etapie subklinicznym [40,41]. W ostatnich latach stworzono model iBox określający ryzyko niewydolności nerki przeszczepionej. Bierze on pod uwagę osiem zmiennych m.in. czas

od przeszczepienia, szacunkowy wskaźnik filtracji kłębuszkowej, białkomocz, dane histopatologiczne oraz obecność anty-HLA DSA z wartością MFI [42]. Nadto wydaje się, że ewolucja DSA po KTx ma wpływ na wyniki przeszczepienia [37]. Dalszych badań wymagają okoliczności, które prowadzą do zaniknięcia bądź przetrwania po KTx preformowanych anty-HLA DSA [31]. Jest to szczególnie ważne w kontekście pojawiania się nowych terapii odczulających, które mogą prowadzić do zaniknięcia preformowanych przeciwciał [26,43].

Przedstawiony cykl publikacji jest wynikiem projektu badawczego dotyczącego oceny przydatności monitorowania anty-HLA DSA u pacjentów po KTx, który został zrealizowany w ramach programu „Diamentowy Grant". Oba artykuły dotyczą tej samej grupy pacjentów po KTx od dawcy zmarłego hospitalizowanych w Klinice Medycyny Transplantacyjnej, Nefrologii i Chorób Wewnętrznych Warszawskiego Uniwersytetu Medycznego.

W pierwszej publikacji (Gniewkiewicz M, Czerwińska K, Zielniok K, Durlik M. Association of Circulating Anti-HLA Donor-Specific Antibodies and Their Characteristics, including C1q-Binding Capacity, in Kidney Transplant Recipients with Long-Term Renal Graft Outcomes. *Journal of Clinical Medicine*. 2023; 12(4):1312. https://doi.org/10.3390/jcm12041312) przedstawiono wyniki analizy wpływu obecnych po KTx anty-HLA DSA oraz ich właściwości (klasa HLA, specyficzność, średnia intensywność fluoroscencji (MFI), zdolność wiązania dopełniacza C1q, subklasa IgG) na wyniki długoterminowe przeszczepienia nerki.

Kolejna publikacja (Gniewkiewicz M, Czerwińska K, Zielniok K, Durlik M. Impact of Resolved Preformed, Persistent Preformed, and De Novo Anti-HLA Donor-Specific Antibodies in Kidney Transplant Recipients on Long-Term Renal Graft Outcomes. *Journal of Clinical Medicine*. 2023; 12(10):3361. https://doi.org/10.3390/jcm12103361) ukazuje wyniki analizy znaczenia ewolucji anty-HLA DSA po KTx (przeciwciała zanikłe preformowane, przetrwałe preformowane, *de novo*) dla odległych wyników przeszczepienia. Ponadto w pracy zidentyfikowano czynniki mające związek z ewolucją przeciwciał po przeszczepieniu.

5. Założenia i cel pracy

Monitorowanie odpowiedzi immunologicznej jest obecnie jednym z ważniejszych aspektów opieki nad pacjentem po przeszczepieniu nerki. Naukowcy i klinicyści starają się wydłużyć przeżycie przeszczepu, co w sytuacji niedoboru narządów do transplantacji jest szczególnie ważne. Przeciwciała przeciwko antygenom zgodności tkankowej dawcy są udowodnionym czynnikiem ryzyka rozwoju procesu odrzucania humoralnego oraz utraty przeszczepu.

Cele pracy:

1. Ocena wpływu krążących przeciwciał przeciwko antygenom zgodności tkankowej dawcy z uwzględnieniem ich właściwości (klasa HLA, specyficzność, MFI, zdolność wiązania dopełniacza C1q, subklasa IgG) na odległe wyniki przeszczepienia nerki od dawcy zmarłego.

2. Określenie znaczenia ewolucji przeciwciał przeciwko antygenom zgodności tkankowej dawcy (zanikłe preformowane, przetrwałe preformowane, *de novo*) dla odległych wyników przeszczepienia nerki od dawcy zmarłego oraz identyfikacja czynników mających wpływ na tę ewolucję.

6. Kopie opublikowanych prac

Journal of
Clinical Medicine

MDPI

Article

Association of Circulating Anti-HLA Donor-Specific Antibodies and Their Characteristics, including C1q-Binding Capacity, in Kidney Transplant Recipients with Long-Term Renal Graft Outcomes

Michal Gniewkiewicz [1,*], Katarzyna Czerwinska [1], Katarzyna Zielniok [2] and Magdalena Durlik [1]

[1] Department of Transplantation Medicine, Nephrology and Internal Diseases, Medical University of Warsaw, Nowogrodzka 59, 02-006 Warsaw, Poland
[2] Department of Clinical Immunology, Medical University of Warsaw, Nowogrodzka 59, 02-006 Warsaw, Poland
* Correspondence: michal.gniewkiewicz@gmail.com

Abstract: Post-transplant antihuman leukocyte antigen donor-specific antibodies (anti-HLA DSAs) monitoring in kidney transplant recipients remains unclear and is currently under investigation. The pathogenicity of anti-HLA DSAs is determined by antibody classes, specificity, mean fluorescent intensity (MFI), C1q-binding capacity, and IgG subclasses. The aim of this study was to investigate the association of circulating DSAs and their characteristics with renal allograft long-term outcomes. The study included 108 consecutive patients from our transplant center who underwent kidney allograft biopsy between November 2018 and November 2020, 3 to 24 months after kidney transplantation. At the time of biopsy, patients' sera were collected for analysis of anti-HLA DSAs. Patients were followed for a median time of 39.0 months (Q1–Q3, 29.8–45.0). Detection of anti-HLA DSAs at the time of biopsy (HR = 5.133, 95% CI 2.150–12.253, $p = 0.0002$) and their C1q-binding capacity (HR = 14.639, 95% CI 5.320–40.283, $p \leq 0.0001$) were independent predictors of the composite of sustained 30% reduction from estimated glomerular filtration rate or death-censored graft failure. Identification of anti-HLA DSAs and their C1q-binding capacity could be useful in identifying kidney transplant recipients at risk for inferior renal allograft function and graft failure. Analysis of C1q is noninvasive, accessible, and should be considered in clinical practice in post-transplant monitoring.

Keywords: antihuman leukocyte antigen donor-specific antibodies; C1q-binding DSA; kidney transplantation

check for updates

Citation: Gniewkiewicz, M.; Czerwinska, K.; Zielniok, K.; Durlik, M. Association of Circulating Anti-HLA Donor-Specific Antibodies and Their Characteristics, including C1q-Binding Capacity, in Kidney Transplant Recipients with Long-Term Renal Graft Outcomes. *J. Clin. Med.* **2023**, *12*, 1312. https://doi.org/10.3390/jcm12041312

Academic Editors: Georgios Tsoulfas and Ioannis N. Boletis

Received: 3 January 2023
Revised: 2 February 2023
Accepted: 5 February 2023
Published: 7 February 2023

Copyright: © 2023 by the authors. Licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (https://creativecommons.org/licenses/by/4.0/).

1. Introduction

Kidney transplantation (KTx) is the treatment of choice for patients with end-stage kidney disease (ESKD) [1]. Despite improvements in short-term outcomes in kidney transplant recipients due to potent immunosuppressive therapy, advanced surgical techniques, and better post-transplant care, long-term outcomes have not improved to a similar extent [2].

Antibody-mediated rejection (ABMR) is the main cause of kidney allograft dysfunction and kidney allograft loss [3]. The presence of donor-specific antibodies (DSAs), particularly those against human leukocyte antigen (HLA), is a proven risk factor for the development of ABMR [4]. The role of nonanti-HLA DSA such as antibodies against angiotensin II type 1 receptor or against endothelin-1 type A receptor has been broadly analyzed [5,6]. Anti-HLA DSAs can be preexisting (preformed) or may develop de novo after transplantation [7]. Preformed DSAs are caused by exposure to the alloantigens during pregnancy, blood transfusion, or previous transplantation [8,9]. The virtual crossmatch is used to aid in renal allograft allocation and to avoid matching donors to recipients with preformed DSAs [10,11].

De novo anti-HLA DSAs develop after KTx in 13–27% of previously nonsensitized patients [12]. Usually, they emerge within 1-year post-transplant and are directed against

HLA class II [13]. DSA screening in recipients with stable renal allograft function remains unclear and is currently under investigation [14,15]. Moreover, there is no consensus regarding the management of renal transplant recipients without allograft dysfunction with circulating de novo DSAs [16]. The impact of de novo anti-HLA DSAs on the development of ABMR is under investigation, as not all DSA-positive patients develop ABMR [17,18].

The pathogenicity of DSAs is determined by several characteristics, including antibody classes, specificity, strength (expressed by mean fluorescent intensity (MFI)), C1q-binding capacity, and IgG subclasses [19]. Antibodies against HLA class II occur more frequently than against HLA class I [20,21]. Patients with both anti-HLA DSA class I and II, or even class alone II, are at increased risk for ABMR [22]. The association between high MFI levels of DSAs and the increased occurrence of ABMR and decreased graft survival has been reported [23,24]. It has been demonstrated that C1q-binding capacity is a predictor of ABMR and correlates with graft survival [25,26]. However, it is not clear whether this increased risk is connected to complement-binding capacity or high MFI levels, as there is a strong correlation between the ability of DSAs to bind C1q and their strength [27,28]. IgG1 and IgG3 subclasses are strong complement-fixing antibodies, whereas IgG2 and IgG4 subclasses are considered noncomplement-fixing [29]. It was found that IgG3 and IgG4 are highly associated with ABMR and correlated with its phenotypes (IgG3 with acute ABMR, IgG4 with subclinical ABMR). Furthermore, IgG3 immunodominant DSAs are strongly and independently associated with allograft failure [19].

The aim of this study was to investigate, in a cohort of kidney transplant recipients from our center, the association of circulating DSAs and their characteristics, including MFI level, C1q-binding capacity, and IgG subclasses, with renal allograft function and long-term outcomes.

2. Materials and Methods

2.1. Study Design

The study included 108 consecutive patients from our transplant center (Department of Medical Transplantation, Nephrology and Internal Medicine, Medical University of Warsaw) who underwent kidney allograft biopsy between November 2018 and November 2020, 3 to 24 months after kidney transplantation from brain-dead deceased donors.

All kidney transplants required ABO blood group compatibility and a negative complement-dependent cytotoxicity crossmatch. All patients were of white ethnicity and had triple maintenance immunosuppression consisting of tacrolimus or cyclosporine, mycophenolate mofetil, and prednisone. The biopsy was performed using an 18-gauge needle with ultrasound guidance. The biopsy specimens were evaluated based on Banff criteria [30]. From all the patients at the time of biopsy, sera were collected for analysis of circulating anti-HLA DSAs and their characteristics (specificity, HLA class, MFI level, C1q-binding capacity, and IgG subclasses).

The primary outcome was the composite of sustained 30% reduction (defined as two consecutive results at least 3 months apart) from estimated glomerular filtration rate (eGFR) at biopsy or death-censored graft failure (defined as return to dialysis or retransplantation). EGFR was calculated using the Chronic Kidney Disease Epidemiology Collaboration 2009 (CKD-EPI) creatinine equation. All participants were followed through November 2022 (unless patient lost graft or died). Clinical data were obtained from the medical records.

The study was conducted in accordance with the Declaration of Helsinki and was approved by the medical ethics committee of the Medical University of Warsaw (Warsaw, Poland). Informed written consent was obtained from all patients.

2.2. Detection of Anti-HLA DSAs and Characterization

Sera samples were collected at the time of biopsy and stored at −80 °C for further analysis. All samples were analyzed for anti-HLA using LABScreen Mixed Class I and II (#LSM12, One Lambda, Inc., Los Angeles, CA, USA). When positive, samples were tested using LABScreen Single Antigen HLA class I and II assays (#LS1A04 and #LS2A01, One

Lambda, Inc., CA, USA) on a Luminex platform. Antibodies with MFI > 500 were considered positive. Donor specificity for anti-HLA antibodies was determined by comparison of the HLA antibody specificities with the HLA of the donor for HLA-A, -B, and -DR.

The presence of C1q-binding anti-HLA DSAs was assessed using single antigen flow bead assays according to the manufacturer's instructions (C1qScreen, #C1Q, One Lambda, Inc., CA, USA).

Detailed analysis of HLA DSA antibody subclasses was performed based on the method published by Hönger et al. [31]. The modification of the standard single antigen flow bead assay involved replacing the reporter PE-conjugated goat antihuman IgG antibody (#LS-AB2, One Lambda, Inc., CA, USA) with one of the monoclonal antibodies specific for the IgG subclass, that is, IgG1 (# mouse antihuman IgG 1 Fc-PE clone HP6001, # 9054-09), IgG2 (mouse antihuman IgG 2 Fc-PE clone 31-7-4, #9060-09), IgG3 (mouse antihuman IgG 3 Hinge-PE clone HP6050, #9210-09), and IgG4 (mouse antihuman IgG 4 Fc-PE clone HP6025, #9200-09) (all Southern Biotech, Birmingham, AL, USA). The detection of IgG subclasses 1-4 was conducted separately for each subclass on the sera of patients positive for HLA class I using the magnetic beads of the LABScreen Single Antigen HLA class I assay kit (Lot 013, #LS1A04, One Lambda, Inc., CA, USA) and the sera of patients positive for HLA class II using the magnetic beads of the LABScreen Single Antigen HLA class II assay kit (Lot 015, #LS2A01, One Lambda, Inc., CA, USA). Directly before analysis, the sera were centrifuged at 10,000× *g* for 10 min to remove aggregates or contamination. The initial procedure was conducted according to the manufacturer's protocol. A 30 min incubation was performed on 20 µL of patient serum with 5 µL of LABScreen beads in V-bottom 96-well microplates. After washing the plate three times, in the modified part of the procedure, the beads were incubated for 30 min with 100 µL of PE-conjugated secondary antibody solution detecting IgG1, IgG2, IgG3, or IgG 4. After incubation, the beads were washed three times, resuspended in 80 µL of sterile phosphate buffered saline, and immediately proceeded to data acquisition on a Luminex LABScan 100 analyzer. The anti-HLA reactivity of the patients' sera, corrected for nonspecific binding of the beads to negative control serum (#LS-NC, One Lambda, Inc., CA, USA), was calculated from the raw MFI values for each HLA-coated bead using HLA Fusion software v. 4.6 (#FUSPGR, One Lambda, Inc., CA, USA).

2.3. Statistical Analysis

The R software was used for statistical analysis. Categorical data were described as numbers (percentages), and continuous data were expressed as mean values with standard deviations or medians with quartiles 1 and 3 (Q1–Q3). The χ^2 test or Fisher exact test was used for categorical variables, and the 2-sample t-test or Mann–Whitney test for continuous variables. The normality of distribution was assessed by the Shapiro–Wilk test. The primary study outcome was composite of sustained 30% reduction from eGFR at biopsy or death-censored graft failure. Event-free survival was estimated with the Kaplan–Meier method and compared according to anti-HLA antibody status with the use of the log-rank test. Cox proportional hazards models were used to estimate hazard ratios and 95% confidence intervals for the study outcomes. Variables with significant contributions in univariate Cox models were entered into the multiple adjusted Cox models to determine the independent association with outcomes. Backward stepwise elimination method with a p-value of <0.05 necessary for retention in the multivariate model was performed. p values of <0.05 were considered significant.

3. Results

3.1. Characteristics of Study Population

In the study, 108 consecutive kidney transplant recipients from brain-dead deceased donors who underwent kidney allograft biopsy 3 to 24 months after transplantation were included. At the time of biopsy, patients with present circulating DSAs were identified (N = 19). The characteristics of the study population at the time of biopsy are summarized

in Table 1. There was no statistical difference between DSA (−) and DSA (+) patients regarding age at biopsy, sex, BMI, renal replacement therapy, cause of end-stage renal disease, cold ischemia time, HLA mismatches, and clinical characteristics, including type of calcineurin inhibitor (tacrolimus versus cyclosporine), eGFR at biopsy, proteinuria at biopsy, protocol biopsy, time from transplantation to biopsy, and occurrence of C4d deposition in biopsy. A higher percentage of patients with DSAs at the time of biopsy compared with DSA (−) patients had received a prior transplant (78.9% vs. 4.4%, $p < 0.0001$), had PRA > 5% (36.8% vs. 9.0%, $p = 0.0048$), had induction therapy (73.7% vs. 21.3%, $p < 0.0001$), had ABMR diagnosed at the time of biopsy (21.0% vs. 5.6%, $p = 0.0492$), and had anti-HLA DSAs before transplantation (47.4% vs. 10.1%, $p = 0.0005$). Out of 18 patients with anti-HLA DSAs before transplantation, 9 patients had them detected post-transplant at the time of biopsy. The characteristics of anti-HLA DSAs before transplantation were not statistically different between groups regarding number, class, and MFI of immunodominant anti-HLA DSAs.

Table 1. Characteristics of the study population.

