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**ROLA BIOPSJI PROTOKOLARNEJ W DIAGNOSTYCE PRZESZCZEPU
NERKOWEGO**

**Rozprawa na stopień doktora nauk medycznych i nauk o zdrowiu
w dyscyplinie nauki medyczne**

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Miejsce wykonywania pracy: Klinika Transplantologii, Immunologii, Nefrologii i Chorób
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SPIS TREŚCI

WYKAZ STOSOWANYCH SKRÓTÓW	9
STRESZCZENIE W JĘZYKU POLSKIM.....	11
STRESZCZENIE W JĘZYKU ANGIELSKIM	17
1. WSTĘP	23
1.2. MONITOROWANIE STANU PRZESZCZEPIONEJ NERKI.....	23
1.2.1. <i>Biopsja protokolarna</i>	23
1.2.2. <i>Biopsja płynna</i>	25
2. CEL PRACY	29
3. KOPIE OPUBLIKOWANYCH PRAC	31
4. PODSUMOWANIE I WNIOSKI.....	71
4.1. ROLA BIOPSJI PROTOKOLARNEJ W MONITOROWANIU FUNKCJI PRZESZCZEPIONEJ NERKI.....	71
4.2. POTENCJAŁ BIOMARKERÓW JAKO ALTERNATYWY DLA BIOPSJI PROTOKOLARNEJ	74
4.3. PORÓWNANIE BIOPSJI PROTOKOLARNEJ Z BIOPSIĄ PŁYNNĄ	80
OŚWIADCZENIA WSPÓŁAUTORÓW	83
SPIS RYCIN	103
BIBLIOGRAFIA.....	105

WYKAZ STOSOWANYCH SKRÓTÓW

ABMR	odrzucenie zależne od przeciwciał
ACR	ostre odrzucenie komórkowe
AKI	ostre uszkodzenie nerek
CNI	inhibitory kalcyneuryny
CXCL10	chemokina CXC ligand 10
DSA	przeciwciała swoiste dla dawcy
H&E	barwienie hematoksyliną i eozyną
IF/TA	śródmiąższowe zwłóknienie i zanik kanalików
IL-18	interleukina 18
KIM-1	cząsteczka uszkodzenia nerek 1
KTx	transplantacja nerki
L-FABP	białko wiążące kwasy tłuszczowe typu wątrobowego
NGAL	białko lipokalina związane z żelatynazą neutrofilów
PAS	barwienie kwasem nadjodowym-Schiffa
PRA	przeciwciała panelowo reaktywne
RT-PCR	reakcja łańcuchowa polimerazy z odwrotną transkryptazą
dd-	wolnokrążące DNA pochodzące od dawcy
cfDNA	
eGFR	szacowany współczynnik filtracji kłębuszkowej
mRNA	informacyjny RNA
miRNA	mikroRNA
suPAR	rozpuszczalny receptor dla urokinazowego aktywatora plazminogenu
γ-GT	gamma-glutamylotransferaza

STRESZCZENIE W JĘZYKU POLSKIM

WSTĘP

Wydłużenie długoterminowego przeżycia przeszczepów nerkowych jest jednym z podstawowych wyzwań współczesnej transplantologii. Główną przyczyną utraty przeszczepu w odległej obserwacji, jest kumulacja nieodwracalnych zmian przewlekłych, będących zejściem nieleczonych lub nie odpowiadających na leczenie procesów związanych z odrzucaniem. Pogorszenie funkcji przeszczepionej nerki, wyrażające się wzrostem stężenia kreatyniny i spadkiem eGFR, jest zwykle późnym objawem rozwijającej się w niej patologii. Znacznie wcześniej zmiany można wykryć w badaniu histopatologicznym wycinka nerki, co daje szansę na wdrożenie terapii przed dokonaniem się nieodwracalnego, przewlekłego uszkodzenia narządu przeszczepionego.

Wykrywaniu patologii na ich wczesnym etapie rozwoju, gdy możliwe jest jeszcze zahamowanie postępu zmian, dedykowane są biopsje protokolarne. Poglądy na przydatność diagnostyczną i rokowniczą biopsji protokolarnej nerki przeszczepionej były w ostatnich 15 latach zróżnicowane. Po wprowadzeniu w latach 90-tych ubiegłego wieku leków o silnym działaniu immunosupresyjnym i związanym z tym spadkiem częstości występowania epizodów ostrego odrzucania T-komórkowego, wśród wielu badaczy zapanowało przekonanie, że wykonywanie biopsji protokolarnych jest nieuzasadnione, nie wnoszą one bowiem informacji, które prowadziłyby do modyfikacji postępowania terapeutycznego. Badania ostatnich lat pokazały jednak, iż częstość występowania procesu odrzucania (szczególnie jego formy zależnej od przeciwciał) jest znacznie większa niż poprzednio uważano i że odrzucanie to może przebiegać w sposób niemy klinicznie. Ustalenia te rzuciły nowe światło na potencjalną użyteczność biopsji protokolarnej jako narzędzia pozwalającego na wykrywanie niemych klinicznie patologii na etapie, gdy możliwe jest powstrzymanie ich progresji. Co ważne, biopsja nerki jest procedurą inwazyjną, a więc może być obciążona powikłaniami. W tym kontekście zasadne wydaje się udowodnienie przydatności klinicznej tej metody poprzez analizę częstości wykrywania zmian w bioptatach pobieranych u bezobjawowych pacjentów po przeszczepieniu nerki i wyodrębnienie grupy chorych którzy mogą odnieść szczególne korzyści z wykonywania u nich biopsji protokolarnych, wobec pojawiających się nowych możliwości diagnostycznych, w tym oceny markerów uszkodzenia nerek we krwi lub moczu, czyli tzw. biopsji płynnej. Biopsja protokolarna jest rzadko stosowanym narzędziem monitorowania przeszczepionej nerki, tak w Polsce, jak i na świecie. Wydaje się, że jest to spowodowane

niedostatkiem badań poświęconych ocenie jej przydatności w prowadzeniu biorców przeszczepów nerkowych. Przedstawiony cykl prac i wnioski z nich płynące stanowią kolejny krok do uznania i zdefiniowania roli biopsji protokolarnej jako narzędzia diagnostycznego w ośrodkach transplantacyjnych.

CEL PRACY

Celem głównym pracy była ocena wartości diagnostycznej biopsji protokolarnej oraz wybranych biomarkerów uszkodzenia nerek (tzw. biopsji płynnej) w wykrywaniu subklinicznych zmian w przeszczepionej nerce. Cele szczegółowe pracy skupiały się wokół następujących zagadnień: (i) oceny skuteczności biopsji protokolarnej w wykrywaniu subklinicznych uszkodzeń nerki przeszczepionej – analiza częstości i charakterystyki histopatologicznych zmian u biorców przeszczepu; (ii) oceny przydatności diagnostycznej biopsji protokolarnej na tle wybranych biomarkerów w identyfikacji wczesnych uszkodzeń przeszczepu – oceny przydatności „liquid biopsy” oraz klasycznych wskaźników laboratoryjnych; (iii) identyfikacji czynników ryzyka niekorzystnego rokowania u biorców nerki przeszczepionej – analizie korelacji między profilami lipidowymi, markerami zapalnymi i histopatologią w biopsji protokolarnej; (iv) ocenie bezpieczeństwa i skuteczności biopsji protokolarnej w porównaniu do standardowych metod monitorowania funkcji przeszczepionej nerki – analizie powikłań i skuteczności klinicznej tej metody w codziennej praktyce transplantologicznej.

MATERIAŁ I METODY

Rozprawa doktorska oparta została na czterech publikacjach naukowych, które łącznie stanowiły ustrukturyzowany fundament do analizy diagnostycznej i prognostycznej wartości biopsji protokolarnej oraz biomarkerów uszkodzenia nerki przeszczepionej. Pierwsze badanie przeprowadzono jako retrospektywną analizę kolejnych 72 pacjentów, będących pod opieką Kliniki Medycyny Transplantacyjnej, Nefrologii i Chorób Wewnętrznych, u których w latach 2014-2018 wykonano przeszczepienie nerki, a następnie w 3 i 12szym miesiącu po zabiegu wykonywano biopsje protokolarne nerki przeszczepionej. Analizowano zarówno dane kliniczne, laboratoryjne, jak również wyniki badania histopatologicznego wycinków pobranych metodą biopsji gruboigłowej nerki. Podkreślenia wymaga, że autor pracy doktorskiej, odbywający w latach 2016-2020r rezydenturę z zakresu nefrologii w Klinice Medycyny Transplantacyjnej, Nefrologii i Chorób Wewnętrznych, aktywnie uczestniczył w wykonywaniu procedur biopsji nerki

przeszczepionej u tej podgrupy analizowanych w pracy pacjentów u których przeszczepienia nerki dokonywano w latach 2016-2018, a także uczestniczył w kwalifikacji, przygotowaniu tych chorych, oraz opiece w okresie po biopsji nerki, jak również w opiece przewlekłej nad tymi chorymi, pozostającymi pod opieką Kliniki. W wynikach pracy zwracała uwagę relatywnie wysoka częstotliwość uwidacznianych zmian chorobowych, gdyż w 40% analizowanych materiałów biopsyjnych wykrywano zmiany histopatologiczne. Co więcej, udowodniono, że niezależnym czynnikiem ryzyka powstawania niemych klinicznie zmian w przeszczepionej nerce jest dyslipidemia. Wyniki badania udowodniły użyteczność stosowania biopsji protokolarnej jako narzędzia diagnostycznego, a także stanowią kolejny krok w kierunku opracowania profilu pacjenta, który osiągnie największe korzyści ze stosowania tej inwazyjnej procedury.

Kolejne dwie prace włączone w cykl publikacji stanowiły poglądowe omówienie dotychczasowego stanu wiedzy o biopsji protokolarnej oraz innych narzędziach diagnostycznych tj. nowoczesnych markerach uszkodzenia nerek czyli tzw. biopsji płynnej, przy uwzględnieniu dotychczasowych doświadczeń klinicznych w ośrodku macierzystym autora.

Czwarta praca została zaprojektowana i przeprowadzona jako przegląd systematyczny z meta-analizą. Celem niniejszej pracy było określenie wartości predykcyjnej rozpuszczalnego receptora dla urokinazowego aktywatora plazminogenu (suPAR) u pacjentów z ostrym uszkodzeniem nerek (AKI). W tym celu za pomocą uprzednio zdefiniowanych słów kluczowych dokonano przeszukania elektronicznych baz piśmienniczych (PubMed Central, EMBASE, Cochrane). Finalne przeszukania powyższych baz danych miało miejsce 10 stycznia 2023 roku. W wyniku przeszukania ujawniono 335 publikacji spośród których po pełnej do meta-analizy włączono 9 badań z łączną liczbą 6151 pacjentów. Udowodniono przydatność suPAR w predykcji ostrego uszkodzenia nerek, co stanowi przykład użyteczności nowoczesnych, nieinwazyjnych markerów uszkodzenia nerek w diagnostyce i może być przykładem zastosowania innych niż biopsja metod diagnostycznych, co w przyszłości mogłoby być ekstrapolowane również na biorców przeszczepów nerkowych.

WYNIKI

Analiza retrospektywna objęła 72 biorców nerki, u których wykonano łącznie 144 biopsje protokolarne w 3. i 12. miesiącu po przeszczepieniu. Nieprawidłowości histopatologiczne stwierdzono w 40% przypadków. Najczęściej występującymi zmianami były: subkliniczne

odrzucenie komórkowe (19,4%), zmiany graniczne (8,3%), infekcja wirusem BK (5,6%) oraz mikroangiopatia zakrzepowa (2,8%). W wielu przypadkach zmiany te występowały przy prawidłowych wartościach stężenia kreatyniny i eGFR, co potwierdza wartość biopsji protokolarnej w wykrywaniu subklinicznych zmian, niewidocznych w rutynowej diagnostyce laboratoryjnej. Analiza danych klinicznych wykazała, że u pacjentów z obecnością zmian organicznych w przeszczepie stwierdzano istotnie niższe stężenia cholesterolu całkowitego ($p = 0,003$), HDL ($p = 0,015$), LDL ($p < 0,001$) oraz triglicerydów ($p = 0,044$) w porównaniu do pacjentów bez zmian histopatologicznych. Czynnikiem ryzyka uszkodzenia przeszczepu okazała się także dyslipidemia. W analizie korelacyjnej najsilniejszą dodatnią korelację z obecnością zmian miały niedokrwienie ($r = 0,22$), BMI ($r = 0,21$) i hipercholesterolemia ($r = 0,20$), natomiast ujemną korelację wykazano dla wartości HDL ($r = -0,33$), LDL ($r = -0,31$) oraz cholesterolu całkowitego ($r = -0,30$).

Uzupełnieniem wyników klinicznych była pogłębiona analiza systematyczna i metaanaliza dotycząca roli wybranych biomarkerów w diagnostyce uszkodzenia przeszczepu nerkowego. Szczególną uwagę poświęcono białku suPAR (soluble urokinase-type plasminogen activator receptor), uznawanemu za wskaźnik aktywacji układu odpornościowego i ryzyka uszkodzenia śródbłonna. Metaanaliza objęła 12 badań klinicznych z udziałem 2210 pacjentów i wykazała istotny związek pomiędzy podwyższonym stężeniem suPAR a występowaniem ostrego uszkodzenia nerek (AKI) – zarówno w populacji ogólnej, jak i wśród biorców przeszczepów. Łączna średnia różnica (MD) poziomu suPAR między grupą z nawrotem AF a bez wynosiła 2,22 (95% CI: 0,84–3,61; $p = 0,002$). W podgrupie badań, w których suPAR oceniano techniką 3D, różnica między grupami również była istotna (MD = 2,20; 95% CI: 0,42–3,98; $p = 0,02$). Wyniki te potwierdzają, że suPAR może być skutecznym nieinwazyjnym markerem wczesnego wykrywania uszkodzenia przeszczepu nerkowego, a także jego rokowania.

WNIOSKI

Biopsja protokolarna stanowi skuteczne narzędzie diagnostyczne umożliwiające wczesne wykrycie subklinicznych zmian w przeszczepionej nerce. Pomimo swojego inwazyjnego charakteru, cechuje się wysokim profilem bezpieczeństwa, a ryzyko wystąpienia powikłań pozostaje niskie. Jej zastosowanie pozwala na wdrożenie odpowiedniej interwencji terapeutycznej jeszcze przed pojawieniem się objawów klinicznych, co może znacząco wpłynąć na poprawę długoterminowej funkcji przeszczepu.

Postęp w dziedzinie biomarkerów uszkodzenia nerek stwarza perspektywę ograniczenia konieczności wykonywania biopsji inwazyjnych w najbliższej przyszłości. W obecnym stanie wiedzy tzw. biopsja płynna, oparta na analizie markerów w krwi i moczu, powinna być traktowana jako metoda uzupełniająca biopsję protokolarną, a nie jej zamiennik. Obie techniki mają potencjał do synergicznego zwiększenia czułości diagnostycznej i umożliwienia bardziej precyzyjnego monitorowania stanu przeszczepu.

Szczególną grupą pacjentów, która może odnosić wyraźne korzyści z wykonywania biopsji protokolarnych, są biorcy z rozpoznaną dyslipidemią – u których częściej obserwuje się subkliniczne zmiany histopatologiczne. Jednocześnie wskazane są dalsze badania nad wartością prognostyczną biomarkerów takich jak suPAR, szczególnie w kontekście ich przydatności w ocenie ryzyka pogorszenia funkcji przeszczepu i długoterminowych wyników transplantacji nerek.

STRESZCZENIE W JĘZYKU ANGIELSKIM

ROLE OF PROTOCOL BIOPSY IN THE DIAGNOSIS OF RENAL TRANSPLANT

BACKGROUND

Improving long-term kidney graft survival remains one of the fundamental challenges in modern transplant medicine. The primary cause of late graft loss is the accumulation of irreversible chronic lesions, typically resulting from untreated or treatment-resistant rejection processes. A decline in kidney graft function, manifested by increased serum creatinine levels and decreased eGFR, usually reflects a late stage of underlying pathological changes. Importantly, such abnormalities can be detected much earlier through histopathological examination of renal biopsy specimens, providing an opportunity to initiate targeted therapy before irreversible organ damage occurs.

Protocol biopsies are designed to detect subclinical pathology at an early stage, when progression may still be preventable. Over the past 15 years, opinions regarding the diagnostic and prognostic value of protocol kidney biopsies have varied significantly. After the introduction of potent immunosuppressive drugs in the 1990s—leading to a decline in the incidence of T-cell-mediated acute rejection—many researchers questioned the rationale for performing protocol biopsies, arguing that they did not provide information that would impact therapeutic decision-making. However, recent studies have demonstrated that the true incidence of rejection, particularly antibody-mediated forms, is higher than previously thought and that such processes can progress silently, without clinical signs. These findings have renewed interest in protocol biopsies as a tool for detecting clinically inapparent pathologies at a stage when therapeutic intervention may alter the disease course.

Nevertheless, kidney biopsy is an invasive procedure and thus carries a risk of complications. In this context, it is essential to demonstrate the clinical utility of protocol biopsies through an analysis of histopathological findings in asymptomatic transplant recipients and to identify subgroups of patients who may benefit most from this strategy. Simultaneously, novel diagnostic options—such as the detection of kidney injury biomarkers in blood or urine (the so-called “liquid biopsy”)—are emerging. Protocol biopsies remain rarely used in kidney transplant monitoring both in Poland and globally. This likely reflects a lack of studies assessing their practical value in post-transplant management. The presented series of studies and derived conclusions represent another

step toward the recognition and definition of the diagnostic role of protocol biopsy in transplant centers.

OBJECTIVE

The main objective of the study was to evaluate the diagnostic value of protocol biopsy and selected biomarkers of kidney damage (so-called liquid biopsy) in detecting subclinical changes in the transplanted kidney. The specific objectives of the study focused on the following issues: (i) evaluation of the efficacy of protocol biopsy in detecting subclinical lesions of the transplanted kidney - analysis of the frequency and histopathological characteristics of lesions in transplant recipients; (ii) evaluation of the diagnostic utility of protocol biopsy against selected biomarkers in identifying early graft damage - evaluation of the utility of “liquid biopsy” and classical laboratory indicators; (iii) identification of risk factors for adverse prognosis in transplanted kidney recipients - analysis of correlations between lipid profiles, inflammatory markers, and histopathology in protocol biopsy; (iv) price of safety and efficacy of protocol biopsy compared to standard methods of monitoring transplanted kidney function - analysis of complications and clinical efficacy of this method in daily transplant practice.

MATERIAL AND METHODS

The dissertation was based on four scientific publications, which together provided a structured foundation for analyzing the diagnostic and prognostic value of protocol biopsy and biomarkers of transplant kidney injury. The first study was conducted as a retrospective analysis of a consecutive series of 72 patients under the care of the Department of Transplant Medicine, Nephrology and Internal Medicine who underwent kidney transplantation between 2014 and 2018, followed by protocol biopsies of the transplanted kidney at the 3rd and 12th months after the procedure. Both clinical and laboratory data were analyzed, as well as the results of histopathologic examination of sections taken by thick-needle biopsy of the kidney. It should be emphasized that the author of the dissertation, doing his residency in nephrology at the Department of Transplant Medicine, Nephrology and Internal Medicine from 2016 to 2020, actively participated in the performance of transplanted kidney biopsy procedures in this subgroup of patients analyzed in the work who underwent kidney transplantation in 2016-2018, and also participated in the qualification and preparation of these patients and care in the period after kidney biopsy, as well as in the chronic care of these patients under the care of the Department. What was

noteworthy about the results of the study was the relatively high frequency of visualized lesions, as 40% of the biopsy materials analyzed detected histopathological changes. Furthermore, the study established dyslipidemia as an independent risk factor for the development of clinically silent lesions in the transplanted kidney. The results of the study have proven the utility of using protocol biopsy as a diagnostic tool and represent a further step toward developing a patient profile that will achieve the greatest benefit from the use of this invasive procedure.

The next two papers included in the publication series were an overview discussion of the current state of knowledge of protocol biopsy and other diagnostic tools, i.e. modern markers of kidney damage, or so-called liquid biopsy, taking into account the previous clinical experience at the author's home center.

The fourth paper was designed and conducted as a systematic review with meta-analysis. The purpose of this study was to determine the predictive value of the soluble receptor for urokinase plasminogen activator (suPAR) in patients with acute kidney injury (AKI). For this purpose, electronic writing databases (PubMed Central, EMBASE, Cochrane) were searched using predefined keywords. The final search of the above databases took place on January 10, 2023. The search revealed 335 publications, out of which 9 studies with a total of 6151 patients were included after full meta-analysis. The utility of suPAR in the prediction of acute kidney injury was demonstrated, which exemplifies the utility of modern, non-invasive markers of kidney injury in diagnosis and may exemplify the use of diagnostic methods other than biopsy, which could also be extrapolated to renal transplant recipients in the future.

RESULTS

The retrospective analysis included 72 kidney recipients who underwent a total of 144 protocol biopsies at the 3rd and 12th months after transplantation. Histopathological abnormalities were found in 40% of cases. The most common lesions were subclinical cellular rejection (19.4%), borderline lesions (8.3%), BK virus infection (5.6%), and thrombotic microangiopathy (2.8%). In many cases, these lesions were present with normal creatinine and eGFR values, confirming the value of protocol biopsy in detecting subclinical lesions not seen by routine laboratory diagnosis. Analysis of clinical data showed that patients with the presence of organic lesions in the graft had significantly lower levels of total cholesterol ($p = 0.003$), HDL ($p = 0.015$), LDL ($p < 0.001$), and triglycerides ($p = 0.044$) compared to patients without histopathological changes. Dyslipidemia was also

found to be a risk factor for graft failure. In correlation analysis, ischemia ($r = 0.22$), BMI ($r = 0.21$), and hypercholesterolemia ($r = 0.20$) had the strongest positive correlation with the presence of lesions, while negative correlation was shown for HDL ($r = -0.33$), LDL ($r = -0.31$), and total cholesterol ($r = -0.30$) values.