	All Patients (N = 108)	DSA (−) (N = 89)	DSA (+) (N = 19)	*p*-Value
Recipient characteristic				
Age at biopsy, years, median (Q1–Q3)	48.5 (38.8–61.0)	46.0 (38.0–61.0)	54.0 (44.0–58.5)	0.6054
Male, n (%)	69 (63.9%)	58 (65.2%)	11 (57.9%)	0.7368
Body mass index at biopsy, kg/m^2, mean ± SD	25.24 ± 3.848	25.52 ± 3.934	23.93 ± 3.188	0.1031
Previous transplantation, n (%)	19 (17.6%)	4 (4.4%)	15 (78.9%)	<0.0001
Renal replacement therapy, n (%)				0.6326
Pre-emptive transplantation	12 (11.1%)	11 (12.4%)	1 (5.3%)	
Hemodialysis	83 (76.9%)	67 (75.2%)	16 (84.1%)	
Peritoneal dialysis	13 (12.0%)	11 (12.4%)	2 (10.6%)	
Cause of ESRD, n (%)				0.2384
Glomerulonephritis	48 (44.4%)	36 (40.4%)	12 (63.2%)	
ADPKD	19 (17.6%)	15 (16.8%)	4 (21.1%)	
Diabetes	16 (14.8%)	14 (15.7%)	2 (10.5%)	
Congenital anomaly	5 (4.6%)	5 (5.6%)	0	
Other	20 (18.5%)	19 (21.5%)	1 (5.2%)	
Diabetes, n (%)	31 (28.7%)	26 (29.2%)	5 (26.3%)	
Donor characteristic				
Age, years, mean ± SD	46.2 ± 14.88	45.0 ± 14.9	52.0 ± 13.8	0.0616
Male, n (%)	68 (63.0%)	58 (65.2%)	10 (52.6%)	0.4439
Transplant characteristic				
Cold ischemia time, minutes, mean ± SD	1268 ± 575.4	1239 ± 582.9	1416 ± 559.8	0.4972
Induction therapy, n (%)				<0.0001
None	75 (69.4%)	70 (78.7%)	5 (26.3%)	
Basiliximab	18 (16.7%)	10 (11.2%)	8 (42.1%)	

Table 1. *Cont.*

	All Patients (N = 108)	DSA (−) (N = 89)	DSA (+) (N = 19)	*p*-Value
ATG	15 (13.9%)	9 (10.1%)	6 (31.6%)	
HLA mismatches, median, (Q1–Q3)				
A	1.0 (1.0–2.0)	1.0 (1.0–2.0)	1.0 (1.0–1.5)	0.5127
B	1.0 (1.0–2.0)	1.0 (1.0–2.0)	1.0 (1.0–2.0)	0.4485
DR	1.0 (1.0–1.0)	1.0 (1.0–1.0)	1.0 (1.0–1.0)	0.1550
Total	3.0 (3.0–4.0)	3.0 (3.0–4.0)	4.0 (3.0–4.0)	0.4000
Panel-reactive antibody >5%, n (%)	15 (13.9%)	8 (9.0%)	7 (36.8%)	0.0048
Panel-reactive antibody, median (Q1–Q3) [min–max]	0 (0–0) [0–86]	0 (0–0) [0–86]	0 (0–18.5) [0–66]	0.0043
Clinical characteristic				
Immunosuppression, n (%)				0.3223
Tacrolimus	106 (98.1%)	88 (89.9%)	18 (94.7%)	
Cyclosporine	2 (1.9%)	1 (1.1%)	1 (5.3%)	
eGFR at biopsy, mL/min/1.73 m^2, mean ± SD	53.2 ± 20.76	54.6 ± 21.24	46.3 ± 17.19	0.1107
Proteinuria at biopsy ≥ 50 mg/dL, n (%)	16 (14.8%)	11 (12.6%)	5 (26.3%)	0.2306
Proteinuria at biopsy, median (Q1–Q3) [min–max]	0 (0–10.0) [0–100]	0 (0–10.0) [0–100]	0 (0–30.0) [0–50]	0.1363
Protocol biopsy n (%)	61 (56.5%)	52 (58.4%)	9 (47.4%)	0.4483
Time from transplantation to biopsy, months, median (Q1–Q3)	5.0 (3.0–12.0)	5.0 (3.0–12.0)	4.0 (3.0–10.5)	0.4374
ABMR at the time of biopsy, n (%)	9 (8.3%)	5 (5.6%)	4 (21.0%)	0.0492
C4d in biopsy, n (%)	14 (13.0%)	10 (11.2%)	4 (21.1%)	0.2651
Anti-HLA DSA before transplantation, n (%)	18 (16.7%)	9 (10.1%)	9 (47.4%)	0.0005
Number, median (Q1–Q3)	1.0 (1.0–1.8)	1.0 (1.0–1.0)	1.0 (1.0–2.0)	0.5008
Anti-HLA DSA class, n				1
I	11	6	5	
II	6	3	3	
I + II	1	0	1	
MFI of immunodominant anti-HLA DSA before transplantation, mean (SD)	2009 ± 1230.5	1807 ± 1347.3	2211 ± 1144.6	0.5022

Abbreviations: DSA, donor-specific antibodies; Q1-Q3, quartile 1–3; SD, standard deviation; ESRD, end-stage renal disease; ADPKD, autosomal dominant polycystic kidney disease; ATG, anti-thymocyte globulin; HLA, human leukocyte antigen; eGFR, estimated glomerular filtration rate; ABMR, antibody-mediated rejection; MFI, mean fluorescent intensity.

3.2. Characteristics of Anti-HLA DSAs at the Time of Biopsy

Characteristics of anti-HLA DSAs at the time of biopsy are depicted in Table 2. Out of 19 patients with identified circulated anti-HLA DSAs at the time of biopsy, 7 patients (36.8%) had anti-HLA DSA class I, 10 patients (52.6%) had anti-HLA DSA class II, and 2 patients (10.5%) had anti-HLA DSA class I and II. The immunodominant anti-HLA DSAs were class I in 8 patients (42.1%) and class II in 11 patients (57.9%), with a median MFI (Q1–Q3) of 2900 (1284–4648). A total of 10 patients (52.6%) had immunodominant anti-HLA DSAs with C1q-banding capacity (3 patients with immunodominant anti-HLA DSA class I and 7 patients with immunodominant anti-HLA DSA class II). The most frequent IgG subclass of immunodominant anti-HLA DSA was IgG1 (14 patients, 73.7%), followed by IgG3 (7 patients, 36.8%), IgG4 (4 patients, 21.1%), and IgG2 (2 patients, 10.5%). The most predominant pattern was IgG1 alone (7 patients, 36.8%), followed by IgG3 alone (4 patients, 21.1%), IgG1 + IgG3 (3 patients, 15.8%), IgG1 + IgG4 (3 patients, 15.8%), IgG1 + IgG2 (1 patient, 5.3%), and IgG2 + IgG4 (1 patient, 5.3%).

Table 2. Characteristics of anti-HLA DSAs.

All Anti-HLA DSA	
Number, median (Q1–Q3)	1.0 (1.0–1.0)
HLA class specificity, n (%)	
I	7 (36.8)
II	10 (52.6)
I + II	2 (10.5)
iDSA	
HLA class specificity, n (%)	
I	8 (42.1)
II	11 (57.9)
MFI, median (Q1–Q3)	2900 (1284–4648)
C1q binding, n (%)	10 (52.6)
IgG subclasses, n (%)	
IgG1	14 (73.7)
IgG2	2 (10.5)
IgG3	7 (36.8)
IgG4	4 (21.1)

Abbreviations: HLA, human leukocyte antigen; DSA, donor-specific antibodies; Q1–Q3, quartile 1–3; iDSA, immunodominant specific antibody; MFI, mean fluorescent intensity.

3.3. Clinical Outcomes

Patients were followed for a median time of 39.0 months (Q1–Q3, 29.8–45.0) following the biopsy after transplantation. Clinical outcomes during follow-up are shown in Table 3. There was a statistical difference between DSA (−) and DSA (+) patients regarding eGFR at the end of follow-up (51.5 mL/min/1.73 m^2 (Q1–Q3, 34.8–63.3) vs. 36.0 mL/min/1.73 m^2 (Q1–Q3, 26.0–42.0), $p = 0.0049$), the occurrence of >30% decline in eGFR (10.1% vs. 57.9%, $p < 0.0001$), and death-censored graft loss (1.1% vs. 15.8%, $p = 0.0168$). There was no statistical difference regarding proteinuria at the end of follow-up. The combined endpoint was reached by 10 patients (11.2%) in the DSA (−) group vs. 11 patients (57.9%) in the DSA (+) group, $p < 0.0001$. The mean time from biopsy to the combined endpoint was 25.0 ± 10.69 months. During follow-up, four patients experienced a death-censored graft loss: one patient in the DSA (−) group and three patients in the DSA (+) group. The causes were ABMR (N = 2), BK virus nephropathy (N = 1), and recurrence of native kidney disease (N = 1). During follow-up, six patients died, of which four patients died with a functioning

graft. The deaths were the result of cardiovascular causes (N = 3), infections (N = 2), and malignancy (N = 1).

Table 3. Clinical outcomes.

	All Patients (N = 108)	DSA (−) (N = 89)	DSA (+) (N = 19)	*p*-Value
Follow-up postbiopsy, months, median (Q1–Q3)	39.0 (29.8–45.0)	37.0 (28.0–44.0)	44.0 (37.5–47.0)	0.0631
eGFR at the end of follow-up, mL/min/1.73 m^2, median (Q1–Q3)	48.0 (33.0–62.0)	51.5 (34.8–63.3)	36.0 (26.0–42.0)	0.0049
Proteinuria at the end of follow-up, ≥50 mg/dL, n (%)	6 (5.6)	3 (3.4)	3 (15.8)	0.1110
Proteinuria at the end of follow-up, median (Q1–Q3) [min–max]	0 (0–0) [0–311.0]	0 (0–0) [0–311.0]	0 (0–6.8) [0–81.0]	0.4113
>30% decline in eGFR, n (%)	20 (18.5)	9 (10.1)	11 (57.9)	<0.0001
Death-censored graft loss, n (%)	4 (3.7)	1 (1.1)	3 (15.8)	0.0168
Combined endpoint, >30% decline in eGFR or graft loss, n (%)	21 (19.4)	10 (11.2)	11 (57.9)	<0.0001
Time from biopsy to combined endpoint, months, mean ± SD	25.0 ± 10.69	26.9 ± 9.79	23.4 ± 11.60	0.482
Death event, n (%)	6 (5.6)	4 (4.4)	2 (10.5)	0.2842

Abbreviations: DSA, donor-specific antibodies; Q1-Q3, quartile 1–3; eGFR, estimated glomerular filtration rate; SD, standard deviation.

3.4. Survival Analysis

Combined endpoint-free survival analysis according to anti-HLA DSA status at the time of biopsy is depicted in Figure 1. Reduced survival was associated with the presence of circulating DSAs ($p < 0.0001$) and C1q-banding capacity (DSA (+) C1q (−) vs. DSA (+) C1q (+), p-value = 0.01). No statistical difference was observed between DSA (+) Kaplan–Meier curves regarding MFI > 2000 vs. MFI ≤ 2000 ($p = 0.4$), HLA class I vs. HLA class II ($p = 0.9$), IgG1 (+) vs. IgG1 (−) ($p = 0.5$), IgG2 (+) vs. IgG2 (−) ($p = 0.2$), IgG3 (+) vs. IgG3 (−) ($p = 0.8$), and IgG4 (+) vs. IgG4 (−) ($p = 0.2$).

Univariate and multivariate Cox regression models for risk of the combined endpoint of >30% decline in eGFR or graft loss is shown in Table 4. The following independent predictors of >30% decline in eGFR or graft loss were identified in all models: age of the donor and proteinuria at the time of biopsy ≥50 mg/dl. Occurrence of circulating anti-HLA DSAs at the time of biopsy (HR = 5.133, 95% CI 2.150–12.253, $p = 0.0002$) and C1q-binding capacity of anti-HLA DSAs at the time of biopsy (HR = 14.639, 95% CI 5.320–40.283, $p < 0.0001$) were independent predictors of the >30% decline in eGFR or graft loss, and each variable was analyzed separately in a correspondent multivariate model.

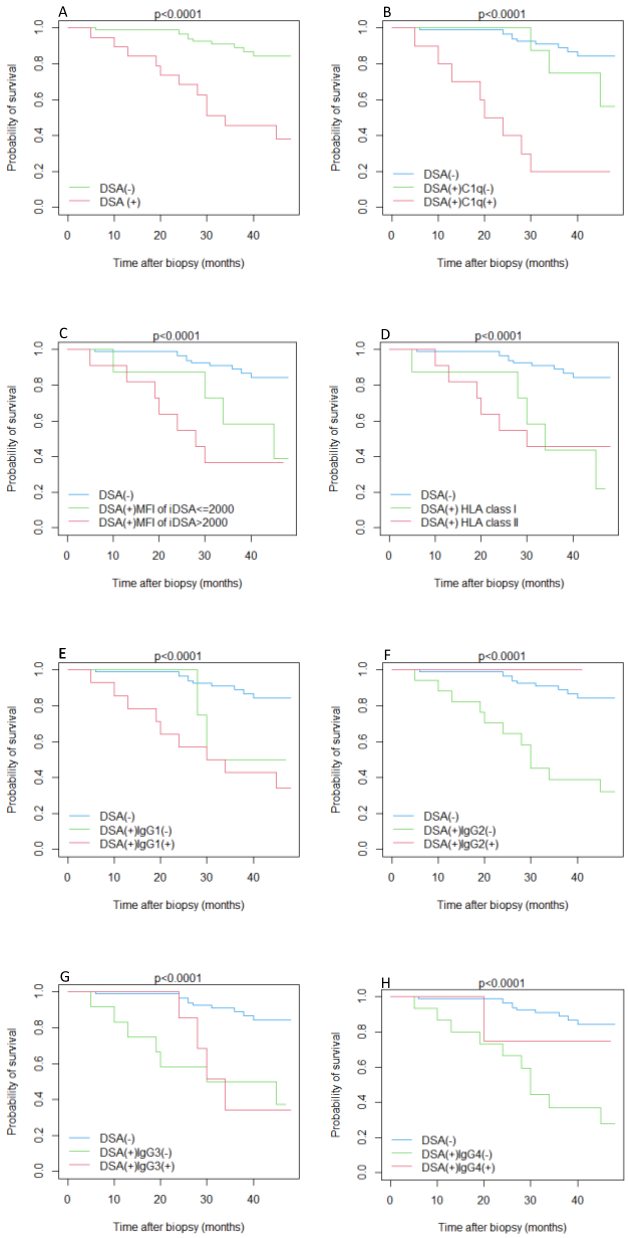

Figure 1. Event-free Kaplan–Meier survival curves according to detection of anti-HLA DSAs at the time of biopsy (**A**) and their characteristics: C1q- binding capacity (**B**), MFI (**C**), HLA class (**D**), IgG subclasses (**E**–**H**). Event defined as composite of sustained 30% reduction from eGFR at biopsy or death-censored graft failure. Abbreviations: DSA, donor-specific antibodies; MFI, mean fluorescent intensity; iDSA, immunodominant donor-specific antibody; HLA, human leukocyte antigen.

Table 4. Univariate and multivariate Cox regression models for risk of the combined endpoint of >30% decline in eGFR or death-censored graft loss.

	Univariate			Multivariate 1			Multivariate 2		
	HR	**95% CI**	***p*-Value**	**HR**	**95% CI**	***p*-Value**	**HR**	**95% CI**	***p*-Value**
Recipient characteristic									
Age at biopsy (per 1-year increase)	1.001	0.969–1.035	0.9486						
Male (vs. female)	1.291	0.518–3.216	0.5835						
Body mass index at biopsy (per 1 kg/m^2 increase)	0.953	0.855–1.063	0.3918						
Previous transplantation (yes vs. no)	3.728	1.568–8.864	0.0029						
Renal replacement therapy									
Pre-emptive	Ref.								
Hemodialysis	0.821	0.239–2.822	0.754						
Peritoneal dialysis	0.761	0.127–4.571	0.765						
Donor characteristic									
Age (per 1-year increase)	1.045	1.010–1.081	0.0106	1.034	1.002–1.067	0.0382	1.040	1.005–1.077	0.0259
Male (vs. female)	0.353	0.146–0.852	0.0205						
Transplant characteristic									
Induction therapy									
No	Ref.								
Basiliximab	3.294	1.224–8.862	0.0182						
ATG	2.925	0.980–8.735	0.0545						
Total HLA mismatches (per 1-mismatch increase)	1.349	0.860–2.116	0.1925						
Panel-reactive antibody >5% (vs. ≤5%)	1.309	0.479–3.579	0.5993						
Clinical characteristic									
eGFR at biopsy (per 1-mL/min/1.73 m^2 increase)	0.992	0.971–1.013	0.4490						
Proteinuria at biopsy ≥50 mg/dL (vs. <50 mg/dL)	2.628	1.019–6.776	0.0457	2.612	1.006–6.783	0.0486	3.504	1.281–9.588	0.0146
ABMR at the time of biopsy (yes vs. no)	2.361	0.692–8.054	0.1698						
C4d in biopsy (yes vs. no)	1.426	0.479–4.245	0.5234						
Anti-HLA DSAs before transplantation (yes vs. no)	3.031	1.221–7.524	0.0168						
Anti-HLA DSAs at biopsy (yes vs. no) *	6.01	2.546–14.190	<0.0001	5.133	2.150–12.253	0.0002			
C1q-binding status									
No anti-HLA DSAs at biopsy **	Ref.						Ref.		
Anti-HLA DSAs (+) C1q (−) at biopsy **	2.501	0.687–9.107	0.164				1.927	0.524–7.084	0.3236
Anti-HLA DSAs (+) C1q (+) at biopsy **	12.844	5.010–32.926	<0.0001				14.639	5.320–40.283	<0.0001

* Included in multivariate model 1. ** Included in multivariate model 2. Abbreviations: HR, hazard ratio; CI, confidence interval; ATG, antithymocyte globulin; HLA, human leukocyte antigen; eGFR, estimated glomerular filtration rate; ABMR, antibody-mediated rejection; DSA, donor-specific antibodies.

4. Discussion

In this study, the utility of the identification of anti-HLAs and their characteristics after kidney transplantation to determine patients at risk for inferior allograft function and allograft failure was analyzed. A total of 19 out of 108 patients (17.6%) were identified with circulating anti-HLA DSAs at the time of biopsy, 3 to 24 months after kidney transplantation. Ten patients (52.6%) had immunodominant anti-HLA DSAs with C1q-banding capacity. The most frequent IgG subclass of immunodominant anti-HLA DSA was IgG1, followed by IgG3. These results are consistent with the available literature [31,32]. The development of anti-HLA DSAs may be caused before transplantation by pregnancy, blood transfusion, or prior transplantation, or anti-HLA DSAs may develop after transplantation [8]. In patients with circulating anti-HLA DSAs, 47.4% had anti-HLA DSAs detected before transplantation, and 78.9% had previous transplantation. There are several clinical events that contribute to the formation of de novo anti-HLA DSAs, including blood transfusion, pregnancy, homograft implantation, nonadherence, and immunosuppression minimization [33–35]. Patients with de novo anti-HLA DSAs display a 25% to 53% incidence of subclinical ABMR at the time of anti-HLA DSA identification, whereas 31% to 50% of patients with preformed anti-HLA DSAs develop subclinical ABMR by 3 months post-transplant [36]. Patients with anti-HLA DSAs have an increased risk of chronic ABMR and kidney allograft loss compared with patients with preformed anti-HLA DSAs [37,38]. However, the detection of circulating anti-HLA DSAs, regardless of their status, is associated with increased expression of rejection transcripts in renal transplant biopsies classified as no rejection [39].

The identification of anti-HLA DSAs was an independent predictor of the composite of sustained 30% reduction from eGFR at biopsy or death-censored graft failure. Previous studies showed an association between circulating anti-HLA DSAs and increased C4d deposition in the peritubular capillaries of the allograft, as well as increased microvascular inflammation, which leads to ABMR and kidney graft failure [40–42]. Moreover, it was discovered that the course of fibrogenesis in renal allograft is significantly accelerated by circulating anti-HLA DSAs independent of ABMR [43]. Recently, the strong association of proinflammatory blood cytokine profiles was demonstrated with the presence of HLA DSAs, even in the absence of histology of rejection [44]. In previous papers, it was reported that the presence of anti-HLA DSAs was associated with inferior graft outcomes and graft failure [45,46]. However, some studies demonstrated contrasting findings [47].

To better evaluate the predictive value of anti-HLA DSAs for renal graft outcomes, their characteristics, including MFI level, C1q-binding capacity, and IgG subclasses, have been broadly analyzed [19]. In this study, the C1q-binding capacity of anti-HLA DSAs was proved to be an independent predictor of inferior renal graft outcomes. This is consistent with what has been found in previous papers [25,26]. A recent meta-analysis by Kang et al., which included relevant studies comparing the clinical outcomes between DSA C1q (+) and DSA C1q (−) kidney transplant recipients, demonstrated that C1q-binding DSAs are associated with increased risks of ABMR, renal allograft failure, and patient death [48]. The pathogenic potential of C1q DSA is explained by the fact that binding complement fraction C1 is the first step in the activation of the classic complement pathway, which results in the formation of a membrane attack complex that contributes to vascular injury and causes target cell damage [49]. Detection of C1q DSA could have therapeutic consequences, as there are available complement-targeting agents, such as eculizumab [26,50]. It has been shown that circulating C1q-binding anti-HLA DSAs after therapeutic intervention for ABMR may reflect the effect of treatment and predict long-term allograft survival [51]. In a recent study, it was demonstrated that timely treatment with an augmented immunosuppressive protocol of C1q-binding anti-HLA DSA-associated ABMR, with early detection and elimination of C1q-binding anti-HLAs, may be associated with better outcomes [52]. A large multicenter randomized clinical trial is needed to assess the role of the C1q-binding capacity of anti-HLA DSAs in kidney transplant recipients.

The study included a relatively small number of patients with DSA positivity; therefore, it was not possible to fully characterize the utility of IgG subclasses on renal allograft

outcomes. IgG subclass assays are not currently commercially available and are not used in clinical practice. In line with previous studies, the IgG1 subclass was the most common [53]. Moreover, it is worth discussing the fact that IgG2 and IgG4 subclasses were identified but only in combination with other subclasses. A similar pattern of results suggesting the evolution of an immune response was obtained by Ponsirenas et al. [54]. It was hypothesized that human B cells follow a programmed sequence of immunoglobulin class switching from IgM to IgG3, then to IgG1 and to IgG2 and, finally, to IgG4 [55]. That could be supported by the finding that IgG2 and IgG4 subclasses of anti-HLA DSAs were detected in eluates of rejected renal allografts [56].

In our study population, patients with immunodominant anti-HLA DSA MFI values of >2000 vs. $\leq$2000 did not have significantly different event-free survival. Analysis of MFI values is challenging, as there is no international threshold for MFI values and a lack of standardization [57]. Therefore, regulatory agencies do not accept single antigen bead assays as quantitative measurements [51].

The results of the study must be interpreted with caution, and several limitations should be borne in mind. This was a single-center study with a relatively small number of patients with DSA positivity. The presence of DSA in patients was tested at different points in time but within 3 to 24 months after kidney transplantation at the time of renal allograft biopsy. The DSAs and their characteristics were not monitored during follow-up. The DSAs against HLA-C, -DP, and -DQ are not included, as HLA-C, -DP, and -DQ typing were not available for kidney donors. The heterogeneity in the induction of immunosuppression treatment could not be avoided.

To conclude, our data reported that the identification of circulating anti-HLA DSAs and their C1q-binding capacity are independent predictors for inferior renal allograft function and graft failure after kidney transplantation. Even though analysis of C1q is limited by its cost, it is noninvasive, easily accessible, and should be considered in routine clinical practice in post-transplant monitoring. This could improve the optimization of post-transplant care. Our research adds to the literature supporting the use of the C1q assay in the immunological stratification of kidney transplant recipients for long-term renal allograft outcomes.

Author Contributions: Conceptualization, M.G. and M.D.; methodology, M.G. and M.D.; validation, K.C. and K.Z.; formal analysis, M.G.; investigation, M.G., K.C. and K.Z.; resources, M.G., K.C., K.Z. and M.D.; data curation, M.G., K.C. and K.Z.; writing—original graft preparation, M.G. and K.Z.; writing—review and editing, M.D.; visualization, M.G.; supervision, M.D.; project administration, M.G.; funding acquisition, M.G. All authors have read and agreed to the published version of the manuscript.

Funding: This research was financed from budget funds for science in the years 2018–2021 as a research project under the "Diamond Grant" program (no. DI2017 002147) supported by the Ministry of Science and Higher Education of Poland.

Institutional Review Board Statement: The study was conducted in accordance with the Declaration of Helsinki and approved by the Institutional Ethics Committee of the Medical University of Warsaw (KB/160/2018).

Informed Consent Statement: Informed consent was obtained from all subjects involved in the study.

Data Availability Statement: The data presented in this study are available on request from the corresponding author.

Conflicts of Interest: The authors declare no conflict of interest.

References

1. McCormick, F.; Held, P.J.; Chertow, G.M. The Terrible Toll of the Kidney Shortage. *J. Am. Soc. Nephrol.* **2018**, *29*, 2775–2776. [CrossRef] [PubMed]

2. Coemans, M.; Süsal, C.; Döhler, B.; Anglicheau, D.; Giral, M.; Bestard, O.; Legendre, C.; Emonds, M.P.; Kuypers, D.; Molenberghs, G.; et al. Analyses of the short- and long-term graft survival after kidney transplantation in Europe between 1986 and 2015. *Kidney Int.* **2018**, *94*, 964–973. [CrossRef]
3. Sellarés, J.; de Freitas, D.G.; Mengel, M.; Reeve, J.; Einecke, G.; Sis, B.; Hidalgo, L.G.; Famulski, K.; Matas, A.; Halloran, P.F. Understanding the causes of kidney transplant failure: The dominant role of antibody-mediated rejection and nonadherence. *Am. J. Transplant.* **2012**, *12*, 388–399. [CrossRef]
4. Cioni, M.; Nocera, A.; Innocente, A.; Tagliamacco, A.; Trivelli, A.; Basso, S.; Quartuccio, G.; Fontana, I.; Magnasco, A.; Drago, F.; et al. De Novo Donor-Specific HLA Antibodies Developing Early or Late after Transplant Are Associated with the Same Risk of Graft Damage and Loss in Nonsensitized Kidney Recipients. *J. Immunol. Res.* **2017**, *2017*, 1747030. [CrossRef]
5. El Band, J.E.K.; Llorente, S.; Martinez-Garcia, P.; Alfaro, R.; Jimenez-Coll, V.; Boix, F.; Galián, J.A.; Martinez-Banaclocha, H.; Botella, C.; Moya-Quiles, M.R.; et al. Evaluation of Antibodies Directed Against Two GPCRs, Anti-AT1R and Anti-ETAR, on Kidney Transplant Outcome. *Curr. Protein Pept. Sci.* **2021**, *22*, 745–757. [CrossRef] [PubMed]
6. Filippone, E.J.; Farber, J.L. Histologic Antibody-mediated Kidney Allograft Rejection in the Absence of Donor-specific HLA Antibodies. *Transplantation* **2021**, *105*, e181–e190. [CrossRef]
7. Hung, S.Y.; Lin, T.M.; Chang, M.Y.; Wang, H.H.; Lee, Y.C.; Ho, L.C.; Chen, Y.T.; Hung, C.M.; Liou, H.H. Risk factors of sensitization to human leukocyte antigen in end-stage renal disease patients. *Hum. Immunol.* **2014**, *75*, 531–535. [CrossRef]
8. Yeung, M.Y. Pre-formed DSA and kidney allograft outcomes. *Braz. J. Nephrol.* **2020**, *42*, 136–137. [CrossRef] [PubMed]
9. Oweira, H.; Ramouz, A.; Ghamarnejad, O.; Khajeh, E.; Ali-Hasan-Al-Saegh, S.; Nikbakhsh, R.; Reißfelder, C.; Rahbari, N.; Mehrabi, A.; Sadeghi, M. Risk Factors of Rejection in Renal Transplant Recipients: A Narrative Review. *J. Clin. Med.* **2022**, *11*, 1392. [CrossRef] [PubMed]
10. Olszowska-Zaremba, N.; Zagożdżon, R.; Gozdowska, J. Accuracy of virtual crossmatch (VXM) prediction of physical crossmatch (PXM) results of donor specific antibody (DSA) in routine pretransplant settings-a single-center experience. *Transpl. Immunol.* **2022**, *72*, 101583. [CrossRef]
11. Leal, R.; Pardinhas, C.; Martinho, A.; Sá, H.O.; Figueiredo, A.; Alves, R. Strategies to Overcome HLA Sensitization and Improve Access to Retransplantation after Kidney Graft Loss. *J. Clin. Med.* **2022**, *11*, 5753. [CrossRef]
12. Matignon, M.; Pilon, C.; Commereuc, M.; Grondin, C.; Leibler, C.; Kofman, T.; Audard, V.; Cohen, J.; Canoui-Poitrine, F.; Grimbert, P. Intravenous immunoglobulin therapy in kidney transplant recipients with de novo DSA: Results of an observational study. *PLoS ONE* **2017**, *12*, e0178572. [CrossRef]
13. Heilman, R.L.; Nijim, A.; Desmarteau, Y.M.; Khamash, H.; Pando, M.J.; Smith, M.L.; Chakkera, H.A.; Huskey, J.; Valdez, R.; Reddy, K.S. De novo donor-specific human leukocyte antigen antibodies early after kidney transplantation. *Transplantation* **2014**, *98*, 1310–1315. [CrossRef] [PubMed]
14. Tambur, A.R.; Campbell, P.; Chong, A.S.; Feng, S.; Ford, M.L.; Gebel, H.; Gill, R.G.; Kelsoe, G.; Kosmoliaptsis, V.; Mannon, R.B.; et al. Sensitization in transplantation: Assessment of risk (STAR) 2019 Working Group Meeting Report. *Am. J. Transplant.* **2020**, *20*, 2652–2668. [CrossRef] [PubMed]
15. Sharma, A.; Jorgensen, D.R.; Mehta, R.B.; Sood, P.; Puttarajappa, C.M.; Wu, C.M.; Tevar, A.D.; Molinari, M.; Zeevi, A.; Hariharan, S. The Clinical Impact of Anti-HLA Donor Specific Antibody Detection Through First Year Screening on Stable Kidney Transplant Recipients. *Transpl. Int.* **2022**, *35*, 10094. [CrossRef] [PubMed]
16. Yamamoto, T.; Watarai, Y.; Takeda, A.; Tsujita, M.; Hiramitsu, T.; Goto, N.; Narumi, S.; Katayama, A.; Morozumi, K.; Uchida, K.; et al. De Novo Anti-HLA DSA Characteristics and Subclinical Antibody-Mediated Kidney Allograft Injury. *Transplantation* **2016**, *100*, 2194–2202. [CrossRef]
17. Nakamura, K.; Sawada, A.; Kita, Y.; Kono, J.; Masui, K.; Sato, T.; Sano, T.; Goto, T.; Akamatsu, S.; Ogawa, O.; et al. Clinical characteristics of renal transplant recipients who developed de novo donor-specific antigen in Kyoto University Hospital: A case series. *Ren. Replace Ther.* **2022**, *8*, 10. [CrossRef]
18. Wu, K.; Schmidt, D.; López Del Moral, C.; Osmanodja, B.; Lachmann, N.; Zhang, Q.; Halleck, F.; Choi, M.; Bachmann, F.; Ronicke, S.; et al. Poor Long-Term Renal Allograft Survival in Patients with Chronic Antibody-Mediated Rejection, Irrespective of Treatment-A Single Center Retrospective Study. *J. Clin. Med.* **2021**, *11*, 199. [CrossRef]
19. Lefaucheur, C.; Viglietti, D.; Bentlejewski, C.; Duong van Huyen, J.P.; Vernerey, D.; Aubert, O.; Verine, J.; Jouven, X.; Legendre, C.; Glotz, D.; et al. IgG Donor-Specific Anti-Human HLA Antibody Subclasses and Kidney Allograft Antibody-Mediated Injury. *J. Am. Soc. Nephrol.* **2016**, *27*, 293–304. [CrossRef]
20. Tatapudi, V.S.; Kopchaliiska, D.; da Gente, G.J.; Buenaventura, O.F.; Singh, M.; Laszik, Z.; Adey, D.B.; Rajalingam, R. Solid-Phase C1q/C3d Fixing Readouts Correlate with High Median Fluorescence Intensity (MFI) De Novo Donor-Specific HLA Antibodies and C4d^{+} Antibody-Mediated Rejection in Kidney Transplant Recipients. *Ann. Transplant.* **2021**, *26*, e934175. [CrossRef]
21. Butiu, M.; Obrisca, B.; Sibulesky, L.; Bakthavatsalam, R.; Smith, K.D.; Gimferrer, I.; Warner, P.; Ismail, G.; Leca, N. Donor-derived Cell-free DNA Complements De Novo Class II DSA in Detecting Late Alloimmune Injury Post Kidney Transplantation. *Transpl. Direct* **2022**, *8*, e1285. [CrossRef] [PubMed]
22. Willicombe, M.; Roufosse, C.; Brookes, P.; Galliford, J.W.; McLean, A.G.; Dorling, A.; Warrens, A.N.; Cook, T.H.; Cairns, T.D.; Taube, D. Antibody-mediated rejection after alemtuzumab induction: Incidence, risk factors, and predictors of poor outcome. *Transplantation* **2011**, *92*, 176–182. [CrossRef] [PubMed]