The clinical results were complemented by an in-depth systematic analysis and meta-analysis on the role of selected biomarkers in the diagnosis of renal allograft injury. Special attention was paid to suPAR (soluble urokinase-type plasminogen activator receptor) protein, considered an indicator of immune activation and risk of endothelial injury. The meta-analysis included 12 clinical trials involving 2,210 patients and showed a significant association between elevated suPAR levels and the incidence of acute kidney injury (AKI) - both in the general population and among transplant recipients. The pooled mean difference (MD) of suPAR levels between the group with and without recurrent AF was 2.22 (95% CI: 0.84-3.61; $p = 0.002$). The difference between groups was also significant in the subgroup of studies that assessed suPAR using the 3D technique (MD = 2.20; 95% CI: 0.42-3.98; $p = 0.02$). These results confirm that suPAR can be an effective non-invasive marker for early detection of renal graft injury, as well as its prognosis.

CONCLUSIONS

Protocol biopsy is an effective diagnostic tool for early detection of subclinical changes in the transplanted kidney. Despite its invasive nature, it has a high safety profile, and the risk of complications remains low. Its use allows for the implementation of appropriate therapeutic intervention even before the appearance of clinical symptoms, which can significantly improve long-term graft function.

Advances in biomarkers of kidney damage offer the prospect of reducing the need for invasive biopsies in the near future. In the current state of knowledge, so-called liquid biopsy, based on the analysis of markers in blood and urine, should be considered as a complementary method to protocol biopsy rather than a replacement for it. Both techniques have the potential to synergistically increase diagnostic sensitivity and enable more precise monitoring of graft status.

A particular group of patients who may benefit from performing protocol biopsies are recipients with known dyslipidemia, in whom subclinical histopathological changes are more likely to be observed. At the same time, further research on the prognostic value of biomarkers such as suPAR is indicated, especially in the context of their usefulness in

assessing the risk of graft function deterioration and long-term renal transplantation outcomes.

1. WSTĘP

Dializoterapia oraz transplantacja nerki stanowią kluczowe metody leczenia nerkozastępczego, ratujące życie i poprawiające jego jakość u pacjentów z niewydolnością nerek. Według danych z 2018 roku, na świecie liczba osób cierpiących na choroby nerek przekraczała 850 milionów, z czego 2,5 miliona żyło dzięki terapii nerkozastępczej.

W 2024 roku w Polsce wykonano 1130 przeszczepień nerki od dawcy zmarłego oraz 82 od dawcy żywego. W pierwszych trzech miesiącach 2025 roku przeprowadzono natomiast odpowiednio 300 i 19 takich zabiegów [1].

Postęp technik chirurgicznych, poprawa doboru immunologicznego dawców i biorców oraz zwiększona dostępność do transplantacji przyczyniają się do dalszego rozwoju transplantologii w Polsce. Współczesne wyzwania w tej dziedzinie koncentrują się na wydłużeniu przeżycia przeszczepu nerkowego oraz wdrażaniu metod zapobiegających jego odrzuceniu. Dlatego tak ważne staje się monitorowanie stanu nerki przeszczepionej poprzez ocenę histopatologiczną bioptatu, jak również użycie badań nieinwazyjnych (tzw. biopsja płynna).

1.2. Monitorowanie stanu przeszczepionej nerki

1.2.1. Biopsja protokolarna

Biopsja protokolarna nerki przeszczepionej (ang. protocol biopsy) jest procedurą diagnostyczną wykonywaną w określonych odstępach czasu po transplantacji, niezależnie od klinicznych objawów dysfunkcji allograftu. Ma na celu identyfikację subklinicznych procesów patologicznych, takich jak ostre odrzucanie komórkowe (ACR, acute cellular rejection), odrzucanie zależne od przeciwciał (ABMR, antibody-mediated rejection), nefrotoksyczność związana z inhibitorami kalcyneuryny (CNI), nefropatia BK, czy nawrót lub rozwój de novo nefropatii. Wczesne wykrycie tych zmian umożliwia interwencję terapeutyczną, co może znacząco wydłużyć czas przeżycia przeszczepu i poprawić jego funkcję w perspektywie długoterminowej [2].

Biopsje protokolarne są rutynowo przeprowadzane w 3., 6. i 12. miesiącu po przeszczepie, choć protokoły różnią się między ośrodkami transplantacyjnymi w zależności od lokalnych wytycznych i profili ryzyka biorców [3]. Procedura jest szczególnie wskazana u pacjentów z podwyższonym ryzykiem immunologicznym, w tym

u osób z preformowanymi przeciwciałami anty-HLA, wysokim poziomem PRA (panel-reactive antibodies), historią retransplantacji lub epizodami ostrego odrzucania w przeszłości [4]. Dodatkowo, biopsje protokolarne mogą być rozważane w późniejszym okresie (np. 2-5 lat po transplantacji) w celu oceny przewlekłych zmian, takich jak śródmiąższowe zwłóknienie i atrofia kanalików (IF/TA, interstitial fibrosis/tubular atrophy) lub diagnostyki przewlekłego ABMR w momencie pojawienia się de novo DSA [5].

Biopsja protokolarna jest wykonywana pod kontrolą ultrasonografii w czasie rzeczywistym, z zastosowaniem igły biopsyjnej o kalibrze 16-18G. Procedura pozwala na pobranie reprezentatywnego fragmentu tkanki korowej nerki, który następnie poddaje się ocenie histopatologicznej zgodnie z aktualną klasyfikacją Banff [6]. Klasyfikacja ta uwzględnia takie parametry, jak nasilenie tubulitis (t), zapalenia śródmiąższowego (i), uszkodzenia naczyń (v) czy obecność kompleksów C4d w naczyniach okołokanalikowych, co umożliwia precyzyjną diagnostykę odrzucania i innych patologii. Materiał biopsyjny jest rutynowo barwiony metodami hematoksylina-eozyna (H&E), PAS, trójbarwnym Massona oraz immunohistochemicznie na obecność C4d a także na obecność antygenu SV40 małego wirusa Polyoma[7,8].

Badania wykazały, że biopsje protokolarne mogą ujawnić cechy subklinicznego odrzucania przeszczepu u 10-25% pacjentów, u których nie doszłoby do wykrycia odchyleń w rutynowych badaniach biochemicznych, takich jak ocena stężenia kreatyniny w surowicy czy współczynnika filtracji kłębuszkowej (eGFR) w pierwszym roku po transplantacji [9]. Ponadto, biopsje protokolarne dostarczają danych o przewlekłych zmianach, takich jak IF/TA czy mikroangiopatia związana z CNI, co może pozwalać na optymalizację schematów immunosupresji, np. poprzez redukcję dawek takrolimusu lub wprowadzenie inhibitorów mTOR [9]. Warto zaznaczyć, że to kumulacja zmian przewlekłych jest dziś jednym z najważniejszych czynników odpowiadających za utratę przeszczepu w odległej obserwacji. Biopsja protokolarna może być również rozważana jako narzędzie prognostyczne – np. w kontekście odrzucania zależnego od przeciwciał (ABMR), wykrycie depozytów C4d i mikrokrażeńiowych zmian zapalnych (glomerulitis, peritubular capillaritis) w biopsjach protokolarnych koreluje z gorszym rokowaniem i wymaga intensywnej terapii, takiej jak plazmafereza czy podanie ludzkich immunoglobulin [10,11].

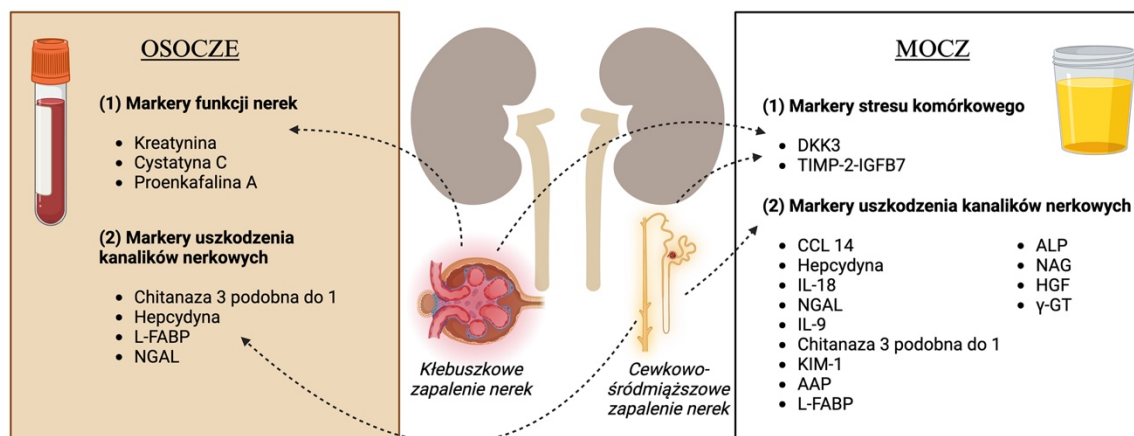
Mimo że biopsja protokolarna jest procedurą stosunkowo bezpieczną, wiąże się z niewielkim ryzykiem powikłań, takich jak makroskopowy krwiomocz (0,5-2%), krwiak

okołonerkowy (1-3%) czy przetoka tętniczo-żylna (<0,1%) [12]. Częstość poważnych komplikacji wymagających interwencji (np. embolizacji) nie przekracza 0,5% [13]. Ograniczenia metody obejmują także potencjalną niespecyficzną zmian histologicznych, co może utrudniać różnicowanie między odrzucaniem a innymi przyczynami uszkodzenia, np. infekcją BK wirusem [14]. Ponadto, koszty procedury, obciążenie psychofizyczne dla pacjenta oraz konieczność posiadania doświadczonego zespołu patologów są argumentami w trwającej debacie nad zasadnością rutynowego stosowania biopsji protokolarnych w porównaniu z biopsjami „ze wskazań” (for-cause biopsy).

Biopsja protokolarna po przeszczepieniu nerki pozostaje kluczowym narzędziem w monitorowaniu stanu allograftu, szczególnie w erze nowoczesnych schematów immunosupresyjnych. Umożliwia wczesną diagnostykę subklinicznych procesów patologicznych, co przekłada się na poprawę przeżycia przeszczepu i jakości życia pacjentów. Jednak jej zastosowanie powinno być indywidualizowane, z uwzględnieniem profilu ryzyka biorcy, dostępnych zasobów oraz potencjalnych korzyści w porównaniu z ryzykiem. Dalsze badania prospektywne są niezbędne, aby ustalić optymalne strategie stosowania biopsji protokolarnych w różnych populacjach pacjentów po transplantacji. Również szybki rozwój analiz biomarkerów zawartych w płynach ustrojowych (tzw.biopsja płynna) pozwalając pośrednio na ocenę czynności przeszczepu, każe zadać pytanie o rolę biopsji protokolarnej w diagnostyce przeszczepu nerkowego i profil pacjenta któremu powinno się proponować tę procedurę jako przynoszącą najwięcej korzyści.

1.2.2. Biopsja płynna

Biopsja płynna (liquid biopsy) to w przeciwieństwie do biopsji protokolarnej nieinwazyjna metoda diagnostyczna oparta na analizie biomarkerów obecnych w płynach ustrojowych, takich jak krew lub mocz, która zyskuje coraz większe znaczenie w monitorowaniu stanu nerki przeszczepionej [15] (Rycina 1). W odróżnieniu od tradycyjnej biopsji tkankowej, biopsja płynna umożliwia wykrywanie subklinicznych zmian w allograftcie, takich jak odrzucanie komórkowe (ACR, acute cellular rejection), odrzucanie zależne od przeciwciał (ABMR, antibody-mediated rejection) czy uszkodzenie związane z nefrotoksycznością, bez konieczności inwazyjnej interwencji. Jej zastosowanie po przeszczepieniu nerki otwiera nowe perspektywy w personalizacji terapii i poprawie rokowania przeszczepu [16].



Rycina 1. Przykłady biomarkerów mających zastosowanie w ocenie funkcji nerki.

Źródło: opracowanie własne.

Biopsja płynna opiera się na analizie szerokiego spektrum biomarkerów, które odzwierciedlają zarówno stan funkcjonalny nerki, jak i procesy patologiczne w allograftcie. Jednym z najczęściej badanych markerów jest wolnokrążące DNA pochodzące od dawcy (donor-derived cell-free DNA, dd-cfDNA), którego poziom w osoczu wzrasta w odpowiedzi na uszkodzenie komórek nerki przeszczepionej. Frakcja dd-cfDNA powyżej 1% jest silnie skorelowana z obecnością ABMR lub ACR, często wyprzedzając zmiany w tradycyjnych parametrach biochemicznych, takich jak stężenie kreatyniny w surowicy czy eGFR (estimated glomerular filtration rate) [17].

Ważną grupę markerów stanowią mikroRNA (miRNA), takie jak miR-142-5p, miR-155 i miR-223, które można wykryć w moczu lub osoczu. Ich zmieniona ekspresja jest związana z procesami zapalnymi i odrzucaniem komórkowym, co pozwala na wczesne rozpoznanie ACR [18]. Kolejnym markerem jest mRNA, w tym transkrypty genów związanych z aktywacją immunologiczną, takich jak CXCL10, perforyna czy granzym B, które można wykryć w moczu metodą RT-PCR. Podwyższone poziomy tych transkryptów są specyficzne dla odrzucania i wspierają diagnostykę różnicową [19,20].

Biopsja płynna obejmuje również markery funkcji nerek i uszkodzenia kanalików nerkowych, które można ocenić w osoczu i moczu. W osoczu kluczowe markery funkcji nerek to kreatynina, cystatyna C oraz proenkefalina A, które odzwierciedlają zdolność filtracyjną nerki. Markery uszkodzenia kanalików nerkowych, takie jak chitanaza 3 podobna do 1 (YKL-40), hepcydyna, białko wiążące kwasy tłuszczowe typu wątrobowego (L-FABP) oraz NGAL (neutrophil gelatinase-associated lipocalin), wskazują na stres oksydacyjny i uszkodzenie kanalików proksymalnych, co może być wczesnym sygnałem odrzucania lub toksyczności leków [21]. W moczu analiza markerów stresu

komórkowego, takich jak DKK3 (dickkopf-3) i TIMP-2/IGFBP7 (test NephroCheck), pozwala na ocenę odpowiedzi komórek na uszkodzenie, natomiast markery uszkodzenia kanalików nerkowych, takie jak CCL14, hepcydyna, IL-18, NGAL, chitanaza 3 podobna do 1, KIM-1 (kidney injury molecule-1), AAP (alanino-aminopeptydaza), ALP (fosfataza alkaliczna), HGF (hepatocyte growth factor) oraz γ -GT (gamma-glutamylotransferaza), dostarczają szczegółowych informacji o stanie kanalików nerkowych i ich regeneracji [22,23].

Białka zapalne, takie jak chemokiny CXCL9 i CXCL10, mierzone w moczu, są markerami rekrutacji limfocytów T i wykazują wysoką czułość w wykrywaniu ACR i ABMR [24,25]. Dodatkowo, analiza metabolitów w moczu, takich jak trimetyloamina-N-tlenek czy kwas hipurowy, za pomocą spektrometrii masowej, pozwala na identyfikację subklinicznych zmian metabolicznych związanych z uszkodzeniem niedokrwiennym lub toksycznością leków [26]. Połączenie tych biomarkerów w panele wielomarkerowe znacząco zwiększa precyzję diagnostyczną biopsji płynnej.

Biopsja płynna jest wykorzystywana zarówno w rutynowym monitorowaniu nerki przeszczepionej, jak i w diagnostyce różnicowej w przypadku podejrzenia odrzucania. Niejednokrotnie, może być również wstępem do wykonywania inwazyjnych badań biopsyjnych i w tym sensie rozważana jako komplementarna metoda diagnostyki przeszczepu nerkowego. Bloom i wsp. wykazali, że pomiar dd-cfDNA pozwala na wykrycie ABMR z czułością 81% i specyficznością 85%, co umożliwia identyfikację pacjentów wymagających dalszej diagnostyki inwazyjnej [27]. Z kolei analiza CXCL10 w moczu wykazała porównywalną skuteczność w przewidywaniu ACR, z wartością predykcyjną zbliżoną do biopsji tkankowej. Markery takie jak NGAL, KIM-1 czy L-FABP w moczu są szczególnie przydatne w wykrywaniu wczesnego uszkodzenia kanalików nerkowych, co może wskazywać na subkliniczne odrzucanie lub toksyczność inhibitorów kalcyneuryny [28]. W porównaniu z biopsją protokolarną, biopsja płynna pozwala na częstsze monitorowanie bez obciążania pacjenta, co ułatwia szybkie dostosowanie terapii, np. poprzez wprowadzenie pulsów metylprednizolonu lub przeciwciał monoklonalnych [29].

2. CEL PRACY

Cel główny:

- Ocena wartości diagnostycznej biopsji protokolarnej oraz wybranych biomarkerów uszkodzenia nerek (tzw.biopsji płynnej) w wykrywaniu subklinicznych zmian w przeszczepionej nerce.

Cele szczegółowe:

- Ocena skuteczności biopsji protokolarnej w wykrywaniu subklinicznych uszkodzeń nerki przeszczepionej – analiza częstości i charakterystyki histopatologicznych zmian u biorców przeszczepu.
- Ocena przydatności diagnostycznej biopsji protokolarnej na tle wybranych biomarkerów w identyfikacji wczesnych uszkodzeń przeszczepu – ocena przydatności „liquid biopsy” oraz klasycznych wskaźników laboratoryjnych.
- Identyfikacja czynników ryzyka niekorzystnego rokowania u biorców nerki przeszczepionej – analiza korelacji między profilami lipidowymi, markerami zapalnymi i histopatologią w biopsji protokolarnej.
- Ocena bezpieczeństwa i skuteczności biopsji protokolarnej w porównaniu do standardowych metod monitorowania funkcji przeszczepionej nerki – analiza powikłań i skuteczności klinicznej tej metody w codziennej praktyce transplantologicznej.

3. KOPIE OPUBLIKOWANYCH PRAC

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

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ABSTRACT

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

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

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

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

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

The epidemiology of kidney diseases shows a growing number of chronic kidney disease (CKD) cases, which in the end stage often necessitate kidney transplantation (KTx) [1]. CKD affects approximately 10–15% of the global population, and its prevalence is higher in older adults, women, and those with underlying conditions like diabetes mellitus and hypertension [2]. In particular, diabetes and hypertension are the two leading causes of kidney failure, contributing together to around 40–60% of all CKD cases globally.

Furthermore, a significant burden of kidney diseases falls on racial minorities, further complicating access to care and outcomes [2]. In Poland, the incidence of kidney transplants has diminished in recent years owing to a scarcity of suitable organs and an older demographic of patients. This decrease aligns with global patterns in organ shortages when the demand for transplants exceeds the availability of viable kidneys. Recent data reveal that preemptive kidney transplantation, which can markedly enhance patient outcomes, constitutes about 9–11% of deceased donor kidney transplants [2]. The durability of graft survival for transplanted kidneys

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has significantly improved over the past thirty years, primarily due to advancements in immunosuppressive treatments and post-transplant management, resulting in a 20–30% rise in graft survival rates compared to previous decades [2].

Prolonging the long-term viability of renal transplants is a primary challenge in contemporary transplantation. Despite substantial advancements in recent decades, the long-term survival rates for kidney transplants continue to pose concerns. Data indicate that the survival rates for kidney transplants at 1, 3, 5, 10, and 15 years are 99%, 97%, 93%, and 70%, respectively, demonstrating a consistent decrease in graft viability over time [3]. The primary reason for graft loss in long-term follow-up is the buildup of permanent chronic alterations, frequently resulting from untreated or treatment-resistant rejection processes. Chronic allograft dysfunction is a primary factor in graft failure, responsible for 35–50% of all kidney transplant losses [4]. The decline in transplanted kidney function, typically indicated by elevated blood creatinine levels and reduced eGFR, frequently represents a late-stage manifestation of underlying conditions. At this point, irreversible harm may have already transpired. Histopathological analysis of kidney biopsy specimens facilitates the identification of early alterations, including inflammation or fibrosis, before the abnormality of clinical indicators such as creatinine levels [4]. Early detection via biopsy provides an opportunity for prompt management and can enhance transplant survival by mitigating problems before the onset of chronic disease. This is why many clinics, including the authors' Clinic of Transplantation Medicine, have implemented a clinical program of protocol biopsies. These biopsies are performed immediately after transplantation and at pre-determined intervals (typically 3 and 12 months after KTx) in clinically asymptomatic patients.

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

biopsies are increasingly considered a crucial instrument in transplant surveillance, particularly in identifying early rejection and histological alterations in high-risk patients [7].

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

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

Material and methods

The authors retrospectively analyzed the clinical and laboratory data of 72 consecutive kidney transplant recipients operated on in 2014–2018, under the care of the Department of Transplantation Medicine, Nephrology, and Internal Diseases of the Medical University of Warsaw. In whom protocol biopsies of the kidney graft were performed. The evaluation included 144 protocol biopsy results, routinely performed 3 and 12 months after the date of transplantation. Every procedure involved taking and analyzing two fragments of renal tissue, and all biopsies used the 16G needle. The biopsy lesions were classified qualitatively and quantitatively using the uniform criteria of the Banff classification. Routinely determined was the presence of complement C4d deposits, polyomavirus SV40 antigen, immunoglobulins, fibrinogen light chains, and complement components C3 and C4 in transplanted kidney biopsies. The Pathology Laboratory of the Clinic assessed all biopsies with the same experienced pathologist. Histopathological data were then correlated with clinical and laboratory data of the renal transplant recipient, including: the degree of immunization before transplantation, assessed by serological method (PRA: panel reactive antibodies) or solid phase method (DSA: donor specific antibodies), comorbidities, cause of end-stage renal disease, duration and method of dialysis therapy; data related to the donor: donor characteristics (age, sex, BMI, smoking status, standard donor, extended criteria donor, living donor), immunological selection of the donor and recipient in terms of HLA A-B- and DR

antigens, immunological selection of the donor and recipient in terms of major blood groups; data characterizing the organ transplantation procedure: cold ischemia time, occurrence of delayed graft function (DGF, defined as the need to perform hemodialysis at least once in the patient in the first week after kidney transplantation); data on the course of the post-transplantation period before the protocol biopsy, including: episodes of acute rejection in the period before the biopsy, immunosuppressive treatment regimen used, ultrasound imaging of the abdominal cavity with Doppler assessment of renal flow. The frequency and type of renal graft pathology were assessed and searched for risk factors that might lead to clinically silent changes in protocol biopsies of the transplanted kidney. Histological findings were, for the sake of statistical analysis, classified into one of the subgroups: 1) normal biopsy; 2) borderline changes; 3) subclinical cell-mediated rejection; 4) subclinical antibody-mediated rejection; 5) glomerulitis; 6) thrombotic microangiopathy; 7) polyomavirus BKV; 8) proliferative arteriopathy.