23. Lefaucheur, C.; Loupy, A.; Hill, G.S.; Andrade, J.; Nochy, D.; Antoine, C.; Gautreau, C.; Charron, D.; Glotz, D.; Suberbielle-Boissel, C. Preexisting donor-specific HLA antibodies predict outcome in kidney transplantation. *J. Am. Soc. Nephrol.* **2010**, *21*, 1398–1406. [CrossRef]
24. Mizutani, K.; Terasaki, P.; Hamdani, E.; Esquenazi, V.; Rosen, A.; Miller, J.; Ozawa, M. The importance of anti-HLA-specific antibody strength in monitoring kidney transplant patients. *Am. J. Transplant.* **2007**, *7*, 1027–1031. [CrossRef]
25. Malheiro, J.; Tafulo, S.; Dias, L.; Martins, S.; Fonseca, I.; Beirão, I.; Castro-Henriques, A.; Cabrita, A. Determining donor-specific antibody C1q-binding ability improves the prediction of antibody-mediated rejection in human leucocyte antigen-incompatible kidney transplantation. *Transpl. Int.* **2017**, *30*, 347–359. [CrossRef] [PubMed]
26. Loupy, A.; Lefaucheur, C.; Vernerey, D.; Prugger, C.; Duong van Huyen, J.P.; Mooney, N.; Suberbielle, C.; Frémeaux-Bacchi, V.; Méjean, A.; Desgrandchamps, F.; et al. Complement-binding anti-HLA antibodies and kidney-allograft survival. *N. Engl. J. Med.* **2013**, *369*, 1215–1226. [CrossRef] [PubMed]
27. Yell, M.; Muth, B.L.; Kaufman, D.B.; Djamali, A.; Ellis, T.M. C1q Binding Activity of De Novo Donor-specific HLA Antibodies in Renal Transplant Recipients With and Without Antibody-mediated Rejection. *Transplantation* **2015**, *99*, 1151–1155. [CrossRef]
28. Navas, A.; Molina, J.; Agüera, M.L.; Guler, I.; Jurado, A.; Rodríguez-Benot, A.; Alonso, C.; Solana, R. Characterization of the C1q-Binding Ability and the IgG1-4 Subclass Profile of Preformed Anti-HLA Antibodies by Solid-Phase Assays. *Front. Immunol.* **2019**, *10*, 1712. [CrossRef]
29. Schaub, S.; Hönger, G.; Amico, P. The complexity of the humoral immune response against HLA antigens. *Transpl. Int.* **2014**, *27*, 249–250. [CrossRef]
30. Haas, M.; Loupy, A.; Lefaucheur, C.; Roufosse, C.; Glotz, D.; Seron, D.; Nankivell, B.J.; Halloran, P.F.; Colvin, R.B.; Akalin, E.; et al. The Banff 2017 Kidney Meeting Report: Revised diagnostic criteria for chronic active T cell-mediated rejection, antibody-mediated rejection, and prospects for integrative endpoints for next-generation clinical trials. *Am. J. Transplant.* **2018**, *18*, 293–307. [CrossRef]
31. Hönger, G.; Hopfer, H.; Arnold, M.L.; Spriewald, B.M.; Schaub, S.; Amico, P. Pretransplant IgG subclasses of donor-specific human leukocyte antigen antibodies and development of antibody-mediated rejection. *Transplantation* **2011**, *92*, 41–47. [CrossRef] [PubMed]
32. Freitas, M.C.; Rebellato, L.M.; Ozawa, M.; Nguyen, A.; Sasaki, N.; Everly, M.; Briley, K.P.; Haisch, C.E.; Bolin, P.; Parker, K.; et al. The role of immunoglobulin-G subclasses and C1q in de novo HLA-DQ donor-specific antibody kidney transplantation outcomes. *Transplantation* **2013**, *95*, 1113–1119. [CrossRef] [PubMed]
33. Snanoudj, R.; Kamar, N.; Cassuto, E.; Caillard, S.; Metzger, M.; Merville, P.; Thierry, A.; Jollet, I.; Grimbert, P.; Anglicheau, D.; et al. Epitope load identifies kidney transplant recipients at risk of allosensitization following minimization of immunosuppression. *Kidney Int.* **2019**, *95*, 1471–1485. [CrossRef] [PubMed]
34. Sharma, A.; Cherukuri, A.; Mehta, R.B.; Sood, P.; Hariharan, S. High Calcineurin Inhibitor Intrapatient Variability Is Associated With Renal Allograft Inflammation, Chronicity, and Graft Loss. *Transplant. Direct* **2019**, *5*, e424. [CrossRef]
35. Coti, I.; Wenda, S.; Andreeva, A.; Kocher, A.; Laufer, G.; Fischer, G.; Andreas, M. Donor-specific HLA antibodies after fresh decellularized vs. cryopreserved native allograft implantation. *Hla* **2020**, *96*, 580–588. [CrossRef]
36. Lefaucheur, C.; Louis, K.; Morris, A.B.; Taupin, J.L.; Nickerson, P.; Tambur, A.R.; Gebel, H.M.; Reed, E.F. Clinical recommendations for posttransplant assessment of anti-HLA (Human Leukocyte Antigen) donor-specific antibodies: A Sensitization in Transplantation: Assessment of Risk consensus document. *Am. J. Transplant.* **2023**, *23*, 115–132. [CrossRef]
37. Aubert, O.; Loupy, A.; Hidalgo, L.; Duong van Huyen, J.-P.; Higgins, S.; Viglietti, D.; Jouven, X.; Glotz, D.; Legendre, C.; Lefaucheur, C.; et al. Antibody-Mediated Rejection Due to Preexisting versus De Novo Donor-Specific Antibodies in Kidney Allograft Recipients. *J. Am. Soc. Nephrol.* **2017**, *28*, 1912–1923. [CrossRef] [PubMed]
38. Haas, M.; Mirocha, J.; Reinsmoen, N.L.; Vo, A.A.; Choi, J.; Kahwaji, J.M.; Peng, A.; Villicana, R.; Jordan, S.C. Differences in pathologic features and graft outcomes in antibody-mediated rejection of renal allografts due to persistent/recurrent versus de novo donor-specific antibodies. *Kidney Int.* **2017**, *91*, 729–737. [CrossRef]
39. Madill-Thomsen, K.S.; Böhmig, G.A.; Bromberg, J.; Einecke, G.; Eskandary, F.; Gupta, G.; Hidalgo, L.G.; Myslak, M.; Viklicky, O.; Perkowska-Ptasinska, A.; et al. Donor-Specific Antibody Is Associated with Increased Expression of Rejection Transcripts in Renal Transplant Biopsies Classified as No Rejection. *J. Am. Soc. Nephrol.* **2021**, *32*, 2743–2758. [CrossRef]
40. Loupy, A.; Hill, G.S.; Suberbielle, C.; Charron, D.; Anglicheau, D.; Zuber, J.; Timsit, M.O.; Duong, J.P.; Bruneval, P.; Vernerey, D.; et al. Significance of C4d Banff scores in early protocol biopsies of kidney transplant recipients with preformed donor-specific antibodies (DSA). *Am. J. Transplant.* **2011**, *11*, 56–65. [CrossRef]
41. Einecke, G.; Sis, B.; Reeve, J.; Mengel, M.; Campbell, P.M.; Hidalgo, L.G.; Kaplan, B.; Halloran, P.F. Antibody-mediated microcirculation injury is the major cause of late kidney transplant failure. *Am. J. Transplant.* **2009**, *9*, 2520–2531. [CrossRef]
42. Lebraud, E.; Eloudzeri, M.; Rabant, M.; Lamarthée, B.; Anglicheau, D. Microvascular Inflammation of the Renal Allograft: A Reappraisal of the Underlying Mechanisms. *Front. Immunol.* **2022**, *13*, 864730. [CrossRef] [PubMed]
43. Gosset, C.; Viglietti, D.; Rabant, M.; Vérine, J.; Aubert, O.; Glotz, D.; Legendre, C.; Taupin, J.L.; Duong Van-Huyen, J.P.; Loupy, A.; et al. Circulating donor-specific anti-HLA antibodies are a major factor in premature and accelerated allograft fibrosis. *Kidney Int.* **2017**, *92*, 729–742. [CrossRef] [PubMed]
44. Van Loon, E.; Lamarthée, B.; Barba, T.; Claes, S.; Coemans, M.; de Loor, H.; Emonds, M.P.; Koshy, P.; Kuypers, D.; Proost, P.; et al. Circulating Donor-Specific Anti-HLA Antibodies Associate With Immune Activation Independent of Kidney Transplant Histopathological Findings. *Front. Immunol.* **2022**, *13*, 818569. [CrossRef] [PubMed]

45. Mohan, S.; Palanisamy, A.; Tsapepas, D.; Tanriover, B.; Crew, R.J.; Dube, G.; Ratner, L.E.; Cohen, D.J.; Radhakrishnan, J. Donor-specific antibodies adversely affect kidney allograft outcomes. *J. Am. Soc. Nephrol.* **2012**, *23*, 2061–2071. [CrossRef]
46. Terasaki, P.I.; Ozawa, M.; Castro, R. Four-year follow-up of a prospective trial of HLA and MICA antibodies on kidney graft survival. *Am. J. Transplant.* **2007**, *7*, 408–415. [CrossRef]
47. Parajuli, S.; Joachim, E.; Alagusundaramoorthy, S.; Aziz, F.; Blazel, J.; Garg, N.; Muth, B.; Mohamed, M.; Redfield, R.R.; Mandelbrot, D.A.; et al. Donor-Specific Antibodies in the Absence of Rejection Are Not a Risk Factor for Allograft Failure. *Kidney Int. Rep.* **2019**, *4*, 1057–1065. [CrossRef]
48. Kang, Z.Y.; Liu, C.; Liu, W.; Li, D.H. Effect of C1q-binding donor-specific anti-HLA antibodies on the clinical outcomes of patients after renal transplantation: A systematic review and meta-analysis. *Transpl. Immunol.* **2022**, *72*, 101566. [CrossRef]
49. Murata, K.; Baldwin, W.M., 3rd. Mechanisms of complement activation, C4d deposition, and their contribution to the pathogenesis of antibody-mediated rejection. *Transplant. Rev.* **2009**, *23*, 139–150. [CrossRef]
50. Patel, J.K.; Coutance, G.; Loupy, A.; Dilibero, D.; Hamilton, M.; Kittleson, M.; Kransdorf, E.; Azarbal, B.; Seguchi, O.; Zhang, X.; et al. Complement inhibition for prevention of antibody-mediated rejection in immunologically high-risk heart allograft recipients. *Am. J. Transplant.* **2021**, *21*, 2479–2488. [CrossRef]
51. Viglietti, D.; Bouatou, Y.; Kheav, V.D.; Aubert, O.; Suberbielle-Boissel, C.; Glotz, D.; Legendre, C.; Taupin, J.L.; Zeevi, A.; Loupy, A.; et al. Complement-binding anti-HLA antibodies are independent predictors of response to treatment in kidney recipients with antibody-mediated rejection. *Kidney Int.* **2018**, *94*, 773–787. [CrossRef] [PubMed]
52. Sigurjonsdottir, V.K.; Purington, N.; Chaudhuri, A.; Zhang, B.M.; Fernandez-Vina, M.; Palsson, R.; Kambham, N.; Charu, V.; Piburn, K.; Maestretti, L.; et al. Complement-Binding Donor-Specific Anti-HLA Antibodies: Biomarker for Immunologic Risk Stratification in Pediatric Kidney Transplantation Recipients. *Transpl. Int.* **2022**, *35*, 10158. [CrossRef]
53. Lowe, D.; Higgins, R.; Zehnder, D.; Briggs, D.C. Significant IgG subclass heterogeneity in HLA-specific antibodies: Implications for pathogenicity, prognosis, and the rejection response. *Hum. Immunol.* **2013**, *74*, 666–672. [CrossRef]
54. Ponsirenas, R.V.G.; Cazarote, H.B.; Araújo, S.A.; Wanderley, D.C.; Shimakura, S.; Valdameri, J.S.; Contieri, F.L.C.; von Glehn, C.; Susin, M.F.; Sotomaior, V.S. Anti-HLA Donor-Specific IgG Subclasses and C1q-binding Evolution in Posttransplant Monitoring. *Transpl. Direct* **2018**, *4*, e385. [CrossRef] [PubMed]
55. Collins, A.M.; Jackson, K.J. A Temporal Model of Human IgE and IgG Antibody Function. *Front. Immunol.* **2013**, *4*, 235. [CrossRef] [PubMed]
56. Heinemann, F.M.; Roth, I.; Rebmann, V.; Arnold, M.L.; Witzke, O.; Wilde, B.; Spriewald, B.M.; Grosse-Wilde, H. Immunoglobulin isotype-specific characterization of anti-human leukocyte antigen antibodies eluted from explanted renal allografts. *Hum. Immunol.* **2007**, *68*, 500–506. [CrossRef]
57. Gu, Y.; Koh, R.W.K.; Lai, M.L.; Pochinco, D.; Teo, R.Z.C.; Chan, M.; Murali, T.M.; Liew, C.W.; Wong, Y.H.; Gascoigne, N.R.J.; et al. Defining the structural basis for human leukocyte antigen reactivity in clinical transplantation. *Sci. Rep.* **2020**, *10*, 18397. [CrossRef]

Disclaimer/Publisher's Note: The statements, opinions and data contained in all publications are solely those of the individual author(s) and contributor(s) and not of MDPI and/or the editor(s). MDPI and/or the editor(s) disclaim responsibility for any injury to people or property resulting from any ideas, methods, instructions or products referred to in the content.

Journal of
Clinical Medicine

MDPI

Article

Impact of Resolved Preformed, Persistent Preformed, and De Novo Anti-HLA Donor-Specific Antibodies in Kidney Transplant Recipients on Long-Term Renal Graft Outcomes

Michal Gniewkiewicz [1,*], Katarzyna Czerwinska [1], Katarzyna Zielniok [2] and Magdalena Durlik [1]

[1] Department of Transplantation Medicine, Nephrology and Internal Diseases, Medical University of Warsaw, Nowogrodzka 59, 02-006 Warsaw, Poland
[2] Department of Clinical Immunology, Medical University of Warsaw, Nowogrodzka 59, 02-006 Warsaw, Poland
* Correspondence: michal.gniewkiewicz@gmail.com

Abstract: The post-transplant evolution of antihuman leukocyte antigen donor-specific antibodies (anti-HLA DSAs) includes three clinical patterns: resolved preformed DSAs, persistent preformed DSAs, and de novo DSAs. The aim of this retrospective study was to analyze the impact of resolved preformed, persistent preformed, and de novo anti-HLA-A, -B, and -DR DSAs in kidney transplant recipients on long-term renal allograft outcomes. This is a post hoc analysis of the study conducted in our transplant center. One hundred eight kidney transplant recipients were included in the study. Patients were followed for a minimum of 24 months after allograft biopsy, which was performed 3 to 24 months after kidney transplantation. The identification of persistent preformed DSAs at the time of biopsy was the most significant predictor of the combined endpoint of the study (>30% decline in estimated glomerular filtration rate or death-censored graft loss; HR = 5.96, 95% CI 2.041–17.431, $p = 0.0011$), followed by the occurrence of de novo DSAs (HR = 4.48, 95% CI 1.483–13.520, $p = 0.0079$). No increased risk was observed in patients with resolved preformed DSAs (HR = 1.10, 95% CI 0.139–8.676, $p = 0.9305$). Patients with resolved preformed DSAs have similar graft prognoses as patients without DSAs, therefore, the persistence of preformed DSAs and development of de novo DSAs are associated with inferior long-term allograft outcomes.

Keywords: resolved preformed DSAs; persistent preformed DSAs; de novo DSAs

check for updates

Citation: Gniewkiewicz, M.; Czerwinska, K.; Zielniok, K.; Durlik, M. Impact of Resolved Preformed, Persistent Preformed, and De Novo Anti-HLA Donor-Specific Antibodies in Kidney Transplant Recipients on Long-Term Renal Graft Outcomes. *J. Clin. Med.* **2023**, *12*, 3361. https://doi.org/10.3390/jcm12103361

Academic Editors: Maria Cappuccilli, Giorgia Comai and Giuseppe Cianciolo

Received: 12 April 2023
Revised: 28 April 2023
Accepted: 8 May 2023
Published: 9 May 2023

Copyright: © 2023 by the authors. Licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (https://creativecommons.org/licenses/by/4.0/).

1. Introduction

The presence of donor-specific antibodies (DSAs) directed against human leukocyte antigens (HLA) is a risk factor for antibody-mediated rejection (ABMR) and allograft loss in kidney transplant (KTx) recipients [1]. Over the last decade, the identification of anti-HLA DSAs and their characteristics has improved due to the use of solid-phase assay technology, particularly single antigen bead (SAB) testing [2]. In addition, the destructive effect of anti-HLA DSAs is determined by antibody characteristics including class, specificity, mean fluorescent intensity (MFI), complement-biding ability, and IgG subclass [3,4].

DSAs may occur before transplantation (preformed) or arise de novo after transplantation. Alloimmunization before KTx can be caused by pregnancy, blood transfusion, or prior transplant [5,6]. The development of de novo DSAs is associated with several clinical events such as pregnancy, blood transfusion, minimization of immunosuppression, nonadherence, and implementation of a homograft [7–9]. Regarding the post-transplant evolution of DSAs, there are three identified clinical patterns: persistent preformed DSAs, resolved preformed DSAs, and de novo DSAs [10]. Patients with persistent preformed DSAs are at a higher risk of ABMR or kidney allograft loss compared to patients with resolved or no preformed DSAs. Factors associated with the persistence of DSAs after transplantation are class II, high MFI, and complement-binding ability [10,11]. However, independently of the evolution status after KTx, preformed DSAs (even with low MFI values) are an important risk factor

for decreased graft survival [12]. Patients with identified circulating de novo DSAs display a significantly increased risk of chronic ABMR and renal allograft loss compared to patients with preexisting DSAs [13,14]. Regardless of the preformed/de novo status, the presence of DSAs corresponds with a higher incidence of diagnosis of subclinical ABMR [15,16]. DSA positivity after KTx is associated with increased ABMR-related gene expression, even in biopsy samples with no molecular or histologic rejection [17].

In solid-organ transplantation, the monitoring of anti-HLA DSAs is currently under investigation. Recently, clinical recommendations for the post-transplant assessment of anti-HLA DSAs were published [18]. Nevertheless, there are some deficits in the existing knowledge that should be addressed. There is a need for a better understanding of the clinical role of persistent preformed anti-HLA DSAs and to investigate factors that lead to the clearance of preformed anti-HLA DSAs after transplantation [11]. As the evolution of DSAs after KTx may modify the outcomes, therapeutic interventions could be implemented [18].

The aim of this retrospective study was to analyze the impact of resolved preformed, persistent preformed, and de novo anti-HLA-A, -B, and -DR DSAs in kidney transplant recipients with long-term renal allograft outcomes.

2. Materials and Methods

2.1. Study Design and Data Collection

This is a post hoc analysis of the study conducted in our transplant center [19]. All 108 consecutive kidney transplant recipients from brain-dead deceased donors from the mentioned study were included in this retrospective study. All patients underwent an ultrasound-guided renal allograft biopsy between 2018 and 2020, 3 to 24 months after KTx, and were followed for a minimum of 24 months after the biopsy (except in cases of death or graft failure).

Patients were divided post hoc into four groups according to their DSA status at the time of biopsy: no anti-HLA DSAs, resolved preformed anti-HLA DSAs, persistent preformed anti-HLA DSAs, and de novo anti-HLA DSAs.

All transplantations were performed with negative complement-dependent cytotoxicity crossmatch. Donor–recipient pairs were ABO blood group compatible. All patients were of white ethnicity. None of the patients received a desensitization protocol before KTx. The decision of whether and what induction therapy (basiliximab or anti-thymocyte globulin (ATG)) to administer was made individually for each patient after the assessment of their immunological risk. Mostly, patients with a high immunological risk received ATG while patients with an intermediate immunological risk received basiliximab. In all patients, the maintenance immunosuppressive regimen consisted of the standard of care agents: a calcineurin inhibitor (tacrolimus or cyclosporine) with mycophenolate mofetil and prednisone. Only two patients were treated with cyclosporine due to tacrolimus intolerance. Biopsies were assessed using Banff classification criteria [20].

At the time of biopsy, serum samples were obtained from all the patients and stored for further analysis. Sera were tested for the presence of anti-HLA DSAs and their characteristics including class, specificity, MFI, C1q-binding capacity, and IgG subclass according to the protocol described previously [19]. Clinical and laboratory data including the DSA status of the patients before KTx were retrospectively extracted from the medical records. All patients were tested for anti-HLA DSAs by SAB assay before transplantation. Antibodies with MFI values above 500 were determined as positive. The identification of donor HLA antibody specificity was limited to HLA-A, HLA-B, and HLA-DR. Donor typing was only available for HLA-A, HLA-B, and HLA-DR; the cross-reactive epitope groups were not considered. The resolved preformed anti-HLA DSAs were defined as antibodies detected before KTx but not detected at the time of the kidney transplant biopsy procedure.

The primary outcome of the study was defined as a permanent 30% decline (two consecutive laboratory assessments $\geq$ 3 months apart) in the estimated glomerular filtration rate (eGFR) compared to the baseline at the time of biopsy or death-censored graft loss (need

for dialysis or retransplantation). The Chronic Kidney Disease Epidemiology Collaboration 2009 (CKD-EPI) creatinine equation was used to determine the eGFR.

Approval of the study was obtained from the Medical University of Warsaw medical ethics committee (Warsaw, Poland). The study was performed according to the principles expressed in the Declaration of Helsinki. All patients provided informed written consent.

2.2. Statistical Analysis

Statistical analysis was conducted using R software. The Shapiro–Wilk test was used to assess the normality of distribution. Continuous variables were presented as the means with standard deviation (SD) or medians with quartiles 1 and 3 (Q1–Q3) as appropriate. Categorical variables are presented as frequencies and percentages. Differences between continuous variables were assessed using the *t*-test, Mann–Whitney U test, and one-way analysis of variance (F-test or Kruskal–Wallis test), depending on the number and distribution of the compared variables. The Chi-square test was used to compare categorical variables. The Yates continuity correction was used when the frequency of events was low (<5). Logistic regression was performed to identify variables associated with DSA's status. Event-free survival was assessed using the Kaplan–Meier method and log-rank test. Univariate and multivariate Cox proportional hazards models were used to quantify the hazard ratios for the study outcome. A backward stepwise elimination method was applied. A p-value < 0.05 was considered statistically significant.

3. Results

3.1. Demographic and Clinical Characteristics

A total of 108 consecutive patients who received kidney transplants from brain-dead deceased donors and underwent renal allograft biopsy 3 to 24 months post-KTx were included in the study. Based on the DSA status at the time of biopsy, four groups were identified: no anti-HLA DSAs (N = 80), resolved preformed anti-HLA DSAs (N = 9), persistent preformed anti-HLA DSAs (N = 9), and de novo anti-HLA DSAs (N = 10). The demographic and clinical characteristics of the groups are provided in Table 1. The groups did not differ in age, sex, body mass index, type of renal replacement therapy, cause of end-stage renal disease, age and sex of the donor, duration of cold ischemia, or number of HLA mismatches. Moreover, no statistical difference was found in terms of clinical characteristics such as the use of tacrolimus versus cyclosporine for maintenance immunosuppression, eGFR and proteinuria at biopsy, protocol biopsy, time from KTx to biopsy procedure, and C4d deposition in biopsy specimens. There was a statistical difference regarding the history of previous renal transplantation, induction of immunosuppression, panel-reactive antibodies, and diagnosis of ABMR at biopsy. The prior KTxs had 2.5% of patients without DSAs, 22.2% of patients with resolved preformed DSAs, 77.8% of patients with persistent preformed DSAs, and 80% of patients with de novo DSAs. The transplant procedure was performed without the induction of immunosuppression in 85% of patients without DSA. In patients with preformed DSAs (resolved and persistent), the induction protocol with anti-thymocyte globulin was the most popular. Half of the patients with de novo DSAs received basiliximab as an induction of immunosuppression. The panel-reactive antibody >5% was the most common in patients with persistent preformed anti-HLA DSAs (55.6%). ABMR was diagnosed in 2.5% of patients without DSAs and in 22.2% and 20% of KTx recipients with preformed and de novo DSAs, respectively.

3.2. Characteristics of Anti-HLA DSAs before and after Transplantation

Table 2 depicts the characteristics of anti-HLA DSAs before transplantation. No significant difference was shown between the number, HLA class specificity, and MFI of resolved and persistent anti-HLA DSAs. Nevertheless, the median MFI level of resolved preformed DSAs was lower compared to the median MFI level of persistent preformed DSAs (983 vs. 1905, not statistically significant).

Table 1. Characteristics of the groups.

	No Anti-HLA DSAs (N = 80)	Resolved Preformed Anti-HLA DSAs (N = 9)	Persistent Preformed Anti-HLA DSAs (N = 9)	De Novo Anti-HLA DSAs (N = 10)	*p*-Value
Recipient characteristic					
Age at biopsy, years, median (Q1–Q3)	45.0 (38.0–62.0)	49.0 (45.0–54.0)	54.0 (44.0–61.0)	51.0 (45.3–55.0)	0.9637
Male, n (%)	53 (66.3)	5 (55.6)	4 (44.4)	7 (70.0)	0.5517
Body mass index at biopsy, kg/m^2, mean ± SD	24.9 ± 4.03	26.6 ± 3.15	24.2 ± 2.82	24.3 ± 3.56	0.1589
Previous transplantation, n (%)	2 (2.5)	2 (22.2)	7 (77.8)	8 (80.0)	<0.0001
Renal replacement therapy, n (%)					0.9640
Pre-emptive transplantation	10 (12.5)	1 (11.1)	0	1 (10.0)	
Hemodialysis	60 (75.0)	7 (77.8)	8 (88.9)	8 (80.0)	
Peritoneal dialysis	10 (12.5)	1 (11.1)	1 (11.1)	1 (10.0)	
Cause of ESRD, n (%)					0.6475
Glomerulonephritis	31 (38.8)	5 (55.6)	5 (55.6)	7 (70.0)	
ADPKD	13 (16.3)	2 (22.2)	2 (22.2)	2 (20.0)	
Diabetes	14 (17.5)	0	1 (11.1)	1 (10.0)	
Congenital anomaly	4 (5.0)	1 (11.1)	0	0	
Other	18 (22.4)	1 (11.1)	1 (11.1)	0	
Diabetes, n (%)	24 (30.0)	2 (22.2)	2 (22.2)	3 (30.0)	0.9311
Donor characteristics					
Age, years, mean ± SD	44.8 ± 15.04	46.9 ± 14.07	57.9 ± 9.24	46.7 ± 15.44	0.1306
Male, n (%)	53 (66.3)	5 (55.6)	5 (55.6)	5 (50.0)	0.6789
Transplant characteristics					
Cold ischemia time, minutes, mean ± SD	1239 ± 607.1	1242 ± 450.9	1271 ± 390.3	1562 ± 753.3	0.4476
Induction therapy, n (%)					<0.0001
None	68 (85.0)	2 (22.2)	1 (11.1)	4 (40.0)	
Basiliximab	8 (10.0)	2 (22.2)	3 (33.3)	5 (50.0)	
ATG	4 (5.0)	5 (55.6)	5 (55.6)	1 (10.0)	
HLA mismatches, median, (Q1–Q3)					
A	1.0 (1.0–2.0)	1.0 (1.0–1.0)	1.0 (1.0–1.0)	1.0 (1.0–1.8)	0.3588
B	1.0 (1.0–2.0)	1.0 (1.0–2.0)	1.0 (1.0–2.0)	1.0 (1.0–2.0)	0.5407
DR	1.0 (0.8–1.0)	1.0 (1.0–1.0)	1.0 (1.0–1.0)	1.0 (1.0–1.0)	0.3914
Total	3.0 (3.0–4.0)	3.0 (3.0–4.0)	3.0 (3.0–4.0)	4.0 (3.0–4.0)	0.7966
Panel-reactive antibody >5%, n (%)	7 (8.8)	1 (11.1)	5 (55.6)	2 (20.0)	0.0017
Panel-reactive antibody, median (Q1–Q3)	0 (0–0)	0 (0–0)	57 (0–33)	0 (0–0)	0.0015

Table 1. *Cont.*

	No Anti-HLA DSAs (N = 80)	Resolved Preformed Anti-HLA DSAs (N = 9)	Persistent Preformed Anti-HLA DSAs (N = 9)	De Novo Anti-HLA DSAs (N = 10)	*p*-Value
Clinical characteristics					
Immunosuppression, n (%)					0.2455
Tacrolimus	79 (98.7)	9 (100.0)	9 (100.0)	9 (90.0)	
Cyclosporine	1 (1.3)	0	0	1 (10.0)	
eGFR at biopsy, mL/min/1.73 m^2, mean ± SD	54.4 ± 21.78	56.8 ± 16.47	40.9 ± 16.23	51.1 ± 17.39	0.2406
Proteinuria at biopsy ≥50 mg/dL, n (%)	10 (12.5)	1 (11.1)	3 (33.3)	2 (20.0)	0.3770
Proteinuria at biopsy, median (Q1–Q3)	0 (0–10)	0 (0–0)	0 (0–50)	5 (0–10)	0.4762
Protocol biopsy n (%)	45 (56.3)	7 (77.8)	5 (55.6)	4 (40.0)	0.4284
Time from transplantation to biopsy, months, median (Q1–Q3)	5 (3–12)	4 (3–12)	4 (3–7)	4 (3–11)	0.8841
ABMR at the time of biopsy, n (%)	3 (3.8)	2 (22.2)	2 (22.2)	2 (20.0)	0.0363
C4d in biopsy, n (%)	10 (12.5)	0	2 (22.2)	2 (20.0)	0.4792

Abbreviations: HLA—human leukocyte antigen; DSAs—donor-specific antibodies; Q1–Q3—quartile 1–3; SD—standard deviation; ESRD—end-stage renal disease; ADPKD—autosomal dominant polycystic kidney disease; ATG—anti-thymocyte globulin; eGFR—estimated glomerular filtration rate; and ABMR—antibody-mediated rejection.

Table 2. Characteristics of anti-HLA DSAs before transplantation.

	Resolved Preformed Anti-HLA DSAs (N = 9)	Persistent Preformed Anti-HLA DSAs (N = 9)	*p*-Value
All anti-HLA DSAs			
Number, median (Q1–Q3)	1.0 (1.0–1.0)	1 (1.0–2.0)	0.5008
HLA class specificity, n (%)			0.5796
I	6 (66.7)	5 (55.6)	
II	3 (33.3)	3 (33.3)	
I + II	0	1 (11.1)	
DSAs with the highest MFI			
HLA class specificity, n (%)			1
I	6 (66.7)	5 (55.6)	
II	3 (33.3)	4 (44.4)	
MFI, median (Q1–Q3)	983 (786–3110)	1905 (1381–2889)	0.4363

Abbreviations: HLA—human leukocyte antigen; DSAs—donor-specific antibodies; Q1–Q3—quartile 1–3; SD—standard deviation; and MFI—mean fluorescent intensity.

Table 3 shows the characteristics of anti-HLA DSAs at the time of biopsy. There was no significant difference between the persistent preformed and de novo anti-HLA DSAs regarding the number, HLA class specificity, MFI, C1q-binding capacity, and IgG subclasses.

However, the median MFI level of persistent preformed anti-HLA DSAs was higher in comparison with de novo anti-HLA DSAs (3843 vs. 1693, not statistically significant).

Table 3. Characteristics of anti-HLA DSAs after transplantation.

	Persistent Preformed Anti-HLA DSAs (N = 9)	De Novo Anti-HLA DSAs (N = 10)	*p*-Value
All anti-HLA DSAs			
Number, median (Q1–Q3)	1.0 (1.0–1.0)	1.0 (1.0–1.0)	0.1457
HLA class specificity, n (%)			0.1572
I	4 (44.5)	3 (30.0)	
II	3 (33.3)	7 (70.0)	
I + II	2 (22.2)	0	
DSAs with the highest MFI			
HLA class specificity, n (%)			0.5085
I	5 (55.5)	3 (30.0)	
II	4 (44.5)	7 (70.0)	
MFI, median (Q1–Q3)	3843 (2900–6206)	1693 (903–3832)	0.1564
C1q binding, n (%)	6 (66.7)	4 (40.0)	0.4825
IgG subclasses, n (%)			
IgG1	7 (77.8)	7 (70.0)	1
IgG2	1 (11.1)	1 (10.0)	1
IgG3	3 (33.3)	4 (40.0)	1
IgG4	1 (11.1)	3 (30.0)	0.6564

Abbreviations: HLA—human leukocyte antigen; DSAs—donor-specific antibodies; Q1–Q3—quartile 1–3; and MFI—mean fluorescent intensity.

3.3. Clinical Outcomes

All patients were followed for a minimum of 24 months after the biopsy procedure. The clinical outcomes are shown in Table 4. The combined endpoint of the study (>30% decline in eGFR or death-censored graft loss) was reached by 66.7% of patients with persistent preformed DSAs, 50% of patients with de novo DSAs, 11.1% of patients with resolved preformed DSAs, and 10% of patients without DSAs ($p < 0.0001$). Two patients (22.2%) with persistent preformed DSAs experienced graft loss. As many as 42.9% of patients with persistent preformed DSAs had proteinuria at the end of follow-up, ≥50 mg/dL ($p = 0.0001$). No statistical difference between the groups was identified regarding the time of follow-up after biopsy, median proteinuria at the end of follow-up, time from biopsy to the primary outcome, and mortality.

Table 4. Clinical outcomes.

	No Anti-HLA DSAs (N = 80)	Resolved Preformed Anti-HLA DSAs (N = 9)	Persistent Preformed Anti-HLA DSAs (N = 9)	De Novo Anti-HLA DSAs (N = 10)	*p*-Value
Follow-up after biopsy, months, median (Q1–Q3)	38.5 (28.0–44.3)	33.0 (29.0–39.0)	45.0 (41.0–47.0)	41.5 (36.3–46.5)	0.1969
eGFR at the end of follow-up, mL/min/1.73 m^2, median (Q1–Q3)	51.0 (34.5–64.5)	54.0 (45.0–62.0)	23.5 (17.8–36.3)	40.0 (32.0–50.0)	0.0188

Table 4. *Cont.*

	No Anti-HLA DSAs (N = 80)	Resolved Preformed Anti-HLA DSAs (N = 9)	Persistent Preformed Anti-HLA DSAs (N = 9)	De Novo Anti-HLA DSAs (N = 10)	*p*-Value
Proteinuria at the end of follow-up ≥50 mg/dL, n (%)	2 (2.5)	1 (12.5)	3 (42.9)	0	0.0001
Proteinuria at the end of follow-up, median (Q1–Q3)	0 (0–0)	0 (0–0)	0 (0–51.5)	0 (0–0)	0.2471
>30% decline in eGFR, n (%)	8 (10.0)	1 (11.1)	6 (66.7)	5 (50.0)	<0.0001
Death-censored graft loss, n (%)	1 (1.3)	0	2 (22.2)	1 (10.0)	0.0095
Combined endpoint: >30% decline in eGFR or graft loss, n (%)	9 (11.3)	1 (11.1)	6 (66.7)	5 (50.0)	<0.0001
Time from biopsy to combined endpoint, months, mean ± SD	25.3 ± 9.05	40	19.5 ± 10.03	28.2 ± 12.60	0.2714
Death event, n (%)	4 (5.0)	0	2 (22.2)	0	0.1151

Abbreviations: HLA—human leukocyte antigen; DSAs—donor-specific antibodies; Q1–Q3—quartile 1–3; eGFR—estimated glomerular filtration rate; and SD—standard deviation.

3.4. Survival Analysis

The event-free survival according to DSA status is shown in Figure 1 and the univariate Cox analysis is demonstrated in Table 5. Compared to patients without anti-HLA DSAs, the persistence of preformed anti-HLA DSAs after KTx and development of de novo anti-HLA DSAs at the time of biopsy were significantly associated with inferior survival ($p < 0.0001$ and $p = 0.0090$, respectively). However, there was no statistical difference regarding the combined endpoint survival between patients with resolved preformed anti-HLA DSAs after transplantation and patients without anti-HLA DSAs ($p = 0.918$).

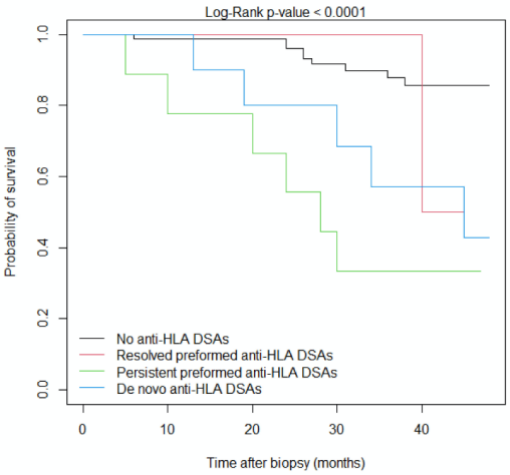

Figure 1. Event-free Kaplan–Meier survival curves according to anti-HLA DSA status at the time of biopsy.

Table 5. Univariate Cox proportional hazards model for the combined endpoint according to the evolution of preformed anti-HLA DSAs and development of de novo anti-HLA DSAs at the time of biopsy.