Statistical analysis

This study utilized both descriptive and inferential statistical approaches to examine the data, to compare different clinical parameters between individuals who have normal kidney function and those who have kidney damage. For continuous variables, the authors computed the mean and standard deviation (SD), as well as the count and percentage for categorical variables. To ascertain the statistical significance of disparities between the two groups (normal kidney vs. kidney injury), the authors employed the independent t-test to compare the means of the two groups for continuous variables, taking into consideration the uneven variances using Welch's t-test. The authors employed the Chi-square test of independence to evaluate the correlation between each categorical variable and kidney function status. In the absence of any link between the variables, the authors compared the observed frequencies in each category with the predicted frequencies. These tests yielded p-values that reveal the statistical significance of the group disparities; a p-value below 0.05 is considered statistically significant. The statistical studies were performed using Python and the appropriate statistical libraries. The table presents the relationship between different parameters and kidney injury, including the sensitivity, specificity, and AUC (area under the curve) values for each variable. The statistical techniques used in this study include correlation analysis, which involves using the Pearson correlation coefficient to measure

the strength and direction of relationships between variables. Additionally, predictive analysis was conducted using logistic regression to evaluate the impact of individual factors on the risk of kidney injury. Logistic regression yields regression coefficients that serve as indicators of the magnitude and direction of the association between independent variables (such as sex, age, BMI, etc.) and the dependent variable (kidney damage).

Results

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

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

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

The variable sex has a minimal correlation with organic kidney damage (0.10), indicating that gender is not a significant factor in this analysis. In contrast, cholesterol-related variables such as HDL, total cholesterol, and LDL have a negative correlation with organic kidney damage. The authors recorded the strongest

Table 1. Baseline characteristics of patients

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

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

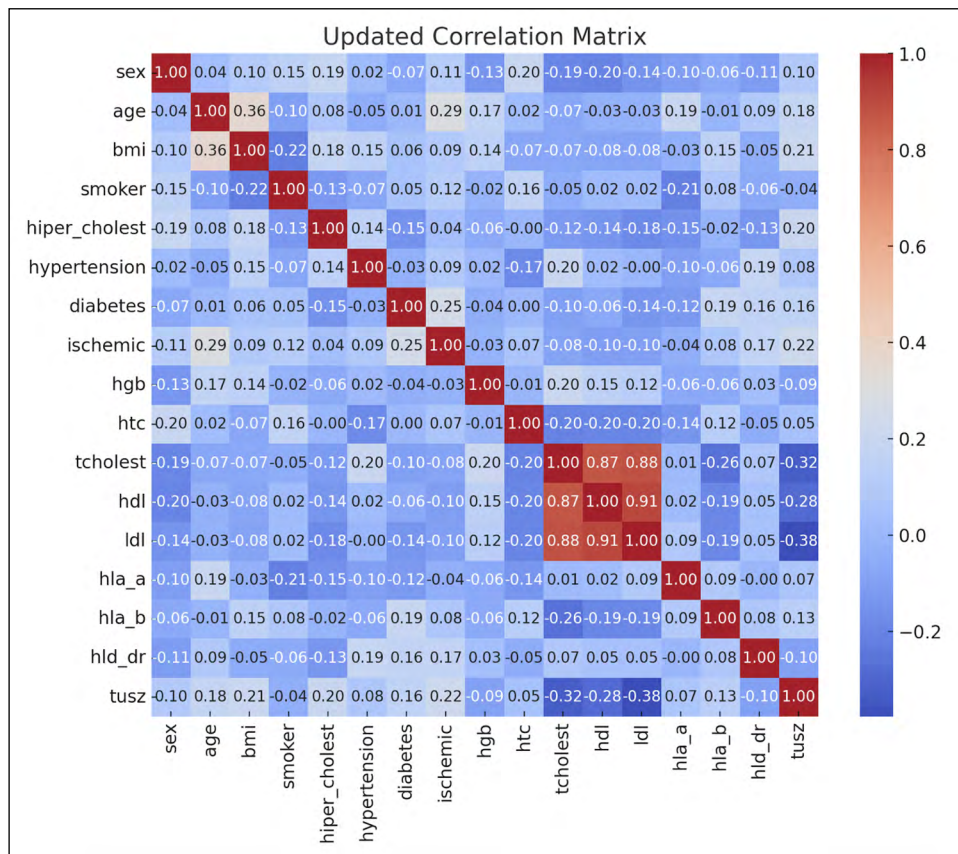


Figure 1. Updated correlation matrix of parameters

negative correlation for LDL (-0.38) and total cholesterol (-0.32), suggesting that higher levels of total cholesterol and LDL may have an inverse relationship with organic kidney damage. The variable HDL (-0.28) also shows a moderate negative correlation.

Discussion

Significant lipid profile differences were found one year after KTx between patients without organic kidney abnormalities and those with organic renal impairment. Statistically, organic renal damage patients had lower total cholesterol than those without kidney abnormalities. HDL and LDL values were lower in the renal injury group, with LDL considerably lower. These findings support prior research on dyslipidemia and chronic kidney disease (CKD) development. Poorer outcomes in CKD patients often result from low HDL and LDL levels, leading to aberrant lipid metabolism. This study's negative connection between LDL and renal damage supports past research linking hypocholesterolemia to inflammation and poorer kidney function [8]. Protocol biopsies detect a high prevalence of subclinical

rejection; therefore, future studies should examine their long-term cost-effectiveness in transplant patient treatment, especially in high-risk groups. This could help determine the biopsies' ideal frequency and time. The study shows substantial links between. Ischaemic heart disease (IHD), BMI, and renal impairment. The positive correlation between BMI and cholesterol levels bolsters the notion that obesity and metabolic syndrome serve as risk factors for chronic kidney disease [9–11]. Due to several factors, creatinine levels before biopsy did not differ significantly between those with and without organic changes. Organic changes in the transplanted kidney may not immediately raise creatinine. Biopsy-detected subclinical rejection or early fibrosis may not affect creatinine levels. The study found that routine biopsies detect these changes before creatinine levels rise, explaining the same results in both groups. Although damaged, the transplanted kidney can maintain normal creatinine levels and correct initial irregularities. Minor or localized injuries often show this. Kidney transplant recipients utilize immunosuppressive medicine to treat early inflammation or rejection, which maintains elevated creatinine levels even when lesions are present on biopsy. Creatinine is not

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

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

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

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

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

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

Protocol biopsies protect kidney transplant recipients by detecting antibody-mediated rejection. Their research revealed that ABMR is on the rise, often progressing asymptotically and going undetected by serum creatinine or eGFR tests [8]. Protocol biopsies provide intervention before permanent rejection in these cases.

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

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

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

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

The study has flaws. The retrospective nature of this study increases selection bias and makes it harder to prove causality between histological alterations and clinical outcomes. The study included 72 kidney transplant recipients with 144 biopsy results, but the sample size may be too small to extrapolate the findings to other populations due to the complexity of kidney transplant patient profiles and immunosuppressive protocol heterogeneity (only 6 patients in the present group have a PRA > 0). The study lacked long-term clinical follow-up beyond biopsy intervals, which is necessary to establish histological findings' prognostic value. The lack of long-term graft survival data makes it difficult to analyze how protocol biopsies affect transplant durability. The present group, however, had a significant rate of aberrant findings (40%), allowing doctors to initiate treatment immediately despite subclinical changes.

Conclusions

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

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Biomarkers identifying deterioration of kidney graft function — usefulness of “liquid biopsy”

Abstract

One of the important challenges of modern transplantology is identifying signs of deterioration in kidney graft function as early as possible and starting effective treatment. While the gold standard in graft monitoring is still the needle biopsy, novel biomarkers with no contraindications and barely any procedure-associated side effects, rise as promising tools.

In this review, we summarize up-to-date knowledge about novel biomarkers that can serve as “liquid biopsy” to identify deterioration in graft function in kidney transplant recipients.

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Keywords: graft biopsy; transplantation; acute kidney transplant rejection

INTRODUCTION

Clinical transplant medicine is a dynamic branch of medicine. Improvements in surgical methods and immunosuppressive medication have enabled efficient organ and tissue transplantation [1]. In addition to solid organ transplants, innovative head and face transplantations are now being explored [2, 3].

Kidney transplantation has become standard clinical practice over the past few decades. The number of transplantation procedures performed globally, including kidney transplantation, increases every year. According to the United Network for Organ Sharing (UNOS), there is an average of 19 000 kidney transplants performed each year in the United States [4]. The World Health Organization estimates that around 170 000 kidney transplants are performed around the world each year [5]. By comparison, according to the Polish Transplantation Registry POLTRANSPLANT, in Poland, where we work, the number of kidney transplants in 2022 amounted to 784 from deceased donors and 73 transplantations from living related donors [6].

The main goal of transplant medicine nowadays is lengthening the time of graft survival and securing appropriate graft function while at the same time taking care of the patient's quality of life. To ensure this, early detection of unfavorable changes in the transplanted kidney and, thus, the implementation of appropriate treatment is important. Monitoring of graft function in kidney transplant recipients is based on laboratory blood tests and needle biopsy, which are the gold standard in kidney transplant disorders. However, the usefulness of needle biopsy, due to the invasive character of the procedure, is limited, and the main contraindications include bleeding disorders, uncontrolled hypertension, infection at the biopsy site, or risk of an allergic reaction to local anesthesia. This diagnostic method puts a patient also at risk of complications, starting with as mild as commonly seen post-procedural hematoma or arterio-venous intrarenal fistula, ending up with a very rare occurrence of graft removal or even the patient's death.

Liquid biopsy, in contrast to traditional biomarkers, such as creatinine or glomerular filtration rate (eGFR) commonly used

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to monitor kidney function, enables earlier detection of graft changes, even in cases of subclinical rejection. Traditional biomarkers often have limited sensitivity, which means that they may fail to detect the early stages of graft rejection, with increases occurring only at more advanced stages of kidney damage [7]. This is why the so-called “liquid biopsy” is important. A liquid biopsy, in general, refers to the molecular examination of non-renal tissue samples and searches for special biomarkers serving as a prognostic tool for patients with renal disorders [8]. Although blood and urine are the most commonly used body fluids in these procedures, saliva, feces, and other body fluids can also be employed as sources for liquid biopsies [9].

In this review, we looked at the most promising markers for early diagnosis and predicting the outcome of deterioration in allogenic kidney graft function.

CLUSTERIN (CLU)

Clusterin (CLU), also known as apolipoprotein J, is a glycosylated multifunctional protein involved in both apoptotic and non-apoptotic processes in various organs, including kidneys. It is observed that low base levels of CLU predispose to more serious deterioration in renal function in ischemia, which suggests a link between CLU and ischemic injury [10]. Elevated CLU levels have been found in the blood of patients with delayed graft function, and the level of CLU started to rise within four hours after surgery. That suggests CLU can be used as an early prognostic marker in kidney transplant recipients, and, going further, it may play a role in the development of graft rejection. Some studies have suggested that CLU levels may be used as a marker for the early detection of kidney transplant rejection, although more research is needed to support these observations.

CLU is not a specific marker associated only with kidney damage, but its level may also be increased in other conditions — so it should not be used as a sole indicator for kidney graft injury. However, some studies showed that elevated serum [11, 12], as well as urine [13] CLU levels, were associated with acute rejection episodes in kidney transplant recipients. Detecting transplant rejection by CLU as a marker has been reported with sensitivity of 40% to 80% and specificity around 70–80% [13–15].

CREATININE

Creatinine is a waste product of muscle metabolism that is generally removed from the bloodstream by the kidneys and excreted in the urine. Creatinine is a popular biomarker used to measure kidney function, notably in the context of kidney transplantation [16]. Creatinine levels alone, however, are not a conclusive diagnostic tool for rejection and should be read in combination with other clinical and laboratory data, including needle biopsy results since even a significant increase in the creatinine level alone does not provide adequate evidence of acute kidney transplant rejection. Also, muscle mass and the patient’s clinical state influence the creatinine level. It has to be noted that these levels may not change significantly in the early stages of rejection, which makes it a less sensitive test for early detection.

The sensitivity and specificity of creatinine as a marker of acute kidney transplant rejection varied in studies from 72% to 81% and 67% to 83%, respectively [17–19]. Creatinine may be measured in both serum and urine. It has to be underlined that creatinine is one of the cheapest and most easily accessible markers of those mentioned in this review.

CYSTATIN-C

Cystatin-C (Cys-C) is a protein produced by all nucleated cells. It regulates the activity of specific proteases. Cys-C is a marker of kidney function because it primarily reflects the amount of the glomerular filtration rate (GFR) [20, 21]. When eGFR drops, Cys-C level in the blood rises [22]. Cys-C is unaffected by muscle mass or other variables, unlike other kidney function indicators such as creatinine. The role of Cys-C in kidney diseases has been investigated well in the available literature. A study by Liu [23] found that serum Cys-C was a better indicator of changes in renal function than serum creatinine, blood urea nitrogen, 2-microglobulin, or uric acid before surgery and at 1, 3, 5, 7, 14, 30, and 90 days after surgery. Koçak et al. [24] found that while Cys-C exhibited high sensitivity in estimating renal function in the early stages after transplantation, its utility as a GFR marker to indirectly assess kidney function after transplantation declined by the end of the first week. Only several studies have indicated that Cys-C has high sensitivity in diagnosing early acute

rejection, with values ranging from 75–90% and specificity ranging from 80–95% [25–27].

In one study, Liu et al. analyzed 160 kidney transplant patients to establish Cys-C accuracy in diagnosing acute rejection. The findings revealed that Cys-C has 90% sensitivity and 95% specificity in predicting acute rejection. The authors cautioned that sensitivity may diminish in later phases of rejection, especially in patients with severe fibrosis or scarring [27]. Urine and serum Cys-C have both been investigated as indicators of renal rejection. However, most research implies that urine Cys-C is a more sensitive measure of renal rejection than serum Cys-C [28, 29]. The timing of Cys-C rise and its prognostic usefulness may be affected by several circumstances; for example, an increase in Cys-C levels may begin several days to weeks before clinical indications of rejection appear [30], while in other cases, Cys-C levels may not change until after rejection has already occurred. Taking this into account, more research has to be performed, and Cys-C cannot be treated as a single marker of graft rejection. Also, the cost-effectiveness of the method is still doubtful.

C-X-C MOTIF CHEMOKINES

C-X-C motif chemokines, such as CXCL9 or CXCL10, are also examined as potential kidney transplant rejection markers.

CXCL9 is also known as a monokine induced by interferon-gamma (MIG). It is primarily produced by immune cells such as T cells, natural killer cells, and dendritic cells and acts as a chemoattractant for activated T cells and natural killer cells. CXCL9 has been implicated in the development of acute cellular transplant rejection. In this process, the immune system recognizes the transplanted organ as foreign and mounts an immune response against it, leading to inflammation and tissue damage. Elevated levels of CXCL9 have been observed in the serum of patients with kidney transplant rejection, and its presence has been associated with higher risk of rejection. Both Moledina et al. [31] and Tinel et al. [32] showed that patients with acute cellular rejection had significantly higher levels of CXCL9 compared to those without rejection. The aforementioned results are also supported by a meta-analysis by Zhou et al., which comprised five trials with a total of 384 kidney transplant recipients [33]. The authors concluded that CXCL9 may have

potential as a biomarker to diagnose acute cellular rejection in kidney transplantation. In general, studies have shown that CXCL9 is a sensitive marker for the diagnosis of acute rejection in kidney transplant recipients, with reported sensitivities ranging from 68% to 94%. The specificity of CXCL9 as a marker of acute rejection is lower, with reported values ranging from 40% to 81% [34, 35].

CXCL10, known as interferon gamma-induced protein 10 (IP-10), is also used as a marker of acute kidney transplant rejection. CXCL10 has been shown to be a more sensitive and specific biomarker for acute cellular rejection compared to other commonly used markers such as serum creatinine and the panel reactive antibody test [35–37]. CXCL10 is a sensitive marker for the diagnosis of acute rejection in kidney transplant recipients, with reported sensitivities ranging from 73% to 91%. The specificity of CXCL10 as a marker of acute rejection is lower, with reported values ranging from 66% to 77% [38]. Additionally, Madhurantakam et al. indicated that CXCL10 levels have been shown to be a predictor of long-term allograft survival in kidney transplant recipients [39]. Notably, CXCL9 and, additionally, CXCL13, due to the mechanism of their production, are also viewed as markers of early infection in patients after transplantation.

DONOR-DELIVERED CELL-FREE DNA

Donor-derived cell-free DNA (cfDNA) is found in a variety of bodily fluids and is mostly derived from blood cells. Circulating cfDNA may be obtained from tumors, donor organs after transplantation, or, in some circumstances, the fetus during pregnancy. Circulating cell-free DNA (cfDNA) is a kind of deoxyribonucleic acid fragment that circulates in the blood and other bodily fluids. Donor-derived cell-free DNA (dd-cfDNA) is cfDNA that comes from a donated organ and is exogenous to the patient. In contrast to an invasive biopsy, dd-cfDNA can be discovered using a non-invasive sample analysis. Studies have found that the level of dd-cfDNA in the blood of kidney transplant recipients is positively correlated with the severity of acute rejection and that measurement of dd-cfDNA levels may be useful in detecting and monitoring acute rejection in those patients. For example, a study by Wijtvliet et al. [40] found that the level of dd-cfDNA in the blood was positively correlated

with the severity of acute rejection in kidney transplant patients. This correlation was also confirmed in studies by Fu et al. [43]. According to Matuszewski et al., the rise in dd-cfDNA concentration occurs before the rise in creatinine, which may allow for early detection of transplant damage and appropriate therapy to minimize premature graft loss [42]. The sensitivity of dd-cfDNA as a marker of acute kidney transplant rejection is relatively high, with values ranging from 60–80%. On the other hand, the specificity of dd-cfDNA as a marker of acute rejection can be lower, with values ranging from 40–60% [43–45]. It should be noted that dd-cfDNA accuracy as a sign of acute rejection can also be affected by the way it is analyzed, type of transplant, and overall health of the patient [46]. That means the method remains experimental.

ENDOCAN

Endocan, formerly known as endothelial cell-specific molecule-1, is a soluble proteoglycan found mostly in vascular endothelial cells of the lungs and kidneys [47]. It is activated by proinflammatory cytokines and is involved in inflammatory, proliferative, and neovascularization processes. Endocan's value as a biomarker in a wide range of disorders is becoming more well-recognized [48–51].

The exact role of endocan in kidney transplant rejection is not fully understood, but the activation of immune cells, including T cells and natural killer cells, is a crucial step in this process, and Endocan has been shown to play a role in this activation. Therefore, many authors think that endocan is a potential target for developing new immunosuppressive drugs [53].

In addition to its role in immune cell activation, Endocan has also been linked to other processes that contribute to transplant rejection, such as oxidative stress and inflammation [50]. The molecule has been shown to increase the production of reactive oxygen species, which can cause oxidative stress, cellular death, and damage to the transplanted kidney. Moreover, Zhao et al. showed that endocan binds to the chemokine receptor CXCR2 on NK T-cells (natural killer T-cells) [53].

Plasma endocan levels appear to give important prognostic information in several kinds of renal failure, including chronic kidney disease, IgA nephropathy, and diabetic nephropathy [54]. Endocan may also aid in early diagnosis of acute kidney illness, chronic renal allograft injury, and acute rejection following

kidney transplantation, hence contributing to prompt endothelial cell injury monitoring. Li et al. showed that endocan may reflect the degree of endothelial cell injury in renal allografts and serve as a highly sensitive and specific marker for acute rejection after renal transplantation [55]. Hence, the exact utility of this marker remains unknown, and more research is needed.

INTERCELLULAR ADHESION MOLECULE 1 (ICAM-1)

ICAM-1 is a cell surface protein that is expressed in the kidneys and has been proposed as a marker of acute kidney transplant rejection. Several studies have demonstrated that increased expression of ICAM-1 is associated with acute rejection and suggested that it may play a role in the recruitment of immune cells to the transplanted kidney [56]. This increased expression can be measured in the blood, providing a potential non-invasive marker of acute rejection.

ICAM-1 is a cell adhesion molecule that is involved in the immune response and has been shown to be upregulated in the kidneys during acute transplant rejection. Several studies have demonstrated that increased expression of ICAM-1 is associated with acute rejection, suggesting that it may play a role in the recruitment of immune cells to the transplanted kidney [57]. This increased expression can be measured in the blood, providing a potential non-invasive marker of acute rejection.

However, while ICAM-1 has shown promise as a marker of acute kidney transplant rejection, more research is needed to fully validate its use in clinical practice. Several studies have shown conflicting results, and the exact role of ICAM-1 in the rejection process is not yet fully understood. It is also possible that ICAM-1 expression may be influenced by other factors, such as infection, ischemia, or chronic allograft injury, making it difficult to differentiate between acute rejection and other causes of kidney dysfunction.

Researchers discovered that measuring ICAM-1 expression was a sensitive indicator of acute rejection in kidney transplant patients. This applies to both blood [58, 59] and urine [60] measures. ICAM-1 has been reported to have excellent sensitivity (capacity to detect true positive cases of rejection) but very low specificity in several investigations (i.e., the ability to rule out false positive cases of rejection). Research has shown that ICAM-1 has

moderate sensitivity and specificity as a marker of kidney transplant rejection [60].

In general, the utilization of ICAM-1 as a biomarker of renal transplant rejection is currently being investigated, and additional research is required to completely comprehend its therapeutic relevance. ICAM-1's diagnostic accuracy may also be affected by the rejection stage, graft status, course of the transplantation procedure, and other variables that influence the immune response to the transplanted kidney.

INTERLEUKINS

Another marker that plays a role in immune response following kidney transplantation is interleukin-2 (IL-2). IL-2 is a cytokine that stimulates the growth and activity of T cells, a mechanism that plays a role in the process of acute rejection. IL-2 receptor antagonist medications, such as basiliximab or daclizumab, are used in selected patients at increased risk of rejection, which helps suppress the immune response and lowers the rejection risk. However, it is important to note that IL-2 also has other important functions in the immune system, and the use of IL-2 receptor antagonists can have potential side effects. Furthermore, Witkowska et al. indicated in their study that IL-2 serum levels might be used as a late marker of unspecific cancers in individuals after kidney transplantation [61]. Moreover, IL-2 and its receptor are central to lymphocyte activation and are the main targets of calcineurin inhibitors. In addition, the anti-IL-2R antibodies inhibit a key target in immune activation. Since these anti-IL-2R antibodies are well tolerated and since calcineurin inhibitors are intrinsically nephrotoxic, anti-IL-2R antibodies have been used to avoid cyclosporin after transplantation [62].

Interleukin-5 (IL-5) is also a cytokine that promotes the survival, activation, and proliferation of eosinophils, a type of white blood cells. In the context of transplantation, eosinophils can contribute to rejection of the transplanted kidney by promoting inflammation and tissue damage. In patients with graft rejection, IL-5 levels have been observed to be higher than normal. However, it is important to note that elevated IL-5 levels are not always a definitive indicator of rejection, as there may be other factors contributing. Additionally, the exact relationship between IL-5 and kidney transplant rejection is still

not fully understood and requires further research. Therefore, IL-5 cannot be treated as an independent marker — but should be used with a combination of clinical and laboratory data to evaluate the possibility of kidney transplant rejection. For example, mitogen-induced peripheral blood lymphocyte IFN-gamma; IL-5 ratios ≥ 15 were highly predictive of allograft failure within 6 months of performing the assay [63].

Interleukin 6 (IL-6) is a multifunctional proinflammatory cytokine that is essential for T cell activation, survival, and differentiation. IL-6 increases rejection and abolishes tolerance by acting as a switch that drives the differentiation of naive T cells into Th17 cells while inhibiting their maturation into regulatory T cells. According to a meta-analysis by Omrani et al., a higher serum IL-6 level in renal transplant recipients compared to healthy controls indicated that the serum level of IL-6 might be utilized to evaluate inflammation in ESRD patients undergoing renal transplantation [64]. Urine but not serum IL-6 values are sensitive indicators of rejection [65]. Waiser et al. showed that the sensitivity of urine measurements was much higher (93%) than serum (54%). The specificity in serum (70%) and urine (60%) was reduced by infection, acute tubular necrosis, and antithymocyte globulin treatment. However, the levels of IL-6 soluble receptor (IL-6sR) in the blood and urine did not correspond with rejection.

Another pro-inflammatory cytokine that has been suggested as a marker of acute kidney transplant rejection is interleukin-8 (IL-8). Several studies have linked increased levels of IL-8 to acute kidney transplant rejection. IL-8 is generated by a variety of cell types, including leukocytes, fibroblasts, and renal tubular epithelial cells, and its levels can rise in response to cellular stress, such as immune system activation. High levels of IL-8 were discovered in the blood and urine of patients with acute kidney transplant rejection [66, 67]. However, IL-8 should not be used as a sole diagnostic marker for acute kidney transplant rejection and should be interpreted in the context of other clinical and laboratory findings.

Interleukin-18 (IL-18) is another pro-inflammatory cytokine that is being studied as a possible sign of acute kidney transplant rejection [68]. IL-18 is involved in immune response regulation as well as inflammation mediation. IL-18 is generated by a variety of cell types, including macrophages, dendritic

cells, and renal tubular epithelial cells, and it can be induced by cellular stress, such as infection or tissue damage [69, 70]. Striz et al. [71] showed that upregulation of epithelial IL-18 plays an important role in immune and immunopathological reactions in renal parenchyma and contributes to rejection mechanisms of kidney allograft. Moreover, Kim et al. [72] indicated that the 137GG genotype of the IL-18 gene, encoding higher IL-18 production, seems to be associated with AR (acute rejection) and may be a useful marker of AR risk in renal transplant recipients.

It is important to remember, however, that the interleukins we have discussed so far are just a few examples of this group of mediators that can be used to predict acute kidney transplant rejection.

KIDNEY INJURY MOLECULE-1

Kidney Injury Molecule-1 (KIM-1) is a transmembrane protein that is upregulated in response to kidney injury [73]. It has been shown to play a role in the development of kidney injury [74], including kidney transplant rejection [75].

Studies have found that elevated levels of KIM-1 in the urine or serum of kidney transplant patients are associated with increased risk of acute rejection. KIM-1 has been identified

as an independent predictor of graft outcomes and can be used to monitor the progression of kidney transplant rejection. KIM-1 is expressed in differentiated proximal renal tubular epithelial cells in damaged regions. It may participate in the progress of renal injury or repair. Many studies have illustrated different functions of KIM-1 in various renal diseases, including protective functions in acute kidney injury and damaging functions in chronic kidney disease. In injured renal cells, KIM-1 may function as a scavenger, with the phosphatidylserine type-1 receptor overseeing apoptotic cell phagocytosis [76] (Fig. 1). Following a renal injury (either ischemic or toxic), elevated KIM-1 levels may help differentiate acute tubular necrosis from prerenal azotemia and chronic kidney disease (CKD). Different authors proposed that elevated KIM-1 levels may also be used to identify patients at risk of progressing from acute kidney injury (AKI) to CKD, based on the observation that levels are constantly elevated in the latter [77, 80].

In addition to KIM-1's role in the response to kidney injury, KIM-1 has been shown to play a role in the regulation of kidney function by modulating the activities of various signaling pathways (ischemia-reperfusion injury pathway and the antibody-mediated injury pathway) involved in the regulation of renal homeostasis [79, 80].

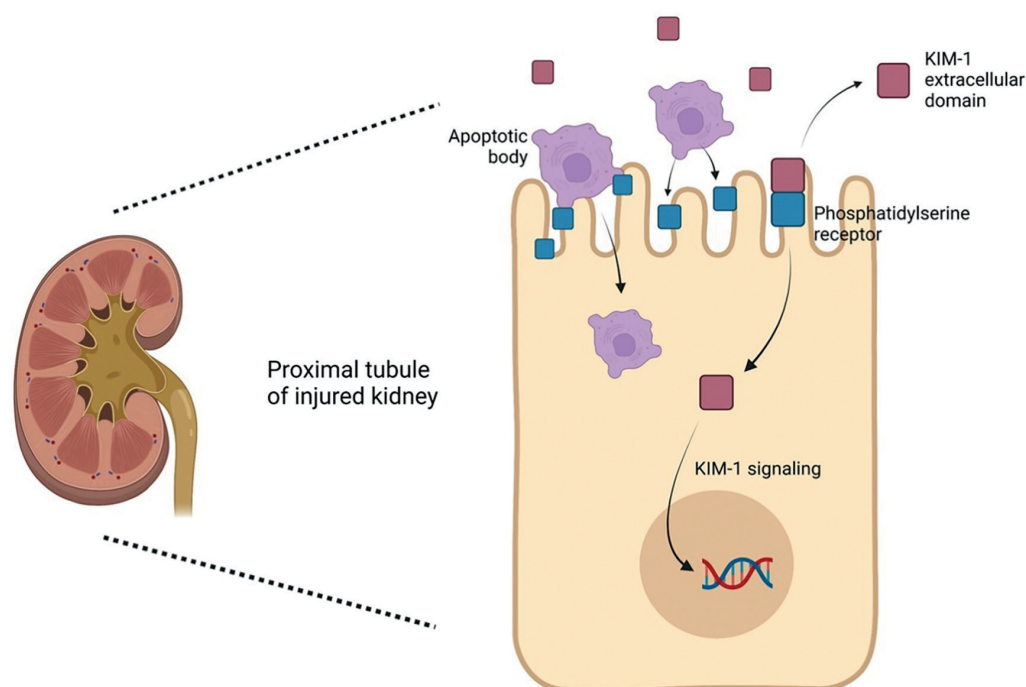


Figure 1. Kidney injury molecule-1 (KIM-1) expression in the proximal convoluted tubule after renal injury with phagocytosis of apoptotic cells

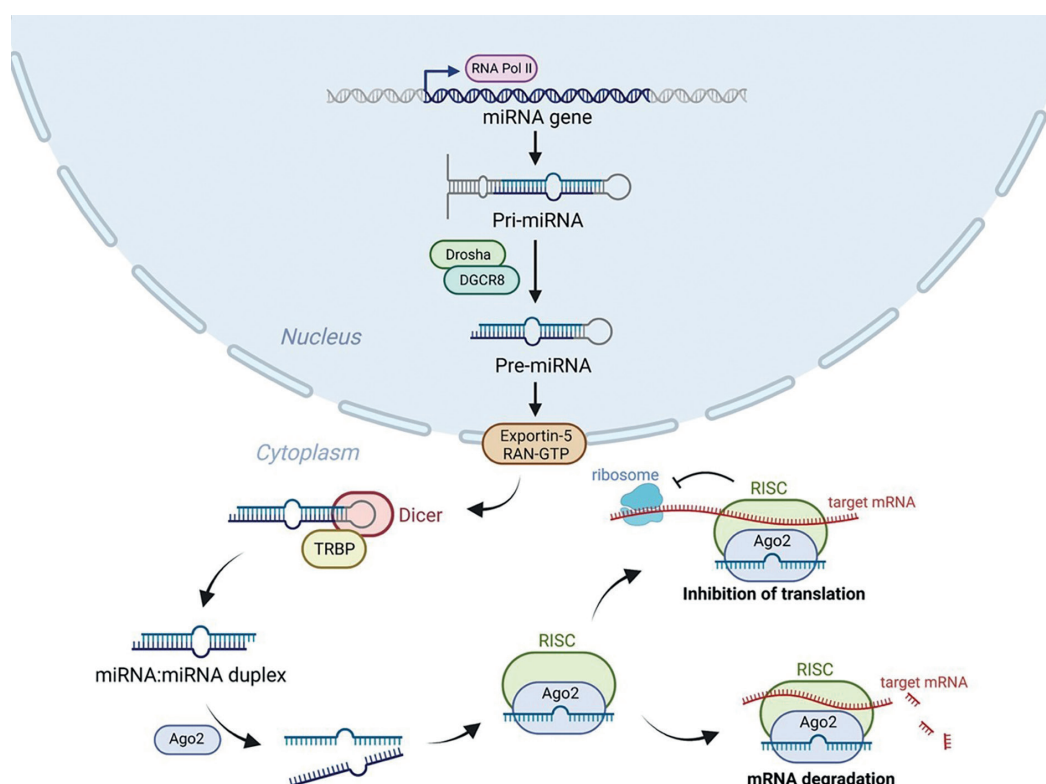


Figure 2. miRNA biogenesis and action mechanisms

Zhang et al. showed that KIM-1 staining sensitively and specifically identified proximal tubular injury and correlated with the degree of renal dysfunction. Moreover, KIM-1 expression is more sensitive than histological assessment treated so far as the gold standard for detecting early tubular injury, and its level of expression in transplant biopsies may indicate the potential for recovery of kidney function [81]. In addition, van Timmeren et al. indicated that urinary excretion of KIM-1 is an independent predictor of long-term graft loss and, therefore, a promising biomarker in the early prediction of graft loss [82]. The same conclusions were also reached by Szeto et al. [83], which indicates that KIM-1 serum may be the most promising and accurate marker for the prediction of early acute kidney allograft rejection.

miRNA

MicroRNAs (miRNAs) are short, non-coding RNA molecules that play a crucial role in regulating gene expression. At the same time, because they are highly stable in blood, urine, and other body fluids, they are thought to have potential as biomarkers and therapeutic targets for various diseases [84, 85], as well

as kidney transplantation [86–88]. However, as Roberts et al. rightly point out, the fact that one miRNA might be implicated in several illnesses remains a concern. Furthermore, standardization of miRNA expression levels throughout analysis remains a challenge [89]. The summary of miRNA biogenesis and mechanism of action is presented in Figure 2. Sui et al. indicated that 20 miRNAs were differentially expressed in three patients with acute kidney allograft rejection [90]. Anglicheau et al. [91] identified several upregulated miRNAs, including miR-142, miR-155, and miR-223, appropriately attributed to graft-involving immune cells and others expressed by resident renal cells that were downregulated (let-7c, miR-10b and miR-30a).

MiR-15a plays a multifaceted role in kidney transplantation, regulating a variety of cellular processes [92]. MiR-15a has been shown to impact the expression of genes implicated in the fibrotic response [93] and to reduce TGF-beta1 production, a key driver of fibrosis [94]. Furthermore, MiR-15a has been shown to affect the expression of other fibrosis-related genes such as type I collagen and alpha-smooth muscle actin. MiR-15a has also been related to immune response regulation, which is critical for the success of kidney

transplants. MiR-15a, for example, has been shown to influence the expression of genes involved in T lymphocyte activation and activity, which play an important role in the immune response. Also, it has been shown that it affects the expression of genes that control the production of cytokines [95].

Also, miR-21 has been identified as a possible biomarker for kidney transplant rejection. There is some evidence that miR-21 is upregulated in response to kidney transplant rejection, and it has been suggested that miR-21 may play a role in the rejection process [96] and that its levels in both blood and urine are linked to rejection [97]. MiR-21 has been shown to control a variety of immune-related cellular processes, including the activation and activity of immune cells such as T cells and macrophages. Furthermore, miR-21 has been discovered to influence the production of cytokines and other signaling molecules involved in the immune response. MiR-21, in particular, has been demonstrated to be a valuable marker for early detection of subclinical rejection, which standard clinical and laboratory approaches might miss. Furthermore, miR-21 levels have been demonstrated to correlate with the degree of rejection, implying that they might be utilized to monitor therapy response. Moreover, Chen et al. found that miR-21-5p, miR-20a-5p, and miR-101-3p all participated in the TGF-beta pathway and can be used as chronic allograft dysfunction-associated miRNAs in the TGF-beta pathway [98].

Elevated miR-125 values also correlate with acute rejection in kidney transplant patients. Sharaby et al. found that increased levels of miR-125 were significantly associated with acute rejection [99]. On the other hand, Zhang et al. found that elevated levels of miR-125 were detectable in urine samples from patients with acute rejection [100].

MicroRNA-142 (miR-142) also plays a role in the development of kidney transplant rejection. According to research, miR-142 levels in the blood of transplant patients experiencing acute rejection are changed. Increased levels of miR-142 have been linked to the activation of immune cells implicated in the rejection process, such as T cells and macrophages [101, 102]. Furthermore, miR-142 has been demonstrated to target genes that govern immune cell activity and contribute to transplant rejection. On the other hand, low levels of miR-142 have been linked to chronic allograft nephropathy, which is common in graft loss.

Another type of miRNA used as a marker in the case of kidney transplant rejection is microRNA-148 (miR-148). According to research, miR-148 expression levels are elevated in the blood and urine of patients who have undergone kidney transplantation and are facing rejection [103, 104]. This shows that miR-148 might be utilized as a non-invasive method for detecting transplant rejection early on.

Studies have shown that elevated levels of miR-155 are associated with kidney transplant rejection as it regulates inflammatory responses by playing a role in activation and differentiation of immune cells, such as T cells and B cells. Boštjančič et al. [105] found that increased levels of miR-155 in peripheral blood mononuclear cells (PBMCs) were associated with acute rejection in kidney transplant patients. Another study found that elevated levels of miR-155 in serum samples were predictive of biopsy-confirmed acute rejection in kidney transplant patients [106].

In turn, Liu et al. [107] indicated that miR-223 might have a significant role in the acute rejection of kidney transplantation. Another study found that mRNA targets of down-regulated miRNAs from serum, such as miR-1224-5p, miR-4508, miR-320, and miR-378a, were universally increased in tissue. When serum miRNA profiles were combined with tissue gene expression, it was discovered that variations in serum miRNAs indicate the function of T-cell mediated pathways in continuous allograft damage [108].

The optimal time for measuring microRNA levels to predict acute rejection in kidney transplantation is currently unknown and subject to ongoing research. Zhang et al. found that elevated levels of miR-125 in urine samples were detectable before the onset of clinical symptoms in patients with acute rejection [109]. This suggests that regular monitoring of miR-125 levels could provide an early warning sign of acute rejection, allowing for prompt intervention and management.

NEUTROPHIL GELATINASE-ASSOCIATED LIPOCAINE

Neutrophil gelatinase-associated lipocalin (NGAL) is an extracellular protein belonging to the lipocalin family. It is also called human neutrophil lipocalin (HNC), lidocaine-2, 24p3, uterocalin, or siderocalin [110]. NGAL has been found to be a reliable

early indicator of acute kidney injury and renal dysfunction, including kidney transplant rejection [10]. NGAL levels are typically measured in the blood or urine of transplant recipients, and a rapid increase in NGAL levels can indicate that the transplant is being rejected and prompt further testing and treatment. Moreover, Li et al. showed that blood NGAL is superior to urine NGAL in the early prediction of delayed graft function (DGF) in kidney transplant recipients [111]. For example, a study by Seeman et al. [112], which involved monitoring the NGAL levels in a cohort of pediatric renal transplant recipients over several months showed that elevated NGAL levels were significantly associated with increased risk of acute allograft rejection. According to a study performed by Lang-Lazdunski et al., NGAL levels were a better predictor of acute rejection than traditional markers such as creatinine levels [113]. However, it is worth noting that NGAL is not a specific marker of transplant rejection, and elevated levels can be seen in other conditions, such as infections, drug toxicity, or kidney injury unrelated to transplantation. This highlights the importance of using NGAL in conjunction with other diagnostic tests to accurately diagnose acute rejection in kidney transplant recipients. This is supported by Cappuccilli et al. [114], who concluded that increased NGAL levels are a strong predictor of acute rejection, especially when combined with other diagnostic assays.

The sensitivity and specificity of NGAL in predicting renal rejection varies according to the research and population under consideration. NGAL has been found in certain studies to have high sensitivity in detecting acute kidney damage and renal transplant rejection. For example, Cho et al. [115] discovered that NGAL has 89.3% sensitivity in detecting acute renal allograft rejection. Bolignano et al. [113] found that the NGAL level was higher in patients with acute renal allograft rejection, but it was also higher in patients with other causes of acute kidney damage. This made it harder to diagnose the exact cause of the increase in the NGAL level. In reaction to severe kidney damage or transplant rejection, NGAL levels might rise within 24–48 hours [113]. NGAL levels greater than 109 mg/mL measured 48 hours after kidney transplantation indicated DGF with 75% sensitivity and 71% specificity [116], which makes it one of the best standardized methods among those discussed in this review.

MONOCLONAL ANTIBODY BINDING PROTEIN (MABP-1)

MABP-1 is a kind of monoclonal antibody that is created in the laboratory using a technique known as hybridoma technology. This method entails combining a specific kind of immune cell (B-cell) with a cancer cell to form a hybrid cell capable of producing an endless amount of a single type of antibody. MABP-1 is made to attach to a specific target on transplanted kidney tissue. This lets it be used as a test to see if a transplant is being rejected quickly.

MABP-1 levels in the patient's blood may be determined using several laboratory techniques, including Western blot analysis or Enzyme-linked Immunosorbent Assay (ELISA) analysis. MABP-1 levels that are elevated may signal that the body is generating an immunological reaction to the transplanted kidney, which is a sign of acute rejection. While MABP-1 can be a valuable marker for acute rejection, increased levels alone may not be sufficient to diagnose rejection. Screening for acute rejection is an important element of post-transplant treatment in kidney transplant patients. MABP-1 levels can be utilized to monitor for acute rejection and to guide treatment decisions, such as immunosuppressive medication adjustments. Regular monitoring of MABP-1 levels can aid in the early detection of rejection and prevent additional damage to the transplanted kidney. Despite that, there is little information on MABP-1's sensitivity and specificity in kidney transplant rejection, MABP-1 may be a sensitive marker for acute rejection, according to some research. MABP-1 exhibited sensitivity of 77% and specificity of 75% in acute rejection [117, 118]. Loga et al. showed in their meta-analysis that MABP-1 had pooled sensitivity of 68.4% and specificity of 84.1% in detecting acute rejection in kidney transplant recipients [119]. It is crucial to note that the sensitivity and specificity of MABP-1 might vary depending on the patient group, test technique, and threshold utilized to identify a positive result [119].

OSTEOPONTIN (OPN)

Osteopontin (OPN) is a glycoprotein that has a role in kidney transplant rejection via a variety of mechanisms. To begin, OPN is a chemoattractant and stimulator of immune cells such as T cells and monocytes, which play a role in transplanted kidney rejection [120]. Furthermore, OPN has been demonstrated

Table 1. Summary of biomarkers used in monitoring transplanted kidney function

Biomarker	Advantages	Disadvantages	Sample type
Clusterin (CLU)	Early detection of delayed graft function; measurable in blood or urine	Not specific to kidney damage, can be elevated in other conditions	Blood; Urine
Creatinine	Widely available, low cost	Low sensitivity in early rejection, influenced by patient's muscle mass	Blood; Urine
Cystatin-C	Not affected by muscle mass, reflects GFR	Sensitivity decreases over time, costly	Blood; Urine
CXCL9/CXCL10	High sensitivity in acute rejection	Low specificity, levels may rise due to infections	Blood; Urine
Donor-Derived Cell-Free DNA	Early detection before rise in creatinine, non-invasive	Low specificity, still experimental	Blood
Endocan	Indicator of endothelial cell injury, involved in immune cell activation	Not fully understood in transplant rejection, more research needed	Blood
ICAM-1	Non-invasive, high sensitivity in rejection	Low specificity, influenced by infection or ischemia	Blood; Urine
Interleukins (IL-2, IL-5, IL-6, IL-8, IL-18)	Sensitive indicators of immune response and inflammation	Non-specific, elevated levels may occur in various conditions	Blood; Urine
KIM-1 (Kidney Injury Molecule-1)	High sensitivity, early detection of tubular injury	May not differentiate between types of injury (acute tubular necrosis vs. rejection)	Blood; Urine
MicroRNAs (miR-15a, miR-21, miR-142, miR-155)	Stable in fluids, non-invasive biomarkers, correlate with rejection severity	Interpretation remains complex, many miRNAs are implicated in various diseases	Blood; Urine
NGAL (Neutrophil Gelatinase-Associated Lipocalin)	Early predictor of acute kidney injury and rejection	Non-specific, can rise due to infections or other kidney injuries	Blood; Urine
Osteopontin (OPN)	Sensitive indicator of acute rejection, involvement in immune response	Requires more studies to validate its clinical utility	Blood; Urine
Procalcitonin	Moderate-to-high specificity in acute rejection, widely available	Can be elevated in other conditions such as sepsis	Blood
TNF-Alpha (Tumor Necrosis Factor Alpha)	Indicator of systemic inflammation and rejection	Moderate sensitivity and specificity, can be elevated in other inflammatory conditions	Blood; Urine

to boost the production of pro-inflammatory cytokines, which are inflammation-promoting chemicals. Inflammation is a major component of the immunological response to a kidney transplant, and it can cause tissue damage and rejection. OPN regulates the extracellular matrix, which is a network of proteins and carbohydrates that gives structural support to cells and tissues. OPN has also been shown to interfere the process of getting the immune system to accept a transplanted organ, which is called “inducing transplant tolerance”.