DSA Status	Hazard Ratio	95% Confidence Interval	*p*-Value
No anti-HLA DSAs	Ref.		
Resolved preformed anti-HLA DSAs	1.07	0.136–8.481	0.9470
Persistent preformed anti-HLA DSAs	8.18	2.902–23.03	<0.0001
De novo anti-HLA DSAs	4.61	1.541–13.793	0.0063

Abbreviations: HLA—human leukocyte antigen; DSAs—donor-specific antibodies.

The multivariate Cox regression model for the risk of the combined endpoint is depicted in Table 6. In this model, the identification of persistent preformed anti-HLA DSAs at the time of biopsy is the most significant predictor of inferior graft outcomes (HR = 5.96, $p = 0.0011$), followed by the occurrence of de novo anti-HLA DSAs (HR = 4.48, $p = 0.0079$), proteinuria at biopsy $\geq$ 50 mg/dL (HR = 1.02, $p = 0.0428$), and increased donor's age, with borderline statistical significance (HR = 1.03, $p = 0.0530$).

Table 6. Multivariate Cox proportional hazards model for the combined endpoint. To build the model, a univariate analysis was undertaken, and significant factors were entered into the multivariate model. A backward stepwise elimination method was used.

Variable	Hazard Ratio	95% Confidence Interval	*p*-Value
Donor's age (per 1-year increase)	1.03	0.999–1.066	0.0530
Proteinuria at biopsy $\geq$50 mg/dL (vs. <50 mg/dL)	1.02	1.001–1.036	0.0428
DSA status			
No anti-HLA DSAs	Ref.		
Resolved preformed anti-HLA DSAs	1.10	0.139–8.676	0.9305
Persistent preformed anti-HLA DSAs	5.96	2.041–17.431	0.0011
De novo anti-HLA DSAs	4.48	1.483–13.520	0.0079

Abbreviations: DSA—donor-specific antibody; HLA—human leukocyte antigen.

3.5. Factors Associated with DSA Status

Multivariate logistic regression analysis revealed several clinical, independent variables significant with DSA status (Table 7). The use of anti-thymocyte globulin therapy was a risk factor for resolved preformed anti-HLA DSAs. The persistence of preformed anti-HLA DSAs was associated with previous transplantation and the increased age of the donor. Moreover, a prior transplant was identified to be a risk factor for the development of de novo anti-HLA DSAs.

Table 7. Factors associated with DSA status.

Variable	Odds Ratio	95% Confidence Interval	*p*-Value
Resolved preformed anti-HLA DSAs			
Induction therapy (ATG vs. None)	18.25	7.40–44.97	0.0013
Induction therapy (Basiliximab vs. None)	4.56	1.61–12.87	0.1434
Persistent preformed anti-HLA DSAs			
Previous transplantation (yes vs. no)	25.77	10.34–64.23	0.0004
Donor's age (per 1-year increase)	1.08	1.04–1.13	0.0424
De novo anti-HLA DSAs			
Previous transplantation (yes vs. no)	31.64	13.48–74.23	<0.0001

Abbreviations: HLA—human leukocyte antigen; DSAs—donor-specific antibodies; and ATG—anti-thymocyte globulin.

4. Discussion

In the study, the clinical impact of the evolution of anti-HLA DSAs in kidney transplant recipients was assessed. In recent years, transplant matching has evolved due to the development of new technologies. Moreover, the characteristics of donors and recipients have changed, which alters KTx outcomes [21]. Currently, in most transplant centers, histocompatibility testing for solid organ transplantation includes ABO blood group compatibility, HLA donor–recipient matching, and crossmatching testing [22]. Anti-HLA antibody screening is crucial for assessing the immunological risk in kidney transplant recipients. However, the interpretation of DSAs should be conducted with great care; it demands an understanding of the complexity of antibodies and the technical aspects of detection assays [23].

In the cohort, 50% of patients with preformed anti-HLA DSAs cleared their antibodies after transplantation. None of the patients received desensitization treatment. This is directly in line with previous findings by Sanev et al. In their large study, which included 924 kidney transplant recipients, they also demonstrated that the persistence of DSAs is associated with higher MFI values and antibodies directed against HLA class II [10]. However, this is not shown in our analysis, probably due to the smaller number of patients.

Our results present that KTx recipients with resolved preformed anti-HLA DSAs have similar renal allograft outcomes regarding the eGFR and graft survival compared to patients without DSAs. Overall, these findings are in accordance with the results reported by other researchers [10,24,25]. The mechanism of clearance of DSAs after transplant without desensitization therapy is not fully explained. It could be hypothesized that an immunosuppressive regimen used after transplantation could decrease the production of weak antibodies [10]. In our study, the clearance of DSAs was associated with the use of ATG. It could be speculated that some preformed DSAs could be clinically irrelevant or in some way may be influenced by ATG. Another circumstance that potentially drives DSA disappearance is the development of graft accommodation, but this process is still under investigation [26].

The detection of persistent preformed anti-HLA DSAs at the time of biopsy and the identification of de novo anti-HLA DSAs are the main independent predictors of worse graft outcomes, defined as a 30% sustained decline of the GFR or graft failure. No significant difference between the characteristics of these antibodies was found. This might imply that current, circulating DSAs are more essential in the prediction of the renal allograft compared to resolved DSAs. A similar conclusion was reached in studies involving not only adult renal transplant recipients but also patients after other solid organ transplantation such as heart, lung, or intestine [27–30]. A history of previous transplantation is independently associated with the persistence of preformed DSAs. This is consistent with what has been found by Caillard et al. [24]. In addition, the persistence of DSAs was associated with increased donor age. It is difficult to explain such results, but it should be mentioned that the donor's age was also determined to be a risk factor for overall graft failure [31]. In our model, prior transplantation is also associated with the development of de novo DSAs. Similar findings were reported before in the liver transplant recipients cohort [32]. These results could suggest that in the case of kidney retransplantation, immunological status and histocompatibility in an individual patient need to be carefully assessed.

Our findings highlight the clinical impact of the clearance of anti-HLA DSAs after renal transplantation. This is particularly important within the context of emerging new desensitization therapies [33,34]. However, factors leading to the clearance or persistence of preformed anti-HLA DSAs should be further investigated. In clinical practice, in kidney transplant recipients, therapeutic decisions should be based on the detected, circulating anti-HLA DSAs rather than considering pre-transplant antibodies. Nevertheless, there is a fundamental need for more studies assessing post-transplant DSA positivity without allograft dysfunction with reference to the utility of kidney allograft biopsies or potential noninvasive biomarkers such as donor-derived cell-free DNA [18].

One of the limitations of the present study is the post hoc, retrospective nature of the analysis. In addition, a relatively modest number of patients had been identified with DSAs in this single-center investigation. The characteristics of pre-transplant antibodies such as C1q-binding capacity and IgG subclasses were not assessed. Donor typing did not include HLA-C, -DP, and -DQ, therefore, antibodies against these antigens were not analyzed and the ABMR risk could not be assessed precisely. The cross-reactive epitope groups were not considered. Patients were tested for post-transplant DSAs only at the time of kidney allograft biopsy, which was performed 3 to 24 months after the transplant. Follow-up biopsies are not available. The use of different agents in the induction of immunosuppressive therapy was inevitable.

To conclude, kidney transplant recipients with resolved preformed anti-HLA DSAs have similar graft prognoses as patients without DSAs. The persistence of preformed anti-HLA DSAs after kidney transplantation and the occurrence of de novo anti-HLA DSAs are independent predictors for inferior long-term allograft outcomes. Candidates for kidney retransplantation should undergo a cautious immunological risk assessment. These findings are valuable in light of the development of new desensitization protocols. Although transplant immunology evolves rapidly, there are many gaps in the current knowledge and further studies are needed to answer clinical questions.

Author Contributions: Conceptualization, M.G. and M.D.; methodology, M.G. and M.D.; validation, K.C. and K.Z.; formal analysis, M.G.; investigation, M.G., K.C. and K.Z.; resources, M.G., K.C., K.Z. and M.D.; data curation, M.G., K.C. and K.Z.; writing—original graft preparation, M.G.; writing—review and editing, M.D.; visualization, M.G.; supervision, M.D.; project administration, M.G.; funding acquisition, M.G. All authors have read and agreed to the published version of the manuscript.

Funding: This research was financed from budget funds for science in the years 2018–2021 as a research project under the "Diamond Grant" program (no. DI2017 002147) supported by the Ministry of Science and Higher Education of Poland.

Institutional Review Board Statement: The study was conducted in accordance with the Declaration of Helsinki and approved by the Institutional Ethics Committee of the Medical University of Warsaw (KB/160/2018).

Informed Consent Statement: Informed consent was obtained from all subjects involved in the study.

Data Availability Statement: The data presented in this study are available on request from the corresponding author.

Conflicts of Interest: The authors declare no conflict of interest. The funders had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript; or in the decision to publish the results.

References

1. Konvalinka, A.; Tinckam, K. Utility of HLA Antibody Testing in Kidney Transplantation. *J. Am. Soc. Nephrol.* **2015**, *26*, 1489–1502. [CrossRef] [PubMed]
2. Sullivan, H.C.; Gebel, H.M.; Bray, R.A. Understanding solid-phase HLA antibody assays and the value of MFI. *Hum. Immunol.* **2017**, *78*, 471–480. [CrossRef] [PubMed]
3. Viglietti, D.; Loupy, A.; Vernerey, D.; Bentlejewski, C.; Gosset, C.; Aubert, O.; Duong van Huyen, J.P.; Jouven, X.; Legendre, C.; Glotz, D.; et al. Value of Donor-Specific Anti-HLA Antibody Monitoring and Characterization for Risk Stratification of Kidney Allograft Loss. *J. Am. Soc. Nephrol.* **2017**, *28*, 702–715. [CrossRef]
4. Muñoz-Herrera, C.M.; Gutiérrez-Bautista, J.F.; López-Nevot, M.Á. Complement Binding Anti-HLA Antibodies and the Survival of Kidney Transplantation. *J. Clin. Med.* **2023**, *12*, 2335. [CrossRef] [PubMed]
5. Zhang, R. Donor-Specific Antibodies in Kidney Transplant Recipients. *Clin. J. Am. Soc. Nephrol.* **2018**, *13*, 182–192. [CrossRef]
6. Oweira, H.; Ramouz, A.; Ghamarnejad, O.; Khajeh, E.; Ali-Hasan-Al-Saegh, S.; Nikbakhsh, R.; Reißfelder, C.; Rahbari, N.; Mehrabi, A.; Sadeghi, M. Risk Factors of Rejection in Renal Transplant Recipients: A Narrative Review. *J. Clin. Med.* **2022**, *11*, 1392. [CrossRef]
7. Sharma, A.; Cherukuri, A.; Mehta, R.B.; Sood, P.; Hariharan, S. High Calcineurin Inhibitor Intrapatient Variability Is Associated with Renal Allograft Inflammation, Chronicity, and Graft Loss. *Transplant. Direct* **2019**, *5*, e424. [CrossRef]
8. Coti, I.; Wenda, S.; Andreeva, A.; Kocher, A.; Laufer, G.; Fischer, G.; Andreas, M. Donor-specific HLA antibodies after fresh decellularized vs cryopreserved native allograft implantation. *Hla* **2020**, *96*, 580–588. [CrossRef]

9. Snanoudj, R.; Kamar, N.; Cassuto, E.; Caillard, S.; Metzger, M.; Merville, P.; Thierry, A.; Jollet, I.; Grimbert, P.; Anglicheau, D.; et al. Epitope load identifies kidney transplant recipients at risk of allosensitization following minimization of immunosuppression. *Kidney Int.* **2019**, *95*, 1471–1485. [CrossRef]
10. Senev, A.; Lerut, E.; Van Sandt, V.; Coemans, M.; Callemeyn, J.; Sprangers, B.; Kuypers, D.; Emonds, M.P.; Naesens, M. Specificity, strength, and evolution of pretransplant donor-specific HLA antibodies determine outcome after kidney transplantation. *Am. J. Transplant.* **2019**, *19*, 3100–3113. [CrossRef]
11. Redondo-Pachón, D.; Pérez-Sáez, M.J.; Mir, M.; Gimeno, J.; Llinás, L.; García, C.; Hernández, J.J.; Yélamos, J.; Pascual, J.; Crespo, M. Impact of persistent and cleared preformed HLA DSA on kidney transplant outcomes. *Hum. Immunol.* **2018**, *79*, 424–431. [CrossRef]
12. Ziemann, M.; Altermann, W.; Angert, K.; Arns, W.; Bachmann, A.; Bakchoul, T.; Banas, B.; von Borstel, A.; Budde, K.; Ditt, V.; et al. Preformed Donor-Specific HLA Antibodies in Living and Deceased Donor Transplantation: A Multicenter Study. *Clin. J. Am. Soc. Nephrol.* **2019**, *14*, 1056–1066. [CrossRef]
13. Aubert, O.; Loupy, A.; Hidalgo, L.; Duong van Huyen, J.-P.; Higgins, S.; Viglietti, D.; Jouven, X.; Glotz, D.; Legendre, C.; Lefaucheur, C.; et al. Antibody-Mediated Rejection Due to Preexisting versus De Novo Donor-Specific Antibodies in Kidney Allograft Recipients. *J. Am. Soc. Nephrol.* **2017**, *28*, 1912–1923. [CrossRef]
14. Haas, M.; Mirocha, J.; Reinsmoen, N.L.; Vo, A.A.; Choi, J.; Kahwaji, J.M.; Peng, A.; Villicana, R.; Jordan, S.C. Differences in pathologic features and graft outcomes in antibody-mediated rejection of renal allografts due to persistent/recurrent versus de novo donor-specific antibodies. *Kidney Int.* **2017**, *91*, 729–737. [CrossRef]
15. Loupy, A.; Suberbielle-Boissel, C.; Hill, G.S.; Lefaucheur, C.; Anglicheau, D.; Zuber, J.; Martinez, F.; Thervet, E.; Méjean, A.; Charron, D.; et al. Outcome of subclinical antibody-mediated rejection in kidney transplant recipients with preformed donor-specific antibodies. *Am. J. Transplant.* **2009**, *9*, 2561–2570. [CrossRef]
16. Yamamoto, T.; Watarai, Y.; Takeda, A.; Tsujita, M.; Hiramitsu, T.; Goto, N.; Narumi, S.; Katayama, A.; Morozumi, K.; Uchida, K.; et al. De Novo Anti-HLA DSA Characteristics and Subclinical Antibody-Mediated Kidney Allograft Injury. *Transplantation* **2016**, *100*, 2194–2202. [CrossRef]
17. Madill-Thomsen, K.S.; Böhmig, G.A.; Bromberg, J.; Einecke, G.; Eskandary, F.; Gupta, G.; Hidalgo, L.G.; Myslak, M.; Viklicky, O.; Perkowska-Ptasinska, A.; et al. Donor-Specific Antibody Is Associated with Increased Expression of Rejection Transcripts in Renal Transplant Biopsies Classified as No Rejection. *J. Am. Soc. Nephrol.* **2021**, *32*, 2743–2758. [CrossRef]
18. Lefaucheur, C.; Louis, K.; Morris, A.B.; Taupin, J.L.; Nickerson, P.; Tambur, A.R.; Gebel, H.M.; Reed, E.F. Clinical recommendations for posttransplant assessment of anti-HLA (Human Leukocyte Antigen) donor-specific antibodies: A Sensitization in Transplantation: Assessment of Risk consensus document. *Am. J. Transplant.* **2023**, *23*, 115–132. [CrossRef]
19. Gniewkiewicz, M.; Czerwinska, K.; Zielniok, K.; Durlik, M. Association of Circulating Anti-HLA Donor-Specific Antibodies and Their Characteristics, including C1q-Binding Capacity, in Kidney Transplant Recipients with Long-Term Renal Graft Outcomes. *J. Clin. Med.* **2023**, *12*, 1312. [CrossRef]
20. Haas, M.; Loupy, A.; Lefaucheur, C.; Roufosse, C.; Glotz, D.; Seron, D.; Nankivell, B.J.; Halloran, P.F.; Colvin, R.B.; Akalin, E.; et al. The Banff 2017 Kidney Meeting Report: Revised diagnostic criteria for chronic active T cell-mediated rejection, antibody-mediated rejection, and prospects for integrative endpoints for next-generation clinical trials. *Am. J. Transplant.* **2018**, *18*, 293–307. [CrossRef]
21. Bestard, O.; Thaunat, O.; Bellini, M.I.; Böhmig, G.A.; Budde, K.; Claas, F.; Couzi, L.; Furian, L.; Heemann, U.; Mamode, N.; et al. Alloimmune Risk Stratification for Kidney Transplant Rejection. *Transplant. Int.* **2022**, *35*, 10138. [CrossRef] [PubMed]
22. Markkinen, S.; Helanterä, I.; Lauronen, J.; Lempinen, M.; Partanen, J.; Hyvärinen, K. Mismatches in Gene Deletions and Kidney-related Proteins as Candidates for Histocompatibility Factors in Kidney Transplantation. *Kidney Int. Rep.* **2022**, *7*, 2484–2494. [CrossRef] [PubMed]
23. Schinstock, C.A.; Gandhi, M.J.; Stegall, M.D. Interpreting Anti-HLA Antibody Testing Data: A Practical Guide for Physicians. *Transplantation* **2016**, *100*, 1619–1628. [CrossRef] [PubMed]
24. Caillard, S.; Becmeur, C.; Gautier-Vargas, G.; Olagne, J.; Muller, C.; Cognard, N.; Perrin, P.; Braun, L.; Heibel, F.; Lefebre, F.; et al. Pre-existing donor-specific antibodies are detrimental to kidney allograft only when persistent after transplantation. *Transplant. Int.* **2017**, *30*, 29–40. [CrossRef]
25. Kimball, P.M.; Baker, M.A.; Wagner, M.B.; King, A. Surveillance of alloantibodies after transplantation identifies the risk of chronic rejection. *Kidney Int.* **2011**, *79*, 1131–1137. [CrossRef]
26. Kenta, I.; Takaaki, K. Molecular Mechanisms of Antibody-Mediated Rejection and Accommodation in Organ Transplantation. *Nephron* **2020**, *144* (Suppl. 1), 2–6. [CrossRef]
27. Irving, C.A.; Carter, V.; Gennery, A.R.; Parry, G.; Griselli, M.; Hasan, A.; Kirk, C.R. Effect of persistent versus transient donor-specific HLA antibodies on graft outcomes in pediatric cardiac transplantation. *J. Heart Lung Transplant.* **2015**, *34*, 1310–1317. [CrossRef]
28. Le Pavec, J.; Suberbielle, C.; Lamrani, L.; Feuillet, S.; Savale, L.; Dorfmüller, P.; Stephan, F.; Mussot, S.; Mercier, O.; Fadel, E. De-novo donor-specific anti-HLA antibodies 30 days after lung transplantation are associated with a worse outcome. *J. Heart Lung Transplant.* **2016**, *35*, 1067–1077. [CrossRef]
29. Abu-Elmagd, K.M.; Wu, G.; Costa, G.; Lunz, J.; Martin, L.; Koritsky, D.A.; Murase, N.; Irish, W.; Zeevi, A. Preformed and de novo donor specific antibodies in visceral transplantation: Long-term outcome with special reference to the liver. *Am. J. Transplant.* **2012**, *12*, 3047–3060. [CrossRef]