Several studies have found that high OPN levels in the blood are linked to increased risk of acute rejection in kidney transplant patients [121, 122]. Ranges of normal values for OPN in blood are typically considered to be below 30 ng/mL and in urine below 20 ng/mL.

Urinary OPN levels were used to diagnose acute rejection in kidney transplant recipients, with sensitivity of 73% to 76% and specificity of 78% to 88% [123]. Yang et al. then assessed blood OPN levels as a biomarker for acute renal allograft rejection [75]. In this study, 82 patients were analyzed, and it was found that serum OPN levels had sensitivity of 60% and specificity of 86% for diagnosing acute rejection.

Some research has also found that blocking or reducing OPN can help minimize the risk of transplant rejection. Researchers have tried a range of ways to suppress OPN in animal trials, including utilizing antibodies that specifically target the protein, decreasing OPN synthesis, and disrupting its interaction with other molecules involved in the immune response.

These studies have shown that inhibiting OPN can minimize the risk of transplant rejection and enhance transplant survival. Wang et al. found in a mouse model of kidney transplantation that suppressing OPN made acute rejection less severe and increased transplant survival [124]. In another study, Wang et al. summarize blocking OPN with an antibody to reduce the incidence of transplant rejection in a mouse model of heart transplantation [121]. Based on these results, it seems likely that targeting OPN could be a way to reduce transplant rejection and improve transplant outcomes.

PROCALCITONIN

Procalcitonin is a biomarker that has been shown to be indicative of acute kidney transplant rejection. Procalcitonin levels in the blood have been linked to the presence of acute rejection in transplant patients. However, procalcitonin is not a particular marker for rejection, and it can be elevated in circumstances other than rejection, such as sepsis and renal damage. Procalcitonin has been reported in certain studies to have moderate sensitivity (60–70%) and moderate to high specificity (80–90%) in identifying acute rejection in kidney transplant recipients [125, 126]. For example, in a meta-analysis conducted by Zhou et al., the overall sensitivity and specificity of procalcitonin for the diagnosis of acute rejection were 0.68 (95% CI: 0.59–0.76) and 0.83 (95% CI: 0.77–0.88), respectively [127]. However, in other trials, procalcitonin's sensitivity and specificity were lower [128], which makes use of this marker highly questionable in everyday practice.

TUMOR NECROSIS FACTOR ALPHA

Tumor necrosis factor alpha (TNF-alpha) has also been linked to acute kidney transplant rejection [129]. TNF-alpha works by promoting inflammation and activating the immune response [130]. In the case of acute kidney transplant rejection, TNF-alpha can trigger an immune response against the transplanted kidney, leading to inflammation and tissue damage [131]. This immune reaction may inflame and damage the graft tissue, limiting its function and possibly leading to graft failure.

TNF-alpha levels may be tested in serum or urine. Blood TNF-alpha levels are a direct indicator of systemic TNF-alpha levels and may be a useful predictor of systemic immune

response and the likelihood of acute kidney transplant rejection. TNF-alpha levels in urine may reflect the local immune response and provide a more accurate evaluation of TNF-alpha in transplanted kidneys [129]. This is especially valuable for tracking acute kidney transplant rejection since it may represent the degree of TNF-alpha-mediated tissue damage in the transplanted kidney.

In clinical studies, TNF-alpha was demonstrated to have moderate to great sensitivity in acute kidney transplant rejection, with sensitivity ranging from 50 to 80%. TNF-specificity alphas, on the other hand, may be moderate as markers for acute kidney transplant rejection, with some studies showing specificity ranging from 30–60% [132, 133].

EXTRACELLULAR VESICLES

Extracellular vesicles (EVs), such as exosomes, are being extensively researched as non-invasive indicators for the detection of kidney transplant rejection. These vesicles transport proteins, lipids, and RNA that indicate the cellular condition of the transplanted organ, providing significant insights into immune activity and tissue injury. We can extract extracellular vesicles from biological fluids such as urine or blood, making them a compelling alternative to conventional biopsy procedures. Their parts, like donor-derived cell-free -DNA, immune-related proteins, and microRNAs, have been linked to acute rejection events. This means that early diagnosis and better monitoring of grafts are possible [134, 135].

GENE EXPRESSION PROFILES IN KIDNEY TRANSPLANT MONITORING

Gene expression profiling (GEP) presents a viable method for assessing kidney transplant outcomes. It enables clinicians to identify immunological activation, inflammation, and tissue damage, before substantial functional deterioration. This strategy emphasizes the identification of alterations in the expression of particular genes associated with rejection, including those that govern T-cell activation and inflammatory responses. Research indicates that GEP testing in peripheral blood can accurately predict transplant rejection. A study showed that gene expression measurement could distinguish between rejection and non-rejection with accuracy of

85% [136]. A study of 155 kidney transplant patients indicated that increased levels of particular gene markers in peripheral blood samples were associated with biopsy-confirmed rejection events. Researchers reported that gene expression patterns can identify mild to severe rejection with sensitivity and specificity scores ranging from 75% to 90% [137]. GEP can evaluate regulatory T-cell activity, which is essential in preventing allograft rejection. A study demonstrated that longitudinal monitoring of T-cell-related gene expression showed a substantial association between lower expression levels and acute rejection, with diagnostic sensitivity of 78% and specificity of 82% [138].

SUMMARY

To conclude, any variations from the commonly used norms of laboratory indicators in patients after transplantation have to be treated with caution as they can suggest graft-threatening circumstances, i.e. the possibility of an adverse immune response, increasing the risk of transplant rejection that is indirectly life-threatening. Moreover, the above-mentioned biomarkers are not always specific for rejection, and their elevated levels might be found in other conditions such as

sepsis and renal diseases. So, they should be used along with other clinical and lab results to spot rejection and figure out how to treat it. Moreover, in our opinion, a cross-sectional prospective research study with a large number of kidney transplant patients and multiple biomarkers is required to identify which biomarkers are most useful in predicting acute kidney transplant rejection (AKTR) and how early rejection can be diagnosed. All in all, according to up-to-date knowledge, needle biopsy remains the gold standard for diagnosing AKTR, while the discussed markers can be valuable indicators for histological assessment.

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A comprehensive review and meta-analysis of suPAR as a predictor of acute kidney injury

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Abstract

Introduction and Objective. The global impact of acute kidney injury (AKI) has not been thoroughly investigated. With the development of new techniques, soluble urokinase plasminogen activator receptor (suPAR) has become increasingly important in the diagnosis of AKI. Therefore, a systematic review and meta-analysis was carried out to evaluate the predictive value of suPAR for AKI.

Materials and method. The review and meta-analysis investigated the relationship between suPAR levels and acute kidney injury. Pubmed, Scopus, Cochrane Controlled Register of Trials, and Embase were searched for relevant studies from inception to 10 January 2023. Stata (Ver. 16 StataCorp, College Station, TX, USA) was used for all statistical analyses. A random effects model using the Mantel-Haenszel approach was employed, and odds ratios (OR) and standard mean differences (SMD) with 95% confidence intervals (CI) were calculated for binary and continuous outcomes, respectively.

Results. Nine studies reported suPAR levels among patients with and without AKI. Pooled analysis showed that suPAR levels in patients with and without AKI varied and amounted to 5.23 ± 4.07 vs. 3.23 ± 0.67 ng/mL (SMD = 3.19; 95%CI: 2.73 to 3.65; $p < 0.001$). The results from the sensitivity analysis did not alter the direction.

Conclusions. This results show that increasing suPAR levels are associated with the occurrence of AKI. SuPAR might act as a novel biomarker for CI-AKI in clinical practice.

Key words

soluble urokinase plasminogen activator receptor, suPAR, acute kidney injury, acute renal failure, biomarker, prediction, meta-analysis

Abbreviations

AKI – acute kidney injury; **ARF** – acute renal failure; **CI** – confidence interval; **NGAL** – neutrophil gelatinase-associated lipid calin; **NOS** – Newcastle Ottawa Scale; **OR** – odds ratio; **ROS** – reactive oxygen species; **SMD** – standard mean difference; **suPAR** – soluble urokinase plasminogen activator receptor

INTRODUCTION

Acute kidney injury (AKI) is a clinical condition characterized by a rapid (hours to days) decrease in renal excretory function and the accumulation of nitrogen metabolism products, such as creatinine and urea, as well as other clinically unmeasured waste products [1, 2]. Other common clinical and laboratory signs include less urine output (which does not always happen), a buildup of metabolic acidosis, and higher potassium and phosphate [3].

To emphasize that there is a continuum of kidney injury that begins long before sufficient loss of excretory kidney function can be evaluated with routine laboratory testing,

the term ‘acute kidney injury’ in global guidelines has replaced the previously used ‘acute renal failure’. AKI can have substantial implications, such as increased morbidity, mortality, and length of hospitalization. For example, AKI has been identified as an independent mortality risk factor in patients treated in intensive care units; therefore, early diagnosis of AKI can be essential in improving the prognosis and treatment of this group of patients. While kidney biopsy, an invasive procedure with possible complications, is not useful and practical in the early identification of AKI, there has been a surge of interest in the use of biomarkers to help in the early diagnosis and management of AKI in recent years. Among others, soluble urokinase plasminogen activator receptor (suPAR) is one such biomarker [4].

SuPAR is a new biomarker that has been found to be an excellent predictor of AKI. It is a protein that a variety of cells, including immune cells, release into the circulation

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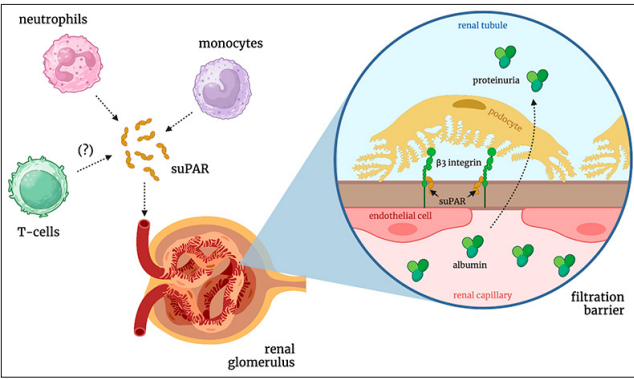


Figure 1. A visualization illustrating the effects of suPAR on the potential development of AKI through the promotion of inflammation, oxidative stress, and apoptosis in the kidneys

and is involved in a variety of physiological processes (Fig. 1). SuPAR levels in the blood of people with AKI have been found to be higher, and the size of this increase has been linked to the severity of the condition [5].

One of the hot spots in kidney disease research in recent years has been the discovery and deployment of new biomarkers for early identification of AKI. A previous study discovered that plasma suPAR was an unanticipated result of neutrophil, monocyte, and macrophage shedding of inflammatory receptors. A mechanistic investigation on kidney disease models indicated that circulating suPAR (derived from inflammatory cells) interacted with v3avβ3 integrins on podocytes, and that higher plasma suPAR levels might predict nephropathy in otherwise healthy persons and those at risk of chronic kidney disease. SuPAR has been shown to be implicated in the pathogenesis of AKI [6, 7]. The causal pathogenic role of suPAR in the development of AKI was recently substantiated by Nussbag et al., using both clinical data and transgenic mouse experiments [8, 9].

In the context of acute kidney injury (AKI), suPAR has been found to play a significant role in the development of the disease. The levels of suPAR in the blood have been shown to be elevated in patients with AKI, and the extent of this elevation has been shown to be related to the severity of the disease, which suggests that suPAR may contribute to the development of AKI by promoting inflammation [10, 11], oxidative stress [12], and apoptosis in the kidneys [13].

SuPAR has been shown to activate immune cells, including monocytes and neutrophils which play a key role in the development of kidney injury [10]. These immune cells release a number of pro-inflammatory cytokines and chemokines that can damage the kidneys and lead to the development of AKI.

As emphasized previously by the authors, suPAR may contribute to AKI through its effects on oxidative stress, a condition in which there is an imbalance between the production of reactive oxygen species (ROS) and the body’s ability to neutralize them. ROS can cause oxidative damage to cells and tissues, leading to the development of various diseases, including AKI. SuPAR has been shown to induce oxidative stress in the kidneys, which may also contribute to the development of AKI.

In addition to its effects on inflammation and oxidative stress, SuPAR may also contribute to AKI by promoting apoptosis [12]. Apoptosis is a type of programmed cell death that occurs in response to various stimuli, including injury, inflammation, and oxidative stress [12]. In the context of AKI, SuPAR has been shown to induce apoptosis in kidney cells, which can lead to further damage and the development of the disease.

The presented study aims to evaluate circulating SuPAR as a prognostic marker of acute kidney injury.

MATERIALS AND METHOD

The PRISMA (Preferred Reporting Items for Systematic Reviews and Meta-Analysis) guidelines were used to carry out this systematic review and meta-analysis [14] (Suppl. Tab. 1). The PROSPERO Registration No. for this systematic review is CRD42023422987.

Data sources and searches. Articles on the relationship of suPAR levels to acute kidney injury were found by searching Pubmed, Scopus, the Cochrane Controlled Register of Trials, and Embase from their inception. The initial searches were conducted on 15 September 2022, and repeated on 20 April 2023. For each database, a specific and effective search method was employed using the following search terms: ‘suPAR’, ‘soluble urokinase plasminogen activator receptor’ AND ‘Acute Kidney Injury’ OR ‘Acute Renal Failure’, ‘AKI’ or

Table 1. Baseline characteristics of included trials

Study	Country	Study design	Acute kidney injury group			No acute kidney injury group			NOS score
			No of patients	Age	Gender, male	No of patients	Age	Gender, male	
Azam et al., 2020	International	Multi-center observational study	91	65(15)	64 (70.3)	261	60(17)	138 (52.9)	9
Gussen et al., 2019	Germany	Prospective single-center non-interventional cohort study	87	NS	NS	27	NS	NS	7
Hayek et al., 2020	USA	Prospective observational cohort study	318	68 (12)	224 (70)	3509	66 (12)	2413 (69)	8
Mossenen et al., 2017	International	Multi-center observational study	21	75 (66-79)	15 (71.4)	86	67 (61-75)	62 (72.1)	8
Qin et al., 2021	China	Prospective study	65	67 (12)	46 (70.8)	334	63 (13)	212 (63.5)	8
Rasmussen et al., 2021	Denmark	Retrospective observational study	327	68 (59-74)	268 (82)	597	67 (59-73)	470 (78.7)	8
Skalec et al., 2022	Poland	Single-centre, prospective observational study	39	69(2)	19 (48.7)	12	60(52)	8 (67)	8
Walls et al., 2021	Denmark	Single centre study	33	75.9 (72.3-83)	8 (24.2)	306	77.9 (70.5-84.5)	119 (38.9)	9
Zhang et al., 2022	China	Case-control study	17	64 (59-69)	13 (76.5)	21	60 (56-66)	13 (61.9)	8

'ARF'. Manual searches were also conducted on the reference lists of the included papers and pertinent systematic reviews. Endnote (X7 for Windows, Clarivate Analytics, Philadelphia, PA, USA) was used to consolidate search results. Duplicates were deleted.

Study selection. All retrieved articles were screened against predetermined selection criteria independently by two investigators (LJ and MP) for the identification of relevant studies. Any differences of opinion were resolved by dialogue with the senior author. Prospective and retrospective observational studies that compared suPAR levels in adult patients, with and without AKI, were included. To avoid overlapping bias in the analysis, only the most detailed report was used when many studies from different institutions included the same groups of people. Moreover, to reduce publication bias, research with a paediatric population, articles with no original data, review papers, conference abstracts or presentations, and editorials or expert opinions were excluded.

Data extraction. Using a predetermined extraction form, three reviewers independently extracted data and evaluated the quality and bias risk of included studies (LJ, MP and AG). The following data was taken from each study: publication data (last name of the first author, year of publication, study design), suPAR levels among patients with and without AKI. When information was uncertain, the authors were contacted. Data from included studies were entered into a pre-defined report form in Microsoft Excel (Microsoft Corporation, Redmond, WA, USA).

Methodological quality and risk of bias assessment. Two authors (LJ and AG) independently evaluated methodological quality and bias risk for publications that satisfied the inclusion criteria. If a decision was contested, a third author served as an adjudicator (LS). The risk of bias within an individual study was determined using the Newcastle Ottawa Scale (NOS) [15] which evaluates the quality of a research study using three criteria: selection, comparability, and exposure. These three variables had maximum scores of 4, 2, and 3, respectively. Studies with NOS scores of 7 were considered high-quality.

Data synthesis and analysis. Stata (Ver. 16 StataCorp, College Station, TX, USA) was used for all statistical analyses. All P values were determined using a two-sided test, and a P value less than 0.05 was considered statistically significant. The incidence of dichotomous data was calculated using the odds ratio (OR) with a 95% CI and analyzed using the Mantel-Haenszel technique. The standard mean difference (SMD) with a 95% confidence interval (CI) was used to represent continuous outcomes. In cases where a research presented a continuous outcome as median, range, and interquartile range, means and standard deviations were approximated using the Hozo et al. method [16].

In all the assessed outcomes, heterogeneity was evaluated by observing forest plots and utilizing I^2 statistics. The I^2 cut-off thresholds of 25%, 50%, and 75% denoted low, moderate, and high degrees of heterogeneity, respectively. If I^2 was greater than 50%, a fixed-effects model was employed; otherwise, a random-effects model was used [17]. Due to the small number of investigations ($n < 10$) a funnel plot was not performed. A sensitivity analysis was performed, in which

one research study was withdrawn at a time, and the others were examined to determine the stability and reproducibility of the amalgamated effects.

RESULTS

The PRISMA flow diagram is presented in Figure 2. A literature search of the four databases (PubMed, Embase, Cochrane, and SCOPUS) yielded 355 articles. After removing 181 duplicates, title and abstract screenings of 174 articles was conducted, excluding a further 154. After excluding 11 articles through full text sieving, nine articles were included in the final meta-analyses [6, 18–25].

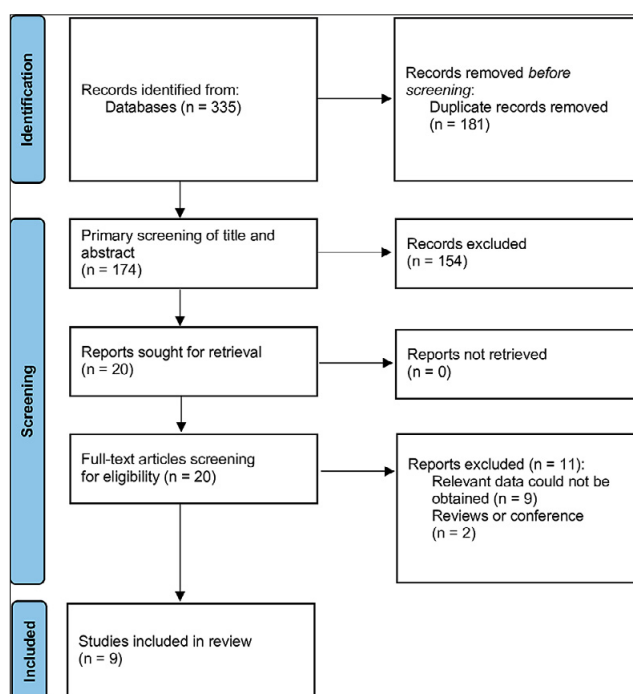


Figure 2. Flow diagram of the search strategy and study selection

The nine trials comprised a combined cohort of 6,151 patients. The participant baseline characteristics of the included studies are shown in Table 1. All selected studies had been published between 2017 – 2022. Of the nine trials, two were performed in Germany and China, and one in each of the following countries: Poland, USA and Denmark. Two studies were international. The NOS scores of the eight included studies were ≥ 7 .

Nine studies reported suPAR levels among patients with and without AKI. Pooled analysis showed that suPAR levels in patients with and without AKI varied and amounted to 5.23(4.07) vs. 3.23(0.67) ng/mL (SMD = 3.19; 95% CI: 2.73 to 3.65; $p < 0.001$) (Fig. 3). The results from the sensitivity analysis did not alter the direction.