30. Fujiyama, N.; Satoh, S.; Saito, M.; Numakura, K.; Inoue, T.; Yamamoto, R.; Saito, T.; Kanda, S.; Narita, S.; Mitobe, Y.; et al. Impact of persistent preformed and de novo donor-specific antibodies detected at 1 year after kidney transplantation on long-term graft survival in Japan: A retrospective study. *Clin. Exp. Nephrol.* **2019**, *23*, 1398–1406. [CrossRef]
31. Betjes, M.G.H.; Sablik, K.S.; Otten, H.G.; Roelen, D.L.; Claas, F.H.; de Weerd, A. Pretransplant Donor-Specific Anti-HLA Antibodies and the Risk for Rejection-Related Graft Failure of Kidney Allografts. *J. Transplant.* **2020**, *2020*, 5694670. [CrossRef]
32. Del Bello, A.; Congy-Jolivet, N.; Muscari, F.; Lavayssière, L.; Esposito, L.; Cardeau-Desangles, I.; Guitard, J.; Dörr, G.; Suc, B.; Duffas, J.P.; et al. Prevalence, Incidence and Risk Factors for Donor-Specific Anti-HLA Antibodies in Maintenance Liver Transplant Patients. *Am. J. Transplant.* **2014**, *14*, 867–875. [CrossRef]
33. Grimaldi, V.; Pagano, M.; Moccia, G.; Maiello, C.; De Rosa, P.; Napoli, C. Novel insights in the clinical management of hyperimmune patients before and after transplantation. *Curr. Res. Immunol.* **2023**, *4*, 100056. [CrossRef]
34. Leal, R.; Pardinhas, C.; Martinho, A.; Sá, H.O.; Figueiredo, A.; Alves, R. Strategies to Overcome HLA Sensitization and Improve Access to Retransplantation after Kidney Graft Loss. *J. Clin. Med.* **2022**, *11*, 5753. [CrossRef]

Disclaimer/Publisher's Note: The statements, opinions and data contained in all publications are solely those of the individual author(s) and contributor(s) and not of MDPI and/or the editor(s). MDPI and/or the editor(s) disclaim responsibility for any injury to people or property resulting from any ideas, methods, instructions or products referred to in the content.

7. Podsumowanie i wnioski

1. U pacjentów po przeszczepieniu nerki od dawcy zmarłego wykrycie przeciwciał przeciwko antygenom zgodności tkankowej dawcy oraz ich zdolność wiązania dopełniacza C1q są predyktorami gorszej czynności narządu przeszczepionego oraz utraty przeszczepu.

   Obecnie analiza zdolności wiązania C1q jest badaniem kosztownym, ale nieinwazyjnym, dostępnym i może być brana pod uwagę w praktyce klinicznej w opiece nad pacjentem po przeszczepieniu nerki. Stwierdzenie u biorcy nerki przeszczepionej anty-HLA DSA, a w szczególności tych wiążących C1q powinno skutkować zwiększeniem czujności lekarza transplantologa, dokładną analizą leczenia immunosupresyjnego czy też zorganizowaniem częstszych kontroli pacjenta. Badanie uzupełnia literaturę podejmującą temat zastosowania oznaczenia zdolności wiązania C1q przeciwciał anty-HLA DSA u biorców nerki przeszczepionej.

2. Pacjenci z zanikłymi preformowanymi anty-HLA DSA po przeszczepieniu nerki mają podobne wyniki odległe jak pacjenci bez nich. Stwierdzenie przetrwałych preformowanych anty-HLA DSA oraz pojawienie się anty-HLA DSA *de novo* po przeszczepieniu nerki są predyktorami gorszej czynności i niewydolności nerki przeszczepionej. Zaniknięcie preformowanych anty-HLA DSA jest związane z użyciem globuliny antytymocytarnej jako indukcji immunosupresji. Przetrwanie preformowanych anty-HLA DSA po transplantacji jest związane ze starszym wiekiem dawcy oraz z poprzednią transplantacją. Czynnikiem ryzyka rozwoju przeciwciał *de novo* po przeszczepieniu jest wcześniejsze przeszczepienie.

   Kandydatów do przeszczepienia nerki, a zwłaszcza do retransplantacji należy poddać starannej ocenie ryzyka immunologicznego. Powyższe badania mogą być szczególnie przydatne w świetle rozwoju nowych protokołów odczulania przed przeszczepieniem.

8. Piśmiennictwo

1. McCormick, F.; Held, P.J.; Chertow, G.M. The Terrible Toll of the Kidney Shortage. *J Am Soc Nephrol* **2018**, *29*, 2775-2776, doi:10.1681/asn.2018101030.
2. Coemans, M.; Süsal, C.; Döhler, B.; Anglicheau, D.; Giral, M.; Bestard, O.; Legendre, C.; Emonds, M.P.; Kuypers, D.; Molenberghs, G., et al. Analyses of the short- and long-term graft survival after kidney transplantation in Europe between 1986 and 2015. *Kidney Int* **2018**, *94*, 964-973, doi:10.1016/j.kint.2018.05.018.
3. Sellarés, J.; de Freitas, D.G.; Mengel, M.; Reeve, J.; Einecke, G.; Sis, B.; Hidalgo, L.G.; Famulski, K.; Matas, A.; Halloran, P.F. Understanding the causes of kidney transplant failure: the dominant role of antibody-mediated rejection and nonadherence. *Am J Transplant* **2012**, *12*, 388-399, doi:10.1111/j.1600-6143.2011.03840.x.
4. Cioni, M.; Nocera, A.; Innocente, A.; Tagliamacco, A.; Trivelli, A.; Basso, S.; Quartuccio, G.; Fontana, I.; Magnasco, A.; Drago, F., et al. De Novo Donor-Specific HLA Antibodies Developing Early or Late after Transplant Are Associated with the Same Risk of Graft Damage and Loss in Nonsensitized Kidney Recipients. *J Immunol Res* **2017**, *2017*, 1747030, doi:10.1155/2017/1747030.
5. El Band, J.E.K.; Llorente, S.; Martinez-Garcia, P.; Alfaro, R.; Jimenez-Coll, V.; Boix, F.; Galián, J.A.; Martinez-Banaclocha, H.; Botella, C.; Moya-Quiles, M.R., et al. Evaluation of Antibodies Directed Against Two GPCRs, Anti-AT1R and Anti-ETAR, on Kidney Transplant Outcome. *Curr Protein Pept Sci* **2021**, *22*, 745-757, doi:10.2174/1389203722666210706163149.
6. Filippone, E.J.; Farber, J.L. Histologic Antibody-mediated Kidney Allograft Rejection in the Absence of Donor-specific HLA Antibodies. *Transplantation* **2021**, *105*, e181-e190, doi:10.1097/tp.0000000000003797.
7. Sullivan, H.C.; Gebel, H.M.; Bray, R.A. Understanding solid-phase HLA antibody assays and the value of MFI. *Hum Immunol* **2017**, *78*, 471-480, doi:10.1016/j.humimm.2017.05.007.
8. Butler, C.L.; Valenzuela, N.M.; Thomas, K.A.; Reed, E.F. Not All Antibodies Are Created Equal: Factors That Influence Antibody Mediated Rejection. *J Immunol Res* **2017**, *2017*, 7903471, doi:10.1155/2017/7903471.
9. Heilman, R.L.; Nijim, A.; Desmarteau, Y.M.; Khamash, H.; Pando, M.J.; Smith, M.L.; Chakkera, H.A.; Huskey, J.; Valdez, R.; Reddy, K.S. De novo donor-specific human leukocyte antigen antibodies early after kidney transplantation. *Transplantation* **2014**, *98*, 1310-1315, doi:10.1097/tp.0000000000000216.

10. Tambur, A.R.; Campbell, P.; Chong, A.S.; Feng, S.; Ford, M.L.; Gebel, H.; Gill, R.G.; Kelsoe, G.; Kosmoliaptsis, V.; Mannon, R.B., et al. Sensitization in transplantation: Assessment of risk (STAR) 2019 Working Group Meeting Report. *Am J Transplant* **2020**, *20*, 2652-2668, doi:10.1111/ajt.15937.
11. Sharma, A.; Jorgensen, D.R.; Mehta, R.B.; Sood, P.; Puttarajappa, C.M.; Wu, C.M.; Tevar, A.D.; Molinari, M.; Zeevi, A.; Hariharan, S. The Clinical Impact of Anti-HLA Donor Specific Antibody Detection Through First Year Screening on Stable Kidney Transplant Recipients. *Transpl Int* **2022**, *35*, 10094, doi:10.3389/ti.2022.10094.
12. Tatapudi, V.S.; Kopchaliiska, D.; da Gente, G.J.; Buenaventura, O.F.; Singh, M.; Laszik, Z.; Adey, D.B.; Rajalingam, R. Solid-Phase C1q/C3d Fixing Readouts Correlate with High Median Fluorescence Intensity (MFI) De Novo Donor-Specific HLA Antibodies and $C4d^{+}$ Antibody-Mediated Rejection in Kidney Transplant Recipients. *Ann Transplant* **2021**, *26*, e934175, doi:10.12659/aot.934175.
13. Butiu, M.; Obrisca, B.; Sibulesky, L.; Bakthavatsalam, R.; Smith, K.D.; Gimferrer, I.; Warner, P.; Ismail, G.; Leca, N. Donor-derived Cell-free DNA Complements De Novo Class II DSA in Detecting Late Alloimmune Injury Post Kidney Transplantation. *Transplant Direct* **2022**, *8*, e1285, doi:10.1097/txd.0000000000001285.
14. Lefaucheur, C.; Loupy, A.; Hill, G.S.; Andrade, J.; Nochy, D.; Antoine, C.; Gautreau, C.; Charron, D.; Glotz, D.; Suberbielle-Boissel, C. Preexisting donor-specific HLA antibodies predict outcome in kidney transplantation. *J Am Soc Nephrol* **2010**, *21*, 1398-1406, doi:10.1681/asn.2009101065.
15. Mizutani, K.; Terasaki, P.; Hamdani, E.; Esquenazi, V.; Rosen, A.; Miller, J.; Ozawa, M. The importance of anti-HLA-specific antibody strength in monitoring kidney transplant patients. *Am J Transplant* **2007**, *7*, 1027-1031, doi:10.1111/j.1600-6143.2006.01721.x.
16. Malheiro, J.; Tafulo, S.; Dias, L.; Martins, S.; Fonseca, I.; Beirão, I.; Castro-Henriques, A.; Cabrita, A. Determining donor-specific antibody C1q-binding ability improves the prediction of antibody-mediated rejection in human leucocyte antigen-incompatible kidney transplantation. *Transpl Int* **2017**, *30*, 347-359, doi:10.1111/tri.12873.
17. Viglietti, D.; Bouatou, Y.; Kheav, V.D.; Aubert, O.; Suberbielle-Boissel, C.; Glotz, D.; Legendre, C.; Taupin, J.L.; Zeevi, A.; Loupy, A., et al. Complement-binding anti-HLA antibodies are independent predictors of response to treatment in kidney recipients with antibody-mediated rejection. *Kidney Int* **2018**, *94*, 773-787, doi:10.1016/j.kint.2018.03.015.

18. Yell, M.; Muth, B.L.; Kaufman, D.B.; Djamali, A.; Ellis, T.M. C1q Binding Activity of De Novo Donor-specific HLA Antibodies in Renal Transplant Recipients With and Without Antibody-mediated Rejection. *Transplantation* **2015**, *99*, 1151-1155, doi:10.1097/tp.0000000000000699.
19. Navas, A.; Molina, J.; Agüera, M.L.; Guler, I.; Jurado, A.; Rodríguez-Benot, A.; Alonso, C.; Solana, R. Characterization of the C1q-Binding Ability and the IgG1-4 Subclass Profile of Preformed Anti-HLA Antibodies by Solid-Phase Assays. *Front Immunol* **2019**, *10*, 1712, doi:10.3389/fimmu.2019.01712.
20. Schaub, S.; Hönger, G.; Amico, P. The complexity of the humoral immune response against HLA antigens. *Transpl Int* **2014**, *27*, 249-250, doi:10.1111/tri.12230.
21. Lefaucheur, C.; Viglietti, D.; Bentlejewski, C.; Duong van Huyen, J.P.; Vernerey, D.; Aubert, O.; Verine, J.; Jouven, X.; Legendre, C.; Glotz, D., et al. IgG Donor-Specific Anti-Human HLA Antibody Subclasses and Kidney Allograft Antibody-Mediated Injury. *J Am Soc Nephrol* **2016**, *27*, 293-304, doi:10.1681/asn.2014111120.
22. Hung, S.Y.; Lin, T.M.; Chang, M.Y.; Wang, H.H.; Lee, Y.C.; Ho, L.C.; Chen, Y.T.; Hung, C.M.; Liou, H.H. Risk factors of sensitization to human leukocyte antigen in end-stage renal disease patients. *Hum Immunol* **2014**, *75*, 531-535, doi:10.1016/j.humimm.2014.02.024.
23. Yeung, M.Y. Pre-formed DSA and kidney allograft outcomes. *J Bras Nefrol* **2020**, *42*, 136-137, doi:10.1590/2175-8239-jbn-2020-0043.
24. Oweira, H.; Ramouz, A.; Ghamarnejad, O.; Khajeh, E.; Ali-Hasan-Al-Saegh, S.; Nikbakhsh, R.; Reißfelder, C.; Rahbari, N.; Mehrabi, A.; Sadeghi, M. Risk Factors of Rejection in Renal Transplant Recipients: A Narrative Review. *J Clin Med* **2022**, *11*, doi:10.3390/jcm11051392.
25. Olszowska-Zaremba, N.; Zagożdżon, R.; Gozdowska, J. Accuracy of virtual crossmatch (VXM) prediction of physical crossmatch (PXM) results of donor specific antibody (DSA) in routine pretransplant settings-a single-center experience. *Transpl Immunol* **2022**, *72*, 101583, doi:10.1016/j.trim.2022.101583.
26. Leal, R.; Pardinhas, C.; Martinho, A.; Sá, H.O.; Figueiredo, A.; Alves, R. Strategies to Overcome HLA Sensitization and Improve Access to Retransplantation after Kidney Graft Loss. *J Clin Med* **2022**, *11*, doi:10.3390/jcm11195753.
27. Sharma, A.; Cherukuri, A.; Mehta, R.B.; Sood, P.; Hariharan, S. High Calcineurin Inhibitor Intrapatient Variability Is Associated With Renal Allograft Inflammation, Chronicity, and Graft Loss. *Transplantation Direct* **2019**, *5*, e424, doi:10.1097/txd.0000000000000862.

28. Coti, I.; Wenda, S.; Andreeva, A.; Kocher, A.; Laufer, G.; Fischer, G.; Andreas, M. Donor-specific HLA antibodies after fresh decellularized vs cryopreserved native allograft implantation. *Hla* **2020**, *96*, 580-588, doi:10.1111/tan.14077.
29. Snanoudj, R.; Kamar, N.; Cassuto, E.; Caillard, S.; Metzger, M.; Merville, P.; Thierry, A.; Jollet, I.; Grimbert, P.; Anglicheau, D., et al. Epitope load identifies kidney transplant recipients at risk of allosensitization following minimization of immunosuppression. *Kidney Int* **2019**, *95*, 1471-1485, doi:10.1016/j.kint.2018.12.029.
30. Senev, A.; Lerut, E.; Van Sandt, V.; Coemans, M.; Callemeyn, J.; Sprangers, B.; Kuypers, D.; Emonds, M.P.; Naesens, M. Specificity, strength, and evolution of pretransplant donor-specific HLA antibodies determine outcome after kidney transplantation. *Am J Transplant* **2019**, *19*, 3100-3113, doi:10.1111/ajt.15414.
31. Redondo-Pachón, D.; Pérez-Sáez, M.J.; Mir, M.; Gimeno, J.; Llinás, L.; García, C.; Hernández, J.J.; Yélamos, J.; Pascual, J.; Crespo, M. Impact of persistent and cleared preformed HLA DSA on kidney transplant outcomes. *Hum Immunol* **2018**, *79*, 424-431, doi:10.1016/j.humimm.2018.02.014.
32. Aubert, O.; Loupy, A.; Hidalgo, L.; Duong van Huyen, J.-P.; Higgins, S.; Viglietti, D.; Jouven, X.; Glotz, D.; Legendre, C.; Lefaucheur, C., et al. Antibody-Mediated Rejection Due to Preexisting versus De Novo Donor-Specific Antibodies in Kidney Allograft Recipients. *Journal of the American Society of Nephrology* **2017**, *28*, 1912-1923, doi:10.1681/asn.2016070797.
33. Haas, M.; Mirocha, J.; Reinsmoen, N.L.; Vo, A.A.; Choi, J.; Kahwaji, J.M.; Peng, A.; Villicana, R.; Jordan, S.C. Differences in pathologic features and graft outcomes in antibody-mediated rejection of renal allografts due to persistent/recurrent versus de novo donor-specific antibodies. *Kidney Int* **2017**, *91*, 729-737, doi:10.1016/j.kint.2016.10.040.
34. Loupy, A.; Suberbielle-Boissel, C.; Hill, G.S.; Lefaucheur, C.; Anglicheau, D.; Zuber, J.; Martinez, F.; Thervet, E.; Méjean, A.; Charron, D., et al. Outcome of subclinical antibody-mediated rejection in kidney transplant recipients with preformed donor-specific antibodies. *Am J Transplant* **2009**, *9*, 2561-2570, doi:10.1111/j.1600-6143.2009.02813.x.
35. Yamamoto, T.; Watarai, Y.; Takeda, A.; Tsujita, M.; Hiramitsu, T.; Goto, N.; Narumi, S.; Katayama, A.; Morozumi, K.; Uchida, K., et al. De Novo Anti-HLA DSA Characteristics and Subclinical Antibody-Mediated Kidney Allograft Injury. *Transplantation* **2016**, *100*, 2194-2202, doi:10.1097/tp.0000000000001012.