DISCUSSION

A systematic and comprehensive meta-analysis that included nine studies was conducted to examine the correlation between suPAR levels and acute kidney injury occurrence. This soluble protein is produced by many different cell types

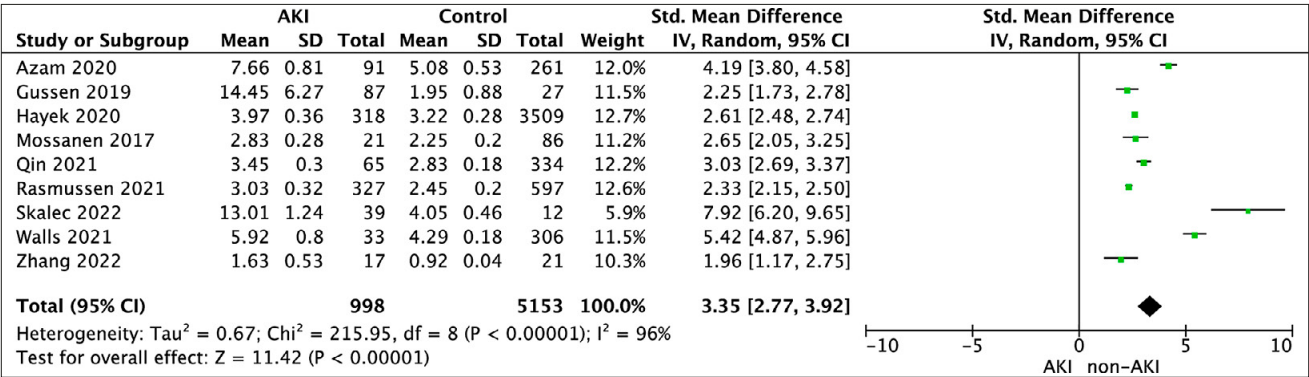


Figure 3. Forest plot of suPAR levels among patients with and without AKI. The centre of each square represents the standardized mean differences for individual trials, and the corresponding horizontal line denotes a 95% confidence interval. The diamonds represent pooled results

and is involved in a number of physiological processes, including inflammation, cell migration and angiogenesis. Studies have shown that elevated levels of suPAR are associated with a variety of diseases, including cancer [26, 27], autoimmune diseases [11, 28], and infectious diseases [29, 30]. In particular, suPAR has been found to be a strong predictor of AKI [31, 32]. The levels of suPAR in the blood have been found to be elevated in patients with AKI, and the extent of this elevation has been shown to be related to the severity of the disease. This is also confirmed by the results obtained in this meta-analysis, which indicate that the level of suPAR closely correlates with the risk of AKI.

SuPAR's high specificity is one of the key reasons it is an excellent predictor of AKI. As indicated by Huang et al., the sensitivity of suPAR in predicting AKI was 0.77 (95% CI 0.67–0.84) and the specificity was 0.64 (95% CI 0.53–0.75) [33]. Moreover, suPAR, unlike other indicators often used to detect AKI, such as creatinine and blood urea nitrogen (BUN), is unaffected by age, muscle mass, or nutrition [34]. As a result, it is a more trustworthy indication of renal function. Furthermore, suPAR has been demonstrated to be higher in individuals with AKI before increases in creatinine or BUN levels are noticed, indicating the start of AKI at an early stage. This early warning is especially important in critically ill patients, who are more likely to develop AKI, and where prompt treatment can make a significant difference in patient outcomes. Moreover, as Azam et al. reported, admission suPAR levels in patients hospitalized for COVID-19 are predictive of in-hospital AKI and the need for dialysis [18]. The prognosis enabling early detection of individuals at risk of developing AKI is highly fascinating and should be one of the focuses of the most recent research on this biomarker. This hypothesis is confirmed by studies that demonstrate that suPAR predicts AKI years before it occurs. Even in acute medical patients with decent eGFR (> 60), if they have a high suPAR, their eGFR is rapidly declining, which shows that suPAR is predictive of eGFR decline [35].

Elevated suPAR levels have been linked to an increased chance of developing AKI as well as a worse prognosis for AKI patients. A study of critically ill patients, for example, discovered that individuals with higher suPAR levels were more likely to need renal replacement treatment, such as dialysis, and had a higher risk of mortality. This emphasizes the need for monitoring suPAR levels in critically ill patients and using the data to guide treatment decisions.

Biomarkers, such as neutrophil gelatinase-associated lipocalin (NGAL), renal injury molecule 1, urine IL 18, and

plasma cystatin C, have also been studied and will continue to be studied in AKI [36]. The area under the curve (AUC) for suPAR and NGAL was 0.69 and 0.78, respectively, with no significant difference ($p = 0.117$). SuPAR in conjunction with NGAL had an AUC of 0.80, which was significantly higher than suPAR alone ($p = 0.032$) [24]. This further underlines the importance of suPAR and its research in the context of AKI.

SuPAR has been found to be a key predictor of renal recovery in addition to its diagnostic and prognostic significance. SuPAR levels have been reported to decline following the beginning of AKI, and patients with lower suPAR levels are more likely to restore renal function. Monitoring suPAR levels may thus be a valuable tool for directing treatment decisions and measuring the efficacy of therapies targeted at improving renal function.

The first paper on the possible causal role of suPAR in the development of AKI was published by Hayek et al. in 2020 [19]. These data have been corroborated in previous studies [8, 9]. The causal role of suPAR opens new therapeutic possibilities for the prevention and treatment of AKI through the direct targeting of suPAR with biological treatment, e.g., anti-suPAR antibodies, and commercial efforts are pursuing these opportunities.

Despite its great potential, suPAR is not frequently employed in clinical practice as an AKI biomarker. This is due to a number of factors, including the small number of standardized techniques for assessing suPAR levels, and the need for additional validation in larger and more diverse patient groups. Currently, just one firm, 'ViroGates' in Birkoroed, Denmark, has created the sole CE/IVD certified methods for suPAR measurement, which include turbidimetric analysis, suPARnostic turbilatex, and POC tech, which may present major prospects for the future widespread application of this biomarker [37]. Furthermore, the expense of suPAR testing may be prohibitive for some healthcare systems. However, as more research is undertaken and the benefits of employing suPAR as a biomarker are understood, its usage is expected to become more prevalent.

CONCLUSIONS

This systematic review and meta-analysis show that increasing suPAR levels are associated with the occurrence of AKI. SuPAR might act as a novel biomarker for CI-AKI in clinical practice.

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The role of protocolar biopsy in the diagnosis of kidney allograft dysfunction

Abstract

Core-needle biopsy in patients with impaired renal transplant function and histopathological evaluation of the obtained tissue samples is a recognized diagnostic method of numerous graft pathologies. Until now the use of this invasive procedure in patients with no revealed signs of transplant pathology and with a stable function of the transplanted kidney at planned intervals after transplantation (the so-called protocol biopsies) has seemed inconclusive.

It is known that changes in the biopsy of the transplanted kidney are an earlier marker of transplant pathology in relation to laboratory abnormalities and the appearance of clinical symptoms, and the accumulation of subclinically progressing chronic changes is currently considered to be the main cause of

renal graft loss. The histopathological evaluation also allows for the assessment of prognosis and the introduction of possible changes in the ongoing treatment. Opponents of protocol biopsy emphasize that it is an invasive procedure and exposes the patient to complications. Due to controversial reports on the usefulness of this method, protocol biopsies are not a routine tool for monitoring transplantation in transplant centers both in Poland and in the world. There is no established regimen for performing them.

This review article summarizes the current state of knowledge concerning the use of protocol biopsies in the diagnosis of transplanted kidney.

Key words: protocolar biopsy, kidney transplantation, subclinical antibody mediated rejection, subclinical T cell mediated rejection

INTRODUCTION

Extending long-term survival of the kidney graft constitutes one of the main challenges in organ transplantation. The main cause of graft loss in the long-term follow-up period is the accumulation of irreversible chronic lesions resulting from untreated or unresponsive to treatment rejection-related processes [1]. Deterioration of the graft kidney function, manifested by increased creatinine levels and decreased eGFR, is usually a late symptom of the developing pathology.

Such lesions may be detected by histopathological examination of a kidney graft specimen at a much earlier stage, which offers a chance to initiate treatment before irreversible chronic graft damage takes place. Protocol biopsy is dedicated to detecting graft pathologies at an early stage, when injury progression may still be halted. In the last 15 years, opinions on the diagnostic and prognostic utility of the

graft kidney biopsy have varied. Following the introduction of potent immunosuppressants in the 1990s and the resulting drop in the incidence rates of acute T cell-mediated rejections (TCMR), many researchers came to believe that protocolar biopsies were unwarranted as they failed to provide information that would lead to therapeutic management modification. However, recent years have shown that graft kidney dysfunction is caused primarily by an antibody-mediated rejection (ABMR) process and is associated with the *de novo* production of donor-specific antibodies (DSA) at any time after transplantation [2]. The rejection may be clinically silent. The findings have shed light on potential utility of protocol biopsy as a tool for detecting clinically silent pathologies at a stage where progression can still be halted. A wealth of information was provided in the 2015 publication, in which Loupy et al. presented the results of 1001 protocol biopsies, performed 12 months after kidney trans-

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plantation (KTx), which revealed subclinical T cell-mediated rejection in 13% and subclinical ABMR in 14% of cases. In the further 8-year follow-up period, patients with subclinical ABMR had significantly worse graft survival (56%) compared with patients with subclinical TCMR (88%) and patients without rejection (90%) ($p < 0.001$). In a multivariate analysis, subclinical ABMR one year after KTx was associated with a 3.5-fold increase in graft loss, decrease in eGFR and proteinuria. As for patients with subclinical TCMR one year after KTx, only those who had developed DSAs and graft glomerulopathy had a higher risk of graft loss, compared with patients without rejection. According to the Authors, subclinical ABMR and TCMR affect graft survival in a different way. Subclinical ABMR was a risk factor for graft function deterioration and loss regardless of baseline DSAs status, eGFR and proteinuria. Subclinical T cell-mediated rejection did not lead to graft function deterioration but increased de novo production of DSAs [3].

The publications available focused also on identifying specific groups of patients who might require intensive histopathological surveillance and would benefit from protocol biopsy as a sensitive diagnostic tool [4]. Despite its likely benefits, protocol biopsy is rarely used to monitor the graft kidney function, either in Poland or worldwide. According to UNOS (United Network for Organ Sharing) survey from 88 transplant centers in US forty percent ($n = 36$) centers reported performing protocol biopsies (20% in all cases and 20% in select cases). The most common time points for performing protocol biopsies were 3- and 12-months (72% each), 6-months (44%), 1-month (31%), and 24-months (25%). Two centers reported performing them at 60 months post transplantation. For diagnosing TCMR, 100% used indication biopsy, 28% used protocol biopsy, 2% used serum biomarkers, and none used urine cytokines. For ABMR, 99% used indication biopsy, 34% used protocol biopsy, 72% used DSA, 21% used C1q positive DSA, and none used gene profiling [5].

TECHNIQUE AND SAFETY OF THE PROCEDURE

The procedure is carried out by nephrologists or surgeons, and occasionally by other specialists. Given the non-anatomical location of the transplanted kidney, most centres per-

form an ultrasound scan immediately before the procedure to accurately assess the graft topography and rule out possible contraindications to the procedure. In patients receiving an anticoagulant or antiplatelet therapy, protocol biopsy may usually be planned in advance or such therapy may be discontinued and, if the patient's condition so requires, low molecular weight heparin may be administered temporarily, which may then be discontinued immediately before the procedure [6].

The most common complications of graft kidney biopsy include perirenal haematomas, while intrarenal arteriovenous fistulas are a little less common. The estimated incidence rates of graft kidney biopsy complications requiring therapeutic management, e.g. blood transfusion or surgical intervention, range from 0% to 4% according to different authors, however, protocol biopsy is associated with an up to 10-fold lower risk of complications compared with biopsy performed "when indicated" [7]. This is related to the planned preparation for the procedure, as well as the patient's good condition at baseline (usually). Taking into account the data available, the prevailing opinion is that protocol biopsy of the graft kidney is a safe procedure, associated with only a low risk of complications, and may be offered to kidney transplant recipients as a routine diagnostic procedure [8].

PRACTICAL UTILITY OF PROTOCOLAR BIOPSIES

Protocolar biopsies are performed at fixed intervals, and the exact schedule depends on the centre's experience and clinical situation. Typically, the first protocolar biopsy is performed on the operating table, during the transplantation procedure, immediately after organ reperfusion. Some authors even propose biopsy "0" (the so-called implantation biopsy), immediately after transplantation, and the so-called biopsy "1 hour," performed one hour after reperfusion, which is supposed to allow for a more accurate assessment of the graft kidney baseline status and the prognosis, taking into account possible early immune reactions and reperfusion-related damage. Such biopsy, in addition to baseline graft assessment, may offer some prognostic information — it has been demonstrated that detection of interstitial fibrosis with tubular atrophy (IF/TA) in specimens collected in the first hours after organ implantation constitutes a negative

prognostic factor and is associated with lower eGFR of the graft [9]. Similarly, the presence of IF/TA, particularly in combination with features of chronic inflammation identified by subsequent biopsies, also constitutes an unfavourable prognostic factor for graft survival. Clearly, the time points may be affected by the patient's individual clinical situation, including the baseline donor-recipient immunological risk status, immunosuppressive and induction therapy, further plans, e.g. minimisation of immunosuppression (IS), and chronically elevated serum levels of calcineurin inhibitors [10]. In general, it is thought that earlier protocolar biopsies are associated with a greater chance of detecting subclinical alloimmune responses (which usually develop within the first three months of transplantation; such biopsy may provide important data that may affect decisions on further IS treatment and possibly minimization of IS), while 1-year biopsies offer a greater chance of detecting graft pathologies such as BK virus infection, recurrence of the underlying disease (glomerulonephritis), lesions resulting from nephrotoxicity of calcineurin inhibitors or signs of chronic inflammation, which has a prognostic value. Subsequently 3-, 5-, 7-, and even 10-year biopsies can be performed to evaluate chronic ABMR, the main cause of graft loss. Annual DSA monitoring is strongly recommended for all kidney transplant recipients. TCMR usually disappears by the 3-year biopsy. Based on detecting pathological changes from protocol biopsy there is possibility of changing diagnosis, changing treatment, reducing immunosuppression dose [11, 12].

Protocol biopsies may be a useful tool to detect viral infections such as BKVN because early diagnosis is necessary to resolve infection and prevent chronic damage. Buehrig et al. demonstrated that all patients with BKVN diagnosed by protocol biopsies and managed by immunosuppression reduction had a satisfactory outcome by 6 months after diagnosis; in contrast, 70% of those with a late diagnosis by indication biopsies had deterioration of kidney function or graft loss. Since many reports support the utility BK virus DNA PCR as a screening strategy for BKVN, protocol biopsies only for BKVN may be unnecessary [13].

Recurrence of native kidney disease following kidney transplantation affects between 10% and 20% of patients, and accounts for up to 8% of graft failures at 10 years post transplant. Subclinical recurrence of both primary

and secondary glomerular diseases is well recognized. Asymptomatic histological recurrence in renal allografts may be missed if protocol biopsies are not available. However the histological diagnosis may be missing because many transplant biopsies are not routinely processed using immunofluorescence and electron microscopy. Another limitations of utility of protocol biopsy for diagnosis of recurrent glomerulonephritis include unknown cause of native kidney disease, donor transmitted glomerulonephritis, lack of histologic features of FSGS in early stage of recurrence. Recurrence of glomerulonephritis in majority of patients is diagnosed in biopsy for cause due to proteinuria [14].

It should be emphasised that both T cell-mediated rejection and antibody-mediated rejection may have subclinical presentation. Early initiation of treatment of these pathologies allows to prevent progression of the lesions as well as the development of IF/TA or chronic graft glomerulopathy, thereby extending graft survival. One cannot omit the psychological aspect of the surveillance biopsies in graft recipients – when presented with the current state of knowledge of graft kidney protocolar biopsy and the benefits associated with the procedure, as well as the risks associated with this invasive procedure, few patients refuse to consent to biopsy and inclusion in the protocolar biopsy programme. This is all the more noteworthy as protocolar biopsy is associated with hospitalisation at the primary centre, which on the one hand constitutes an inconvenience, especially that protocolar biopsy is not performed because of any indications, but on the other — means an opportunity of medical surveillance in the inpatient settings.

It is important to dispel doubts about the eligibility of specific patient groups to protocolar biopsy. There have been reports on groups of patients in whom protocolar biopsies do not provide significant benefits with respect to the risk associated with the procedure. Biopsies performed within the first two weeks of transplantation appear to be of no benefit to low-risk patients in whom immunosuppression protocols with induction are used and who subsequently receive calcineurin inhibitors, even if delayed graft function (DGF) is the indirect indication for such a procedure [15]. This is supported by the predominant opinion that this invasive procedure is not necessary in the case of patients with low immunological risk. Many of the publications available em-

phasise the need for individualised assessment of eligibility to biopsy, taking into account not only the immunological factors concerning the donor-recipient relationship but also the clinical profile of the recipient. Factors that should be taken into account in the eligibility assessment include the patient's age, cardiovascular diseases (heart failure before/after transplantation, atherosclerosis), type 2 diabetes mellitus, post-transplant urinary tract infections, serious infections, rejection episodes and cancer [16]. In each case, the decision to propose protocolar biopsy to a patient should be made on a case-by-case basis, taking into account a wide range of factors as well as the centre's experience in this area.

RECENT LITERATURE REVIEW

Researchers from Taiwan analysed the results of protocolar biopsies in 68 kidney recipients and compared them with the results of biopsies in 122 stable recipients two years after transplantation. The rejection process was identified by 13 protocolar biopsies, and in 11 cases borderline lesions were detected. Patients were administered glucocorticoid pulses. Over the 5-year follow-up period, graft survival was better in the protocolar biopsy group ($p = 0.0143$). In four and 17 recipients in the protocolar biopsy group and non-protocolar biopsy group, respectively, a biopsy performed because of indications confirmed the rejection process. In the recipients with the rejection process detected, the graft function was better in the protocolar biopsy group compared with the non-biopsy group. However, no difference in graft survival were observed in the 12-year follow-up period. In addition, in nine protocolar biopsies different types of glomerulopathy were identified, the most common (in four cases) being IgA glomerulopathy. No patient lost the graft because of GN. The Authors conclude that protocolar biopsy allows to detect subclinical rejection, and early intervention increase 5-year graft survival rates [17].

In a retrospective study, French researchers from Grenoble assessed the role of protocolar biopsy performed in 333 kidney transplant recipients in 2007–2013; 282 subjects had not undergone kidney biopsy, they constituted the control group. In patients who had undergone a kidney biopsy, 5-year graft survival rates were better regardless of the patient survival rates ($p < 0.001$), compared with patients who had not undergone

protocolar biopsy. As for graft kidney specimens, 212 (64%) were normal, 87 (26%) showed IF/TA of varying grade and 24 (7%) showed features of subclinical rejection, including borderline lesions in 20; the patients were effectively treated with GS pulses. Nine biopsies revealed: recurrence or de novo GN in five patients, BKV nephropathy in two patients, acute CNI nephrotoxicity in one patient and features of pyelonephritis in one patient. Among patients who had undergone biopsy, 87 (26%) had IF/TA score of > 0 , and recipients with IF/TA score of 3 had the worst graft survival rates. One hundred and forty-four patients (44%) presented cv lesions (*fibrosis endarteritis*); cv2 and cv3 lesions were associated with the worst 5-year graft survival rates. According to the Authors, protocolar biopsy performed at three months improves graft survival rates, primarily thanks to early treatment of immune-mediated lesions [18].

Korean authors assessed safety and feasibility of protocolar biopsy two weeks and twelve months after KTx. In 2012–2019, 842 protocolar biopsies were performed two weeks after KTx and 399 biopsies – one year after KTx. Biopsies were technically successful and safe; the complication rates were 0.3% in the case of biopsies performed two weeks and 0.2% in the case of biopsies performed twelve months after KTx. The incidence rates of subclinical rejection were 15.4% (130/842) and 33.6% (134/399) for biopsied performed two weeks and twelve months after KTx, respectively ($p < 0.001$). The authors do not provide long-term results but emphasise that protocolar biopsy is safe and can detect the subclinical rejection process (19).

The authors from Malaysia evaluated protocolar biopsies performed in 147 recipients (334 biopsies were performed between one month and 22 years after KTx, each recipient had undergone 1–7 biopsies) between 2012 and 2017. No rejection was detected in 161 (48.2%) cases, borderline lesions were found in 145 (43.4%) cases, and subclinical rejection — in 28 (8.4%) cases. Immune-mediated lesions were more common in the first five years after KTx. Borderline lesions were identified in 59 (36.4%), 64 (54.2%) and 22 (40.7%) biopsies at < 1 year, 1–5 years and > 5 years, respectively ($p = 0.011$). Subclinical rejection was found in six (3.7%) biopsies at < 1 year, 18 (15.3%) biopsies in the period of 1–5 years and four (7.4%) biopsies at > 5 years after KTx ($p = 0.003$). IF/TA, *de novo* or recur-

rent glomerulopathy and other unexpected lesions were found in 40 (12%), 10 (3%) and 12 (3.6%) biopsies, respectively. Recipients of kidney transplants from living donors had significantly lower rates of subclinical rejection ($p = 0.007$). The authors emphasised that in spite of stable graft function, morphological examination relatively frequently revealed subclinical rejection [20].

Another publication from Spain concerns the analysis of protocolar biopsies performed 4–6 months and 12 months after KTx, in 2015–2021; 134 biopsies were performed in 100 patients — 71 biopsies 4–6 months and 63 biopsies 12 months after KTx. The biopsies revealed 19 (14%) cases of subclinical rejection and 10 (7.4%) cases of borderline lesions. In addition, nephrocalcinosis was reported in 4.4% patients, IgA nephropathy in 2.2% patients and BK virus nephropathy in 1.5% patients. Protocolar biopsy findings lead to a therapeutic intervention in 45 patients (in 33% of all biopsies), most commonly the administration of methylprednisolone pulses (12.6%) and conversion to mTOR inhibitors (8.9%). In the Authors' opinion, protocolar biopsy is a useful tool for graft function monitoring as well as early detection and treatment of subclinical lesions [21].

Mareena S. Zachariah et al. presented 5-year results of 261 protocolar biopsies in 159 kidney recipients (2004–2012), performed 3–9 months (early) and subsequently 12–24 months (late) after KTx. The morphological image was classified as: IF/TA (interstitial fibrosis/tubular atrophy), subclinical acute rejection with IF/TA and border lesions with IF/TA. The effect of these lesions on glomerular filtration rate (eGFR) was assessed with respect to eGFR 12 months after KTx. In early biopsies, normal kidney was found in 105 (66%) recipients while in the remaining 54 (34%) subjects the following pathologies were identified: subclinical acute rejection plus IF/TA in seven recipients (4.4%), borderline lesions plus IF/TA in 17 (10.69%) recipients and IF/TA in 30 (18.87%) recipients. Late biopsies were performed in 102 recipients — in 59 (58%) no pathology was identified while in 43 (42%), the findings were as follows: subclinical acute rejection plus IF/TA in four (4%) recipients, borderline lesions plus IF/TA in 8 (9%) recipients and IF/TA in 30 (29%) recipients. Glomerular filtration rate at 12 months was related to eGFR at three months, the donor's age, delayed graft function and early pro-

tocolar biopsy findings. Changes in eGFR over time were associated with IF/TA in early biopsies and subclinical rejection and borderline lesions in late biopsies. In the long-term follow-up, the final eGFR values were related to IF/TA in early biopsies and subclinical rejection in late biopsies. Early protocolar biopsies allowed to predict eGFR at 12 months, while late biopsies — graft function over time. The presence of borderline lesions in the protocolar biopsy was predictive of long-term graft function [22].

Observational study from Author's transplant centre included 61 patients who underwent protocol biopsy 12 months after the transplantation. The biopsy results revealed abnormal histologic material in 37 patients (60%), mild inflammatory lesions in 21 patients, interstitial fibrosis and tubular atrophy (IFTA) grade II to III in 12 and BK virus nephropathy in 4. Immunosuppressive treatment was modified in the group with mild inflammatory changes and in the BKV group after the biopsy result. In the group with mild inflammatory lesions, renal function was stable during 5-years follow-up. In the BKV nephropathy group, there was a significant reduction in serum creatine levels. Protocol biopsies are useful for detecting early pathologies and preventing allograft failure. Patients with detectable pathology that can be treated or in whom therapy modification is possible will benefit from protocol biopsies [23].

Naumnik et al. from another polish transplant center reported results of a prospective observational study involving seventeen kidney recipients transplanted who underwent “zero”, 3-month and 12-month allograft biopsies as well as DSA assessment. Histologic analysis of the biopsies showed subclinical acute cellular rejection in 17.6% of patients at 3-months post transplantation, and additional case of borderline rejection at the 12-month point. Moreover, two cases (11.8%) of polyomavirus BK nephropathy were diagnosed (one at 3 and one at 12 month point). None of the patients developed de novo DSA. Protocol biopsies allowed Authors' to detect significant proportion of patients with subclinical, but histologically relevant acute cellular rejection and BK nephropathy. Early therapeutic intervention had beneficial effects in a 4-year follow up [24].

The Authors from Korea evaluated the 504 patients who underwent protocolar biopsies and 350 who did not undergo protocolar biopsy.

Biopsies were performed 2 weeks and one year after transplantation, 207 recipients underwent single biopsy and 297 recipients the double biopsy. The double protocol biopsy group had advantages in 5-year graft survival, CKD progression, and new-onset CKD. Authors conclude, that protocol biopsy can play a protective role in the maintenance of kidney grafts in kidney transplant recipients [25].

Mehta et al. evaluated the long-term impact of early subclinical inflammation through surveillance biopsy in a prospective observational cohort of 586 patients who underwent protocol biopsy in their first year post-transplant. Patients were classified based on their biopsy findings: 282 with no significant inflammation and 304 with subclinical inflammation and tubulitis (182 with subclinical borderline changes and 122 with subclinical T Cell mediated rejection). Adjusted odds of having a subsequent clinical biopsy proven acute rejection and death-censored graft loss were significantly higher in the subclinical inflammation group compared to no subclinical inflammation during 5-year follow-up. Overall, Authors highlighted the need for identifying patients with subclinical inflammation through surveillance biopsy and develop strategies to prevent further alloimmune injuries [26].

De novo donor-specific antibodies (dnDSAs) are associated with the development of ABMR and graft loss. A multicentre (nine centres) French study retrospectively assessed whether or not regular monitoring for *de novo* DSAs combined with biopsy should become a routine practice. In patients with *de novo* DSAs (MFI > 1000) and stable kidney function biopsies were performed. Biopsies were performed in 123 patients, on average 65.3 (median) months after KTx. Renal function had remained stable for the three preceding months. Subclinical ABMR was found in 51 (41.4%) patients, including 32 (26%) cases of active ABMR and 19 (15.5%) cases of chronic active subclinical ABMR. No ABMR was identified in 72 biopsies (58.5%). The predictors for active subclinical ABMR were as follows: dominant DSAs MFI > 4,000; MFI of the sum of DSAs > 6300, recipient's age < 45 years, and no use of GS at the time of biopsy. Proteinuria of > 200 mg/g was a predictor of chronic active subclinical ABMR. Patients with active ABMR had greater declines in GFR within five years of biopsy and worse graft survival. Biopsy in patients with *de novo* DSAs allowed to detect ABMR in 40% of cas-

es, but the Authors did not see any improvement after treatment [27].

Early diagnosis and treatment of subclinical ABMR based on the donor-specific antibody (DSA) testing may result in better outcomes. Filippone and Faber reviewed the literature on subclinical antibody-mediated rejection (ABMR) associated with donor specific antibodies. Subclinical ABMR occurs in up to 40% of patients transplanted with pre-existing DSA routinely having biopsies within the first year following transplantation and subclinical ABMR occurs in up to 40% of patients with dnDSA if biopsied by protocol at the time of initial dnDSA detection. Subclinical AMR portends adverse outcomes (worse kidney function and graft loss) whether associated with preexisting DSA or dnDSA. They recommend to perform protocol biopsies within the first year following transplantation in all patients transplanted with preexisting DSA and in all patients with dnDSA at initial detection [28].

Recently published by ESOT Working Group on Subclinical DSA Monitoring "The Clinical Utility of Post-Transplant Monitoring of Donor-Specific Antibodies in Stable Renal Transplant Recipients: A Consensus Report With Guideline Statements for Clinical Practice" recommends a routine antibody monitoring at three to six months post-transplant and annually thereafter. Monitoring for dnDSA during functional graft life is a continuous process and should not change upon detection of dnDSA [29].

All the publications presented concern retrospective observational studies, often single-centre studies, involving various study populations and biopsies performed at different post-transplantation time points; also objectives were different; however, they show that protocolar biopsies can detect subclinical rejection or borderline lesions, which may have a beneficial effect on the preservation of good graft function. Early diagnosis of subclinical antibody-mediated rejection has an additional prognostic value, although no effective therapies for this pathology are available today. Large prospective studies are necessary to fully assess the utility of protocolar biopsy.

SUMMARY

Protocol biopsy of the graft kidney is a safe diagnostic tool serving to detect pathologies at an early stage. No doubt, the introduc-

tion of protocol biopsy into clinical practice has also allowed to broaden our the knowledge of the pathophysiology of the graft kidney lesions. However, at present, the role of protocol biopsy as a routine diagnostic tool is still under discussion, therefore it is not performed in all centres. Based on the experience gained so far, it seems possible to limit this examination to the groups of patients who would derive the greatest clinical benefit. Such groups would include primarily patients with an increased risk of rejection, higher sensitisation degree and after incompatible transplantation (immunologic or blood type incompatibilities), as well as patients in whom IS minimisation protocols are used, with lower doses of calcineurin inhibitors or steroids. However, this requires further analyses. Certainly, the decision to provide surveillance via protocol biopsy should always be made on a case-by-case basis, taking into account not only immunological but also clinical factors, as well as the centre's experience. With time and with the development of the immunosuppressants segment and noninvasive diagnostic techniques, the role of graft kidney biopsy, including protocol biopsy, will decrease. There have already been reports of non-invasive tests with similar sensitivity and specificity in diagnosing graft rejection. However, their

introduction into routine clinical practice will require time and further testing. Noninvasive biomarkers include urine chemokines, TTV replication, gene profiling, proteomics and dd cf DNA. The latter seems to be the most promising biomarker and currently commercially available in some countries [30, 31].

AUTHOR CONTRIBUTIONS

Łukasz Jankowski — research concept and design, collection and/or assembly of data, data analysis and interpretation, writing the article, critical revision of the article, final approval of the article;
 Julia Stępień — writing the article, final approval of the article;
 Marcin Jurczak — writing the article, final approval of the article;
 Zuzanna Sala — writing the article, final approval of the article;
 Magdalena Durlík — critical revision of the article, final approval of the article.

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CONFLICT OF INTEREST

Authors declare no conflict of interest.

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4. PODSUMOWANIE I WNIOSKI

Monitorowanie funkcji przeszczepionej nerki jest kluczowym elementem opieki nad pacjentami po transplantacji. Wczesne wykrycie subklinicznych zmian w alloprzeszczepie pozwala na wdrożenie odpowiednich interwencji, które mogą zapobiec jego niewydolności. W ostatnich latach duże zainteresowanie budzą zarówno biopsja protokolarna, jak i nowe biomarkery diagnostyczne (biopsja płynna), które pozwalają na nieinwazyjną ocenę funkcji nerki.

W skład monotematycznego cyklu publikacji weszły cztery publikacje, spośród których dwie prace to prace poglądowe [23,30], jedna została zaprojektowana i przeprowadzona jako badanie retrospektywne [31], zaś kolejna jako przegląd systematyczny z meta-analizą [32].

4.1. Rola biopsji protokolarnej w monitorowaniu funkcji przeszczepionej nerki

Biopsja protokolarna nerki po transplantacji to procedura diagnostyczna polegająca na planowym pobraniu tkanki w określonych odstępach czasu, niezależnie od objawów klinicznych wskazujących na zaburzenia funkcji przeszczepu [33,34]. Ma na celu identyfikację ukrytych zmian patologicznych, takich jak odrzucanie zależne od limfocytów T (TCMR) czy przeciwciał (ABMR), które mogą prowadzić do stopniowego pogorszenia funkcji nerki i jej utraty [35]. Wraz z postępem w immunologii transplantacyjnej oraz dostępnością zaawansowanych terapii immunosupresyjnych, znaczenie tej metody w nadzorze nad przeszczepem jest szeroko dyskutowane.

Transplantacja pozostaje najefektywniejszym sposobem leczenia niewydolności nerek w stadium krańcowym, znacząco poprawiając rokowania i komfort życia pacjentów w porównaniu z dializami [36,37]. Jednak długotrwałe utrzymanie funkcji przeszczepu jest wyzwaniem, głównie z powodu postępujących zmian wynikających z reakcji odrzucania. Kliniczne oznaki pogorszenia, takie jak wzrost kreatyniny czy spadek eGFR, pojawiają się relatywnie późno, co utrudnia szybką reakcję terapeutyczną [38]. Biopsja protokolarna umożliwia wykrycie zmian, takich jak zwłóknienie śródmiąższowe i zanik kanalików (IF/TA) czy subkliniczne odrzucanie na wczesnym etapie, zanim zaawansowanie tych zmian lub ich kumulacja doprowadzi do istotnego klinicznie pogorszenia funkcji przeszczepu, aż do jego utraty.

Włączone w cykl publikacji badanie retrospektywne w którym analizowano 144 biopsje u 72 pacjentów w 3. i 12. miesiącu po przeszczepie wykazało zmiany histopatologiczne u 40% badanych, w tym subkliniczne odrzucanie komórkowe i zmiany graniczne [31].

Podobne wnioski płyną z pracy Loupy i wsp., gdzie w 1001 biopsjach rok po transplantacji stwierdzono subkliniczne TCMR w 13% i ABMR w 14%, co wiązało się z gorszym rokowaniem w perspektywie 8 lat [39]. Te obserwacje wskazują, że biopsje protokolarne mogą być kluczowe w identyfikacji pacjentów potrzebujących szybkiej interwencji, a także umożliwiają dostosowanie leczenia, co może wydłużyć funkcjonowanie przeszczepu. W analizowanej w pracy doktorskiej grupie chorych, w biopsji po 3 miesiącach odchylenia od normy stwierdzono u 22 pacjentów, z czego u 7 z nich było to subkliniczne odrzucanie komórkowe, u jednej osoby ABMR, u 5 chorych zmiany graniczne, u 3 cechy infekcji wirusem BKV, u 2 zmiany zakrzepowe, a u 4 arteriopatía proliferacyjna. W każdym przypadku stwierdzanych nieprawidłowości rozpoczynano terapię wg zaleceń (m.in. modyfikacja immunosupresji, pulsy glikokortykosteroidów, podawanie immunoglobulin; zależnie od przyczyny). W badaniach biopsyjnych po 12 miesiącach stwierdzono, że u 15 z 22 omawianych pacjentów w wyniku leczenia nastąpiła normalizacja bioptatu, co świadczy o istotnych korzyściach odniesionych przez tych chorych z wykonania procedury biopsyjnej. U 7 pacjentów diagnoza patologii przeszczepu w obu badaniach biopsyjnych nie uległa zmianie (1 pacjent z TCMR, 2 z ABMR, po jednym chorym ze zmianami zakrzepowymi, nawrotem kłębuszkowego zapalenia w nerce przeszczepionej, zmianami granicznymi i BKV). Dodatkowo w biopsji po 12 miesiącach wykryto zmiany de novo u 7 pacjentów (po 2 przypadki zmian granicznych, arteriopatii proliferacyjnej i nawrotu kłębuszkowego zapalenia nerek i jeden chory z infekcją BKV).

Biopsja protokolarna jest zwykle przeprowadzana w ustalonych momentach, takich jak moment bezpośrednio po przeszczepieniu (biopsja zerowa), a następnie po 3, 6, 12 i 24 miesiącach, zależnie od protokołu ośrodka i stanu pacjenta. Wczesne biopsje pomagają wychwycić początkowe reakcje immunologiczne, natomiast późniejsze mogą ujawnić skutki toksyczności leków, infekcji wirusowych czy nawrotu choroby podstawowej. Analizując rozkład wyników wśród analizowanych chorych można uznać, że nie odbiega on od opisanych powyżej celów – z dużą przewagą rozpoznań subklinicznego odrzucania komórkowego po 3 miesiącach, z kolei istotnym procentowo udziałem nawrotu choroby podstawowej po 12 miesiącach. Zauważyć należy jednak, że ze względu na długi okres

między badaniami (9 miesięcy), nieznany jest przebieg zaburzeń u pacjentów z rozpoznanymi w biopsji po 3 miesiącach od KTx patologiami graftu u których wdrażano terapie tych patologii, a w kolejnej biopsji zmiany utrzymywały się (remisja i powtórny nawrót lub utrzymywanie się zmian pomimo leczenia).

Choć biopsja protokolarne jest zabiegiem inwazyjnym, ryzyko powikłań, takich jak krwaki czy przetoki naczyniowe, jest dobrze kontrolowane. Badanie Lim i wsp. podkreśla bezpieczeństwo tej metody, wskazując na minimalny odsetek powikłań (0,3% w drugim tygodniu i 0,2% po roku) przy wysokiej skuteczności w wykrywaniu subklinicznych procesów odrzucania [40]. Furness i wsp. w przeprowadzonej analizie wieloośrodkowej wykazali, że poważne komplikacje wymagające interwencji występują w 0–4% przypadków, a biopsje planowe są nawet 10-krotnie bezpieczniejsze od tych wykonywanych w trybie nagłym [41]. Zastosowanie ultrasonografii i odpowiednie przygotowanie pacjenta dodatkowo zwiększają bezpieczeństwo procedury.

Również wśród analizowanych w przywołanej pracy retrospektywnej 144 procedur biopsyjnych, przeprowadzanych w Klinice Medycyny Transplantacyjnej i Nefrologii WUM nie odnotowano istotnych powikłań związanych z tym zabiegiem, stwierdzając jedynie niewielkie krwaki okołonerkowe w 4 przypadkach (2,7% przypadków).

W przywołanej pracy, wchodzącej w skład pracy doktorskiej zauważono, że u bezobjawowych pacjentów z subklinicznymi zmianami wykrywanymi w ciągu pierwszego roku po transplantacji w badaniu biopsji protokolarnej, profil lipidowy różnił się od normy (obserwowano niższe poziomy cholesterolu i frakcji HDL oraz LDL), co może sugerować związek między metabolizmem lipidów a uszkodzeniem nerki przeszczepionej. Wszyscy analizowani pacjenci otrzymywali standardowe leczenie immunosupresyjne (glikokortykosteroidy, takrolimus, mykofenolan mofetylu). Jednocześnie, choć najczęstszą manifestacją dyslipidemii u osób po przeszczepieniu nerki jest hipercholesterolemia, powodowana najczęściej leczeniem immunosupresyjnym, to z obserwacji chorych z przewlekłą chorobą nerek (CKD) wiadomo, że podobny, jak stwierdzany u chorych w pracy wchodzącej w skład pracy doktorskiej, profil lipidowy może korelować z nasileniem procesów zapalnych i pogorszeniem funkcji nerki. Chorych z przewlekłą niewydolnością nerek i gorszym rokowaniem najczęściej cechuje wysoki poziom trójglicerydów, niski poziom lipoprotein o dużej gęstości (HDL) oraz prawidłowy lub obniżony poziom całkowitego cholesterolu i cholesterolu lipoprotein o małej gęstości (LDL), co znajduje wyjaśnienie w fakcie, że za takim spektrum zaburzeń lipidogramu kryje

się potencjał aterogeny, powodowany przez wzrost lipoprotein o średniej i bardzo małej gęstości, a także zwiększone tworzenie utlenionych LDL [42].

Należy zauważyć, że uzyskane przez autora pracy doktorskiej wyniki wskazują na możliwość identyfikacji dodatkowych czynników ryzyka wymagających szczególnej uwagi i skłaniają do wnikliwego nadzoru nad takimi chorymi po przeszczepieniu z uwzględnieniem biopsji protokolarnej jako potencjalnej opcji diagnostycznej. Do czynników tych zaliczono otyłość i chorobę niedokrwienną serca, wskazując na zespół metaboliczny i zaburzenia perfuzji naczyń jako wiodące.

Wiadomym jest, że największe korzyści z biopsji protokolarnych odnoszą pacjenci o podwyższonym ryzyku immunologicznym, np. z wysokim poziomem przeciwciał (PRA > 0), obecnością DSA czy po przeszczepach od dawców marginalnych. Huang i wsp. zalecają biopsje protokolarne w takich przypadkach oraz przy minimalizacji immunosupresji [43]. Z kolei Bertrand i wsp. wykazali, że u pacjentów z nowopowstałymi DSA i stabilną funkcją nerki biopsje ujawniły subkliniczne ABMR w 41,4% przypadków, co miało znaczenie prognostyczne, choć nie zawsze pozwalało na skuteczne leczenie [44]. Wobec braku istotnych różnic pomiędzy analizowanymi grupami w zakresie ryzyka immunologicznego, wyników tych nie potwierdzono w analizowanej pracy.

Mimo zalet, biopsja protokolarna nie jest powszechnie stosowana z powodu swojej inwazyjności, kosztów i braku ujednoliconych standardów. Jak wskazano w jednym z artykułów wchodzących w skład cyklu prac konieczna jest potrzeba większych badań prospektywnych, aby w pełni ocenić jej skuteczność. Rozwój nieinwazyjnych metod, takich jak analiza dd-cfDNA, może w przyszłości ograniczyć konieczność biopsji, choć obecnie ich precyzja ustępuje tradycyjnej histopatologii, co znajduje potwierdzenie w doniesieniach naukowych [45].

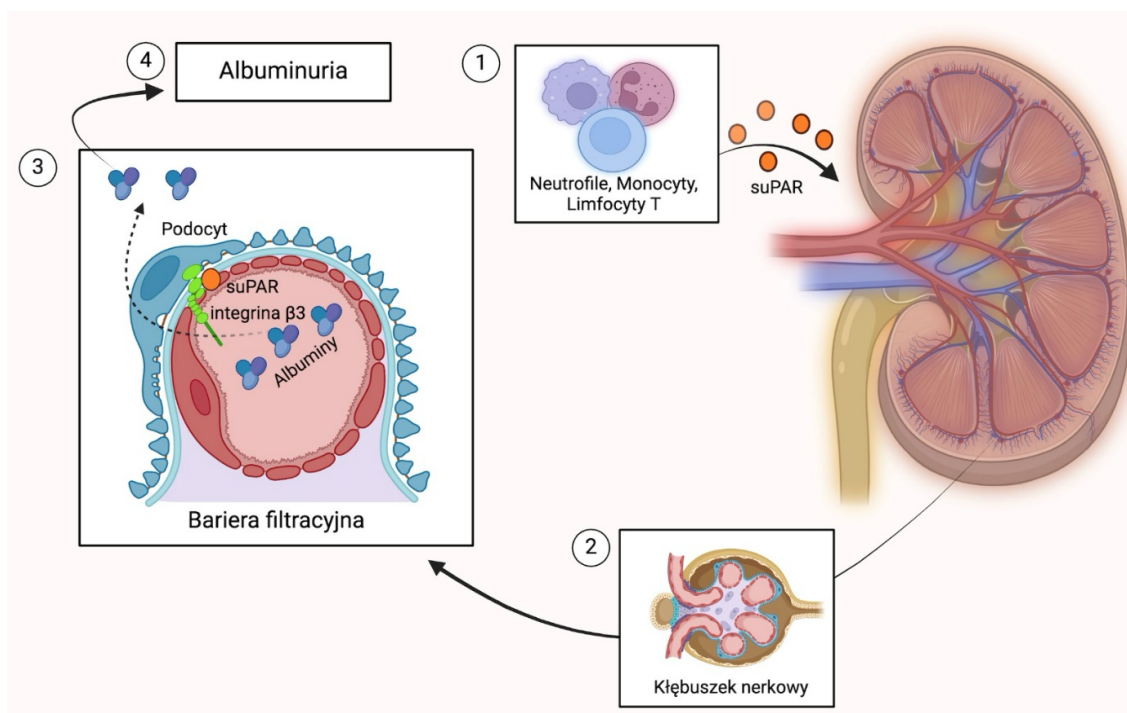
4.2. Potencjał biomarkerów jako alternatywy dla biopsji protokolarnej

W kontekście ograniczeń biopsji protokolarnej, takich jak jej inwazyjność, ryzyko powikłań oraz brak powszechnej implementacji w wielu ośrodkach, rośnie zainteresowanie biomarkerami pozwalającymi na nieinwazyjne monitorowanie funkcji przeszczepionej nerki. W ostatnich latach badania koncentrują się na wykorzystaniu biomarkerów krążących we krwi oraz obecnych w moczu, które mogą sygnalizować wczesne stadium uszkodzenia nerki, a zatem być w przyszłości szerzej używane również jako marker uszkodzenia nerki przeszczepionej.