36. Madill-Thomsen, K.S.; Böhmig, G.A.; Bromberg, J.; Einecke, G.; Eskandary, F.; Gupta, G.; Hidalgo, L.G.; Myslak, M.; Viklicky, O.; Perkowska-Ptasinska, A., et al. Donor-Specific Antibody Is Associated with Increased Expression of Rejection Transcripts in Renal Transplant Biopsies Classified as No Rejection. *J Am Soc Nephrol* **2021**, *32*, 2743-2758, doi:10.1681/asn.2021040433.

37. Lefaucheur, C.; Louis, K.; Morris, A.B.; Taupin, J.L.; Nickerson, P.; Tambur, A.R.; Gebel, H.M.; Reed, E.F. Clinical recommendations for posttransplant assessment of anti-HLA (Human Leukocyte Antigen) donor-specific antibodies: A Sensitization in Transplantation: Assessment of Risk consensus document. *Am J Transplant* **2023**, *23*, 115-132, doi:10.1016/j.ajt.2022.11.013.

38. Nakamura, K.; Sawada, A.; Kita, Y.; Kono, J.; Masui, K.; Sato, T.; Sano, T.; Goto, T.; Akamatsu, S.; Ogawa, O., et al. Clinical characteristics of renal transplant recipients who developed de novo donor-specific antigen in Kyoto University Hospital: a case series. *Renal Replacement Therapy* **2022**, *8*, 10, doi:10.1186/s41100-022-00401-y.

39. Wu, K.; Schmidt, D.; López Del Moral, C.; Osmanodja, B.; Lachmann, N.; Zhang, Q.; Halleck, F.; Choi, M.; Bachmann, F.; Ronicke, S., et al. Poor Long-Term Renal Allograft Survival in Patients with Chronic Antibody-Mediated Rejection, Irrespective of Treatment-A Single Center Retrospective Study. *J Clin Med* **2021**, *11*, doi:10.3390/jcm11010199.

40. Waldecker, C.B.; Zgoura, P.; Seibert, F.S.; Gall, S.; Schenker, P.; Bauer, F.; Rohn, B.; Viebahn, R.; Babel, N.; Westhoff, T.H. Biopsy findings after detection of de novo donor-specific antibodies in renal transplant recipients: a single center experience. *J Nephrol* **2021**, *34*, 2017-2026, doi:10.1007/s40620-021-01040-y.

41. Kim, M.Y.; Brennan, D.C. Therapies for Chronic Allograft Rejection. *Front Pharmacol* **2021**, *12*, 651222, doi:10.3389/fphar.2021.651222.

42. Aubert, O.; Divard, G.; Pascual, J.; Oppenheimer, F.; Sommerer, C.; Citterio, F.; Tedesco, H.; Chadban, S.; Henry, M.; Vincenti, F., et al. Application of the iBox prognostication system as a surrogate endpoint in the TRANSFORM randomised controlled trial: proof-of-concept study. *BMJ Open* **2021**, *11*, e052138, doi:10.1136/bmjopen-2021-052138.

43. Grimaldi, V.; Pagano, M.; Moccia, G.; Maiello, C.; De Rosa, P.; Napoli, C. Novel insights in the clinical management of hyperimmune patients before and after transplantation. *Curr Res Immunol* **2023**, *4*, 100056, doi:10.1016/j.crimmu.2023.100056.

9. Opinia Komisji Bioetycznej

Komisja Bioetyczna
przy Warszawskim Uniwersytecie Medycznym

Tel.: 022/ 57 - 20 -303
Fax: 022/ 57 - 20 -165

ul. Żwirki i Wigury nr 61
02-091 Warszawa

e-mail: komisja.bioetyczna@wum.edu.pl
www.komisja-bioetyczna.wum.edu.pl

KB/..160../2018

Komisja Bioetyczna przy Warszawskim Uniwersytecie Medycznym
w dniu 17 września 2018 r. po zapoznaniu się z wnioskiem:

Prof.dr hab. n.med.Magdalena Durlik
Klinika Medycyny Transplantacyjnej ,Nefrologii
i Chorób Wewnętrznych,
ul. Nowogrodzka 59, 02-006 Warszawa

dotyczącym: wyrażenia opinii w sprawie badania pt. „ Ocena przydatności monitorowania przeciwciał przeciwko antygenom zgodności tkankowej dawcy u pacjentów po transplantacji nerki w stratyfikacji ryzyka immunologicznego utraty przeszczepu."

wyraża następującą
o p i n i ę

- stwierdza, że jest ono dopuszczalne i zgodne z zasadami naukowo-etycznymi*.
- ~~stwierdza, że jest ono niedopuszczalne i niezgodne z zasadami naukowo-etycznymi.*~~

Uwagi Komisji – *verte*

Komisja działa na podstawie art.29 ustawy z dnia 5.12.1996r. o zawodzie lekarza /Dz.U.nr 28/97 poz.152 wraz z późn.zm./, zarządzenia MZiOS z dn.11.05.1999r. w sprawie szczegółowych zasad powoływania i finansowania oraz trybu działania komisji bioetycznych /Dz.U.nr 47 poz.480/, Ustawy prawo farmaceutyczne z dnia 6 września 2001r. (Dz.U.Nr 126, poz. 1381 z późn. zm.) oraz Zarządzenie nr 56/2007 z dnia 15 października 2007r. w sprawie działania Komisji Bioetycznej przy Warszawskim Uniwersytecie Medycznym /Regulamin Komisji Bioetycznej przy Warszawskim Uniwersytecie Medycznym/.
Komisja działa zgodnie z zasadami GCP .
W załączeniu: skład komisji oraz lista obecności

Przewodnicząca Komisji Bioetycznej
Prof. dr hab. n. med. Magdalena Kuźma-Kozakiewicz

*niepotrzebne skreślić

10. Oświadczenia współautorów

Warszawa, 05.06.2023
(miejscowość, data)

Lek. Michał Gniewkiewicz
(imię i nazwisko)

OŚWIADCZENIE

Jako współautor pracy pt. „Association of Circulating Anti-HLA Donor-Specific Antibodies and Their Characteristics, including C1q-Binding Capacity, in Kidney Transplant Recipients with Long-Term Renal Graft Outcomes" oświadczam, iż mój własny wkład merytoryczny w przygotowanie, przeprowadzenie i opracowanie badań oraz przedstawienie pracy w formie publikacji stanowi: przygotowanie koncepcji artykułu, metodyki, zebranie i analizę danych, interpretację wyników, napisanie manuskryptu.
Mój udział procentowy w przygotowaniu publikacji określam jako 70%.

.....

(podpis oświadczającego)

Warszawa, 05.06.2023
(miejscowość, data)

Prof. dr hab. n. med. Magdalena Durlik
(imię i nazwisko)

OŚWIADCZENIE

Jako współautor pracy pt. „Association of Circulating Anti-HLA Donor-Specific Antibodies and Their Characteristics, including C1q-Binding Capacity, in Kidney Transplant Recipients with Long-Term Renal Graft Outcomes" oświadczam, iż mój własny wkład merytoryczny w przygotowanie, przeprowadzenie i opracowanie badań oraz przedstawienie pracy w formie publikacji stanowi: nadzór merytoryczny nad koncepcją publikacji, metodyką oraz interpretacją wyników, edycja i ostateczna akceptacja manuskryptu.
Mój udział procentowy w przygotowaniu publikacji określam jako 15%.

Wkład lek. Michała Gniewkiewicza w powstawanie publikacji określam jako 70%,
(imię i nazwisko kandydata do stopnia)
obejmował on przygotowanie koncepcji artykułu, metodyki, zebranie i analizę danych, interpretację wyników, napisanie manuskryptu.
(merytoryczny opis wkładu kandydata do stopnia w powstanie publikacji)*

Jednocześnie wyrażam zgodę na wykorzystanie w/w pracy jako część rozprawy doktorskiej lek. Michała Gniewkiewicza
(imię i nazwisko kandydata do stopnia)

Kierownik
Kliniki Medycyny Transplantacyjnej, Nefrologii
i Chorób Wewnętrznych
Prof. dr hab. n. med. Magdalena Durlik
(podpis oświadczającego)

*w szczególności udziału w przygotowaniu koncepcji, metodyki, wykonaniu badań, interpretacji wyników

Warszawa, 05.06.2023
(miejscowość, data)

Mgr Katarzyna Czerwińska
(imię i nazwisko)

OŚWIADCZENIE

Jako współautor pracy pt. „Association of Circulating Anti-HLA Donor-Specific Antibodies and Their Characteristics, including C1q-Binding Capacity, in Kidney Transplant Recipients with Long-Term Renal Graft Outcomes" oświadczam, iż mój własny wkład merytoryczny w przygotowanie, przeprowadzenie i opracowanie badań oraz przedstawienie pracy w formie publikacji stanowi: wykonanie badań.
Mój udział procentowy w przygotowaniu publikacji określam jako 10%.

Wkład lek. Michała Gniewkiewicza w powstawanie publikacji określam jako 70%,
(imię i nazwisko kandydata do stopnia)
obejmował on przygotowanie koncepcji artykułu, metodyki, zebranie i analizę danych, interpretację wyników, napisanie manuskryptu.
(merytoryczny opis wkładu kandydata do stopnia w powstanie publikacji)*

Jednocześnie wyrażam zgodę na wykorzystanie w/w pracy jako część rozprawy doktorskiej lek. Michała Gniewkiewicza
(imię i nazwisko kandydata do stopnia)

Katarzyna Czerwińska
(podpis oświadczającego)

*w szczególności udziału w przygotowaniu koncepcji, metodyki, wykonaniu badań, interpretacji wyników

Warszawa, 05.06.2023
(miejscowość, data)

Dr Katarzyna Zielniok
(imię i nazwisko)

OŚWIADCZENIE

Jako współautor pracy pt. „Association of Circulating Anti-HLA Donor-Specific Antibodies and Their Characteristics, including C1q-Binding Capacity, in Kidney Transplant Recipients with Long-Term Renal Graft Outcomes" oświadczam, iż mój własny wkład merytoryczny w przygotowanie, przeprowadzenie i opracowanie badań oraz przedstawienie pracy w formie publikacji stanowi: wykonanie badań, udział w pisaniu manuskryptu.
Mój udział procentowy w przygotowaniu publikacji określam jako 5%.

Wkład lek. Michała Gniewkiewicza w powstawanie publikacji określam jako 70%,
(imię i nazwisko kandydata do stopnia)
obejmował on przygotowanie koncepcji artykułu, metodyki, zebranie i analizę danych, interpretację wyników, napisanie manuskryptu.
(merytoryczny opis wkładu kandydata do stopnia w powstanie publikacji)*

Jednocześnie wyrażam zgodę na wykorzystanie w/w pracy jako część rozprawy doktorskiej lek. Michała Gniewkiewicza
(imię i nazwisko kandydata do stopnia)

Katarzyna Zielniok
.....
(podpis oświadczającego)

*w szczególności udziału w przygotowaniu koncepcji, metodyki, wykonaniu badań, interpretacji wyników

Warszawa, 05.06.2023
(miejscowość, data)

Lek. Michał Gniewkiewicz
(imię i nazwisko)

OŚWIADCZENIE

Jako współautor pracy pt. „Impact of Resolved Preformed, Persistent Preformed, and De Novo Anti-HLA Donor-Specific Antibodies in Kidney Transplant Recipients on Long-Term Renal Graft Outcomes" oświadczam, iż mój własny wkład merytoryczny w przygotowanie, przeprowadzenie i opracowanie badań oraz przedstawienie pracy w formie publikacji stanowi: przygotowanie koncepcji artykułu, metodyki, zebranie i analizę danych, interpretację wyników, napisanie manuskryptu

Mój udział procentowy w przygotowaniu publikacji określam jako 70%.

.....

(podpis oświadczającego)

Warszawa, 05.06.2023
(miejscowość, data)

Prof. dr hab. n. med. Magdalena Durlik
(imię i nazwisko)

OŚWIADCZENIE

Jako współautor pracy pt. „Impact of Resolved Preformed, Persistent Preformed, and De Novo Anti-HLA Donor-Specific Antibodies in Kidney Transplant Recipients on Long-Term Renal Graft Outcomes" oświadczam, iż mój własny wkład merytoryczny w przygotowanie, przeprowadzenie i opracowanie badań oraz przedstawienie pracy w formie publikacji stanowi: nadzór merytoryczny nad koncepcją publikacji, metodyką oraz interpretacją wyników, edycja i ostateczna akceptacja manuskryptu.
Mój udział procentowy w przygotowaniu publikacji określam jako 15%.

Wkład lek. Michała Gniewkiewicza w powstawanie publikacji określam jako 70%,
(imię i nazwisko kandydata do stopnia)
obejmował on przygotowanie koncepcji artykułu, metodyki, zebranie i analizę danych, interpretację wyników, napisanie manuskryptu.
(merytoryczny opis wkładu kandydata do stopnia w powstanie publikacji)*

Jednocześnie wyrażam zgodę na wykorzystanie w/w pracy jako część rozprawy doktorskiej lek. Michała Gniewkiewicza
(imię i nazwisko kandydata do stopnia)

Kierownik
Kliniki Medycyny Transplantacyjnej, Nefrologii
i Chorób Wewnętrznych
Prof. dr hab. n. med. Magdalena Durlik
(podpis oświadczającego)

*w szczególności udziału w przygotowaniu koncepcji, metodyki, wykonaniu badań, interpretacji wyników

Warszawa, 05.06.2023
(miejscowość, data)

Mgr Katarzyna Czerwińska
(imię i nazwisko)

OŚWIADCZENIE

Jako współautor pracy pt. „Impact of Resolved Preformed, Persistent Preformed, and De Novo Anti-HLA Donor-Specific Antibodies in Kidney Transplant Recipients on Long-Term Renal Graft Outcomes" oświadczam, iż mój własny wkład merytoryczny w przygotowanie, przeprowadzenie i opracowanie badań oraz przedstawienie pracy w formie publikacji stanowi: wykonanie badań.
Mój udział procentowy w przygotowaniu publikacji określam jako 10%.

Wkład lek. Michała Gniewkiewicza w powstawanie publikacji określam jako 70%,
(imię i nazwisko kandydata do stopnia)
obejmował on przygotowanie koncepcji artykułu, metodyki, zebranie i analizę danych, interpretację wyników, napisanie manuskryptu.
(merytoryczny opis wkładu kandydata do stopnia w powstanie publikacji)*

Jednocześnie wyrażam zgodę na wykorzystanie w/w pracy jako część rozprawy doktorskiej lek. Michała Gniewkiewicza
(imię i nazwisko kandydata do stopnia)

Katarzyna Czerwińska
(podpis oświadczającego)

*w szczególności udziału w przygotowaniu koncepcji, metodyki, wykonaniu badań, interpretacji wyników

Warszawa, 05.06.2023
(miejscowość, data)

Dr Katarzyna Zielniok
(imię i nazwisko)

OŚWIADCZENIE

Jako współautor pracy pt. „Impact of Resolved Preformed, Persistent Preformed, and De Novo Anti-HLA Donor-Specific Antibodies in Kidney Transplant Recipients on Long-Term Renal Graft Outcomes" oświadczam, iż mój własny wkład merytoryczny w przygotowanie, przeprowadzenie i opracowanie badań oraz przedstawienie pracy w formie publikacji stanowi: wykonanie badań.
Mój udział procentowy w przygotowaniu publikacji określam jako 5%.

Wkład lek. Michała Gniewkiewicza w powstawanie publikacji określam jako 70%,
(imię i nazwisko kandydata do stopnia)
obejmował on przygotowanie koncepcji artykułu, metodyki, zebranie i analizę danych, interpretację wyników, napisanie manuskryptu.
(merytoryczny opis wkładu kandydata do stopnia w powstanie publikacji)*

Jednocześnie wyrażam zgodę na wykorzystanie w/w pracy jako część rozprawy doktorskiej lek. Michała Gniewkiewicza
(imię i nazwisko kandydata do stopnia)

Katarzyna Zielniok
.....
(podpis oświadczającego)

*w szczególności udziału w przygotowaniu koncepcji, metodyki, wykonaniu badań, interpretacji wyników