W przeprowadzonym przeglądzie systematycznym i meta-analizie dotyczącej roli predykcyjnej rozpuszczalny receptor aktywatora plazminogenu typu urokinazy (suPAR, ang. soluble urokinase plasminogen activator receptor) u pacjentów z ostrym uszkodzeniem nerek wykazano, że poziomy suPAR u pacjentów z AKI były znacząco wyższe w porównaniu z grupą kontrolną bez AKI ($5,23 \pm 4,07$ ng/mL vs. $3,23 \pm 0,67$ ng/mL; SMD = 3,19; 95% CI: 2,73–3,65; $p < 0,001$) [32]. Wyniki te wskazują na silny związek między podwyższonym stężeniem suPAR w osoczu a wystąpieniem AKI, co sugeruje, że suPAR może pełnić rolę predykcyjnego biomarkera w tym schorzeniu. Analiza wrażliwości potwierdziła stabilność tych wyników, co dodatkowo wzmacnia ich wiarygodność. Warto zauważyć, że heterogeniczność między badaniami była wysoka ($I^2 = 96\%$), co może wynikać z różnic w populacjach badanych, metodach pomiaru suPAR czy definicjach AKI stosowanych w poszczególnych studiach.

Podwyższone poziomy suPAR w AKI mogą odzwierciedlać jego rolę patofizjologiczną w uszkodzeniu nerek (Rycina 2). SuPAR jest białkiem uwalnianym przez różne typy komórek, w tym komórki układu odpornościowego (np. monocyty, neutrofile), zaś sam mechanizm działania suPAR w patogenezie AKI możemy zgrupować w czterech płaszczyznach, które są kluczowe w rozwoju AKI:

- Aktywacja Stanu Zapalnego: suPAR nasila migrację makrofagów i neutrofilów do nerek poprzez stymulację szlaków sygnałowych zależnych od NF- κ B i MAPK. Prowadzi to do zwiększonej produkcji cytokin prozapalnych (TNF- α , IL-6) i chemokin, które uszkodzają struktury kłębuszków i kanalików.
- Stres Oksydacyjny: suPAR upośledza funkcję mitochondriów w komórkach nabłonka kanalikowego, zwiększając produkcję reaktywnych form tlenu (ROS). ROS uszkodzają lipidy błonowe, białka i DNA, prowadząc do martwicy komórek.
- Apoptoza Podocytów: W kłębuszkach nerkowych suPAR wiąże się z integryną $\alpha V\beta 3$ na podocytach, zaburzając ich przyleganie do błony podstawnej. To wywołuje reorganizację cytoszkieletu, utratę białek sygnałowych (np. nephryny) i zwiększoną przepuszczalność błony filtracyjnej, co objawia się białkomoczem.
- Współdziałanie z Układem Dopełniacza: suPAR aktywuje alternatywną drogę dopełniacza, prowadząc do depozycji składników C3 i C5b-9 w śródbłonku, co nasila mikrozakrzepy i niedokrwienie miąższu nerki.



Rycina 2. Mechanizm działania SuPAR.

Źródło: Opracowanie własne.

Badania wskazują, że suPAR może aktywować integryny na podocytach (al. $\alpha\beta3$), co prowadzi do uszkodzenia bariery filtracyjnej kłębuszków nerkowych, a tym samym przyspiesza progresję uszkodzenia nerek [46]. Wyniki metaanalizy są zgodne z tymi obserwacjami, sugerując, że suPAR nie tylko sygnalizuje obecność AKI, ale może być również zaangażowany w jego patogenezę. Zatem podwyższone poziomy suPAR są skorelowane z ciężkością AKI, co potwierdza wcześniejsze doniesienia. Na przykład Azam i wsp. Wykazali, że poziomy suPAR u pacjentów hospitalizowanych z powodu COVID-19 były predykcyjne dla wystąpienia AKI w trakcie hospitalizacji oraz potrzeby dializoterapii [47]. Te obserwacje sugerują, że suPAR może być nie tylko markerem diagnostycznym, ale także prognostycznym, wskazującym na ryzyko powikłań i zły wynik kliniczny [48].

Diagnostyka ostrej niewydolności nerek, w tym również ocena funkcji nerki przeszczepionej, opiera się tradycyjnie na ocenie stężenia kreatyniny w surowicy oraz monitorowaniu produkcji moczu, zgodnie z kryteriami KDIGO (Kidney Disease: Improving Global Outcomes). Jednakże te klasyczne markery mają poważne ograniczenia, takie jak opóźniona reakcja na uszkodzenie nerek, brak specyficzności wobec przyczyn AKI oraz podatność na wpływ czynników pozanerkowych, takich jak masa mięśniowa, wiek, płeć, stan odżywienia czy choroby współistniejące. Ograniczenia te, w połączeniu z

potrzebą wczesnego rozpoznania AKI dla poprawy rokowania pacjentów, skłoniły badaczy do poszukiwania bardziej precyzyjnych i czułych biomarkerów. Wśród najczęściej badanych alternatyw wymienia się NGAL (neutrophil gelatinase-associated lipocalin), cząsteczkę uszkodzenia nerek 1 (KIM-1, ang. Kidney injury molecule-1), interleukinę 18 (IL-18), cystatynę C oraz opisywany SuPAR. SuPAR, jako stosunkowo nowy biomarker, wyróżnia się unikalnymi właściwościami, które mogą uczynić go wartościowym narzędziem w diagnostyce i predykcji AKI.

Kreatynina w surowicy jest podstawowym parametrem stosowanym w diagnostyce AKI, jednak jej wzrost następuje dopiero po utracie co najmniej 50% przesączania kłębuszkowego (GFR, ang. glomerular filtration rate), co może opóźniać rozpoznanie o 24–48 godzin od momentu uszkodzenia nerek [49]. Waikar i Bonventre oraz inni autorzy wskazują, iż poziom kreatyniny jest zależny od wielu czynników pozanerkowych, takich jak masa mięśniowa, dieta bogata w białko, wiek czy płeć, co obniża jej swoistość i czułość jako markera ostrego uszkodzenia [50–53]. Jak wskazuje Prowle i wsp. produkcja moczu, choć prosta w ocenie, również nie jest niezawodnym wskaźnikiem, gdyż oliguria może być wynikiem odwodnienia lub niewydolności serca, a jej brak nie wyklucza AKI [54].

W kontraście do tych tradycyjnych markerów, suPAR wykazuje zdolność do wczesnego sygnalizowania uszkodzenia nerek. Hayek i wsp. wykazali, że podwyższone stężenie suPAR w osoczu (mediana 5,6 ng/mL u pacjentów z AKI vs. 3,2 ng/mL w grupie kontrolnej; $p < 0,001$) jest wykrywalne przed wzrostem kreatyniny, co sugeruje jego przydatność w identyfikacji subklinicznego stadium AKI [46]. Przeprowadzona potwierdza tę obserwację, wskazując, że średnie stężenie suPAR u pacjentów z AKI wynosiło $5,23 \pm 4,07$ ng/mL, w porównaniu z $3,23 \pm 0,67$ ng/mL u pacjentów bez AKI (SMD = 3,19; 95% CI: 2,73–3,65; $p < 0,001$)[32]. Ta statystycznie istotna różnica podkreśla potencjał suPAR jako markera predykcyjnego, zdolnego do wykrywania AKI na etapie, w którym interwencje terapeutyczne mogą jeszcze zapobiec progresji uszkodzenia.

Kolejnym jednym z najlepiej przebadanych biomarkerów AKI jest NGAL, szczególnie w kontekście uszkodzenia kanalików nerkowych, które dominuje w takich scenariuszach jak AKI pooperacyjne czy wywołane niedotlenieniem [55,56]. NGAL jest białkiem uwalnianym przez neutrofile i komórki kanalików nerkowych w odpowiedzi na stres komórkowy, co czyni go czułym wskaźnikiem ostrej fazy AKI. Haase i wsp. w metaanalizie obejmującej 19 badań i ponad 2500 pacjentów wykazali, że NGAL w moczu i osoczu osiąga AUC w zakresie 0,78–0,82 w predykcji AKI, z czułością 0,76 (95% CI: 0,71–0,80) i swoistością 0,81 (95% CI: 0,76–0,85) w populacjach po zabiegach

kardiochirurgicznych [57]. Podobne zależności opisał Devarajan wskazując, że NGAL w moczu wzrasta już w ciągu 2–4 godzin po ekspozycji na czynnik uszkadzający nerki, co daje mu przewagę nad kreatyniną [58]. Z kolei Marakala w swoim przeglądzie systematycznym wskazuje, iż stężenie NGAL w moczu i surowicy wydaje się być niezależnym czynnikiem prognostycznym nie tylko powikłań nerkowych, ale także chorób sercowo-naczyniowych i związanych z uszkodzeniem wątroby [59].

W porównaniu z NGAL, suPAR wykazuje nieco niższą pojedynczą skuteczność diagnostyczną. W przeprowadzonej meta-analizie [32] wykazano, że AUC dla suPAR wynosi 0,69, co potwierdzają również inne badania, w tym badanie Walls i wsp. w którym suPAR miał czułość 94%, swoistość 40% i zdolność dyskryminacyjną (AUC) 0,69 dla rozwoju AKI przy punkcie odcięcia 4,26 ng / ml [60]. Wyniki te są również zbieżne z wynikami Mossanena i wsp. którzy analizowali pacjentów poddawanych zabiegom kardiochirurgicznym, w którym odnotowano AUC wynoszące 0,65 dla rozwoju AKI przy wartości odcięcia suPAR wynoszącej 2,45 ng / ml [61]. Z kolei Rasmussen i wsp. oceniali moc dyskryminacyjną suPAR dla AKI u pacjentów poddawanych zabiegom kardiochirurgicznym i odnotowali AUC na poziomie 0,60 [62]. NGAL jest głównie markerem lokalnego uszkodzenia kanalików, podczas gdy suPAR, jak wskazują Hayek i wsp. [46], odzwierciedla szersze procesy patofizjologiczne, w tym ogólnoustrojową reakcję zapalną i interakcje z podocytami poprzez integryny $\alpha\beta3$. Ta różnica w mechanizmach działania może tłumaczyć, dlaczego suPAR jest bardziej uniwersalny w heterogenicznych populacjach z AKI o różnorodnej etiologii, al. np. w AKI związanym z sepsą czy kontrastem (CI-AKI).

Cząsteczka uszkodzenia nerek 1 (KIM-1) jest transbłonowym białkiem ekspresjonowanym na komórkach kanalików proksymalnych w odpowiedzi na uszkodzenie, szczególnie w AKI wywołanym niedotlenieniem, toksynami czy zapaleniem. Han i wsp. w badaniu wykazali, że KIM-1 w moczu osiąga AUC = 0,83 (95% CI: 0,77–0,89) w diagnostyce AKI u pacjentów po operacjach kardiochirurgicznych, z czułością 0,74 i swoistością 0,86 [63]. W porównaniu z kreatyniną, KIM-1 reaguje znacznie szybciej – jego poziom wzrasta w ciągu 12–24 godzin po uszkodzeniu, co czyni go bardziej czułym markerem wczesnej fazy AKI [64].

W porównaniu z KIM-1, suPAR charakteryzuje się niższą specyficznością dla konkretnego typu uszkodzenia nerek. Huang et al. w metaanalizie obejmującej 7 badań podają, że czułość suPAR w predykcji AKI wynosi 0,77 (95% CI: 0,67–0,84), a swoistość 0,64 (95% CI: 0,53–0,75), co plasuje go poniżej KIM-1 pod względem dokładności

diagnostycznej w ostrym uszkodzeniu kanalików [65]. Jednak suPAR przewyższa KIM-1 w kontekście predykcji długoterminowej. Han i wsp. wskazują, że podwyższone stężenie suPAR (np. $>4,0$ ng/mL) u pacjentów z prawidłowym eGFR (>60 mL/min/1,73 m²) koreluje z szybkim spadkiem funkcji nerek, co sugeruje, że suPAR może identyfikować subkliniczne uszkodzenie nerek na etapie, którego KIM-1 nie jest w stanie wykryć [63].

Interleukina 18 (IL-18) jest prozapalną cytokiną uwalnianą przez komórki nerek i układu odpornościowego w odpowiedzi na uszkodzenie. Parikh et al. wykazali, że IL-18 w moczu osiąga AUC = 0,73 (95% CI: 0,66–0,80) w predykcji AKI u pacjentów w stanie krytycznym, z czułością 0,68 i swoistością 0,75 [66]. IL-18 jest szczególnie przydatna w AKI związanym z niedotlenieniem, gdzie procesy zapalne odgrywają kluczową rolę, a jej poziom wzrasta w ciągu 6–12 godzin po ekspozycji na czynnik uszkadzający [67]. SuPAR, podobnie jak IL-18, jest związany z procesami zapalnymi, ale jego działanie jest bardziej systemowe. Huang i wsp. wskazują, że suPAR przewyższa IL-18 w czułości (0,77 vs. 0,68), choć ma niższą swoistość (0,64 vs. 0,75) [65]. Kluczową przewagą suPAR jest jego zdolność do przewidywania AKI przed wystąpieniem objawów klinicznych – Azam i wsp. wykazali, że stężenie suPAR przy przyjęciu do szpitala (mediana 7,66 ng/mL w grupie z AKI) było predykcyjne dla AKI u pacjentów z COVID-19 (OR = 1,45; 95% CI: 1,22–1,73; $p < 0,001$), podczas gdy IL-18 jest bardziej reaktywnym markerem ostrej fazy [47].

Kolejnym potencjalnym biomarkerem mającym zastosowanie w diagnostyce i prognostyce AKI jest Cystatyna C, będąca niskocząsteczkowym białkiem (13 kDa) produkowanym przez wszystkie komórki jądrzaste, filtrowanym przez kłębuszki i reabsorbowanym w kanalikach. Jest ono swobodnie filtrowane przez kłębuszki nerkowe, a następnie w pełni reabsorbowane i katabolizowane w komórkach kanalików proksymalnych, bez wydzielania do moczu w warunkach prawidłowych. Jej stężenie w osoczu jest mniej zależne od masy mięśniowej niż kreatynina, co czyni ją bardziej niezawodnym wskaźnikiem GFR [68]. W przypadku AKI, spadek przesączania kłębuszkowego prowadzi do zmniejszonej filtracji cystatyny C, co powoduje jej akumulację w osoczu. Mechanizm działania cystatyny C jako biomarkera opiera się więc na zmianie funkcji filtracyjnej nerek – jest to marker pośredni, odzwierciedlający utratę zdolności kłębuszków do oczyszczania krwi, a nie bezpośrednie uszkodzenie komórek nerkowych. Soto i wsp. w badaniu pacjentów na oddziałach ratunkowych wykazali, że cystatyna C osiąga AUC = 0,76 (95% CI: 0,70–0,82) w predykcji AKI, z czułością 0,71 i swoistością 0,78, reagując w ciągu 12–24 godzin od uszkodzenia [69].

SuPAR wyróżnia się kilkoma unikalnymi właściwościami w porównaniu z innymi biomarkerami. Po pierwsze, jego stężenie nie zależy od masy mięśniowej, wieku ani diety, co eliminuje zmienne zakłócające charakterystyczne dla kreatyniny czy cystatyny C [70]. Po drugie, suPAR wykazuje zdolność do przewidywania AKI na długo przed jego klinicznym ujawnieniem – Po trzecie, w odróżnieniu od NGAL, KIM-1 i IL-18, które są specyficzne dla uszkodzenia kanalików, suPAR odzwierciedla ogólnoustrojową reakcję zapalną i uszkodzenie podocytów, co może być kluczowe w AKI o etiologii kłębuszkowej lub mieszanej [71].

4.3. Porównanie biopsji protokolarnej z biopsją płynną

Monitorowanie funkcji przeszczepionej nerki i wczesne wykrywanie procesu odrzucania jak wykazano uprzednio są ważnymi elementami opieki nad biorcami przeszczepów. Biopsja protokolarna lub tkankowa oraz biopsja płynna stanowią dwa uzupełniające się narzędzia diagnostyczne w tym zakresie, mające różne metody, zastosowania i ograniczenia. Biopsja protokolarna, powszechnie uważana za „złoty standard” w diagnostyce graftu, umożliwia dokładne ilościowe określenie typu odrzucania (komórkowy vs. humoralny), szacowanie uszkodzenia kłębuszków nerkowych i włóknienia, gdy są obecne. Chociaż cechuje ją bardzo wysoki stopień dokładności, jest to technika inwazyjna, która wiąże się z ryzykiem powikłań i jest ograniczona przez niemożność przeprowadzania wielu powtórzeń w krótkich odstępach czasowych.

W świetle aktualnych doniesień wydaje się, że do wczesnego wykrywania procesów odrzucania można również użyć markerów – biopsji płynnej. Ostatnie badania wskazują, że chemokiny CXCL9 i CXCL10 odgrywają istotną rolę w różnicowaniu typów odrzucania i mogą nawet ułatwiać wykrywanie epizodów odrzucania przed pojawieniem się objawów klinicznych [72,73]. Z kolei profilowanie metabolomiczne umożliwiło odkrycie zestawów biomarkerów specyficznych dla uszkodzenia nerki, które mogą być wykorzystane w różnicowej diagnostyce odrzucania oraz innych patologii nerek, takich jak nefropatia wirusa BK czy toksyczność inhibitorów kalcyneuryny.

Coraz częściej zwraca się uwagę, że skuteczność nowych biomarkerów wzrasta, gdy są stosowane w modelach wieloczynnikowych łączących wymiary kliniczne, laboratoryjne i molekularne. Jednym z takich modeli jest model CXCL9 + CXCL10 wraz z przeciwciałami specyficznymi dla dawcy (DSA) i parametrami funkcji przeszczepu nerki,

co znacznie zwiększa dokładność diagnostyczną i pozwala na lepsze ukierunkowanie terapii immunosupresyjnej.

Pomimo licznych zalet, biopsja płynna ma także pewne ograniczenia: nie dostarcza informacji o morfologii tkanek i jest mniej wrażliwa w przypadku subtelnych zmian komórkowych. Ponadto wyniki mogą być zafałszowane przez współistniejące infekcje lub procesy zapalne. W konsekwencji zintegrowane podejście, w ramach którego połączone są diagnostyka molekularna, biopsja płynna i tradycyjne badanie histologiczne, może stać się nowym standardem w przeszczepach nerek. Wdrożenie tych technologii do rutynowej praktyki klinicznej umożliwi dokładniejsze diagnozowanie odrzucania i jego patogenезы oraz dostosowanie leczenia immunosupresyjnego, co zwiększy szanse na długoterminowe przeżycie przeszczepionych organów.

Reasumując, w świetle przedstawionych wyników prac stwierdzić należy, że biopsja protokolarna stanowi użyteczne narzędzie diagnostyczne, szczególnie w konkretnych grupach pacjentów. Jednak szybki rozwój innych metod diagnostycznych, w tym tzw. biopsji płynnej, a także inwazyjność metody sprawia, że każdorazowo pacjent musi być poinformowany o możliwych korzyściach i zagrożeniach związanych z metodą. Obserwacje własne autora wskazują na fakt, że pacjenci po przeszczepieniu nerki chętnie uczestniczą w procedurach które mają potencjał zapewnienia dłuższego przeżycia przeszczepu nerkowego i wykrycia patologii na wczesnym etapie. Doświadczenia z literatury medycznej, a także doświadczenia własne autorów opisane w pracy doktorskiej wskazują, że pacjent może odnieść istotne korzyści z zastosowania tej metody diagnostycznej pod warunkiem indywidualizacji wskazań do wykonania procedury. Jednym z wykrytych w ramach prowadzenia pracy doktorskiej czynników ryzyka wczesnych zmian w przeszczepie nerkowym który uzasadniałby wykonywania biopsji protokolarnej jest zaburzenie profilu lipidowego, uwidoczniono również trend sugerujący że za czynnik ryzyka można uznać podwyższone BMI oraz obecność u chorego choroby niedokrwiennej serca. Wiedza ta może pozwolić na dodatkową indywidualizację procedury i wnieść istotny wkład w praktyczne postępowanie z biorca przeszczepu nerkowego po procedurze przeszczepienia.

WNIOSKI

1. Biopsja protokolarna jest skutecznym narzędziem diagnostycznym służącym wykrywaniu subklinicznych zmian w nerce przeszczepionej. Pomimo swojej inwazyjności jest również procedurą bezpieczną, obarczoną niskim ryzykiem powikłań.
2. Rozwój nowoczesnych markerów uszkodzenia nerek sprawia, że prawdopodobna jest rezygnacja z inwazyjnych metod diagnostycznych w przeciągu kilku/kilkunastu lat. W chwili obecnej tzw. biopsję płynną i biopsje protokolarne można traktować jako metody komplementarne.
3. Szczególne korzyści z biopsji protokolarnej odnoszą chorzy z dyslipidemią. Wymagane są dalsze badania nad użytecznością markerów klinicznych takich jak suPAR, w predykcji pogorszenia funkcji nerki, także u chorych będących biorcami przeszczepu nerkowego.

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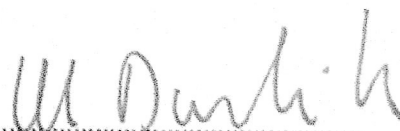
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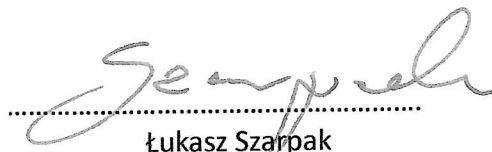
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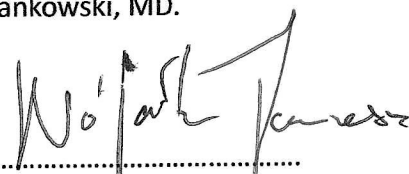
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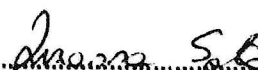
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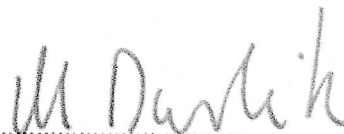
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6. SPIS RYCIN

Rycina 1. Przykłady biomarkerów mających zastosowanie w ocenie funkcji nerki..	26
Rycina 2. Mechanizm działania SuPAR.	76

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