POMORSKI UNIWERSYTET MEDYCZNY
W SZCZECINIE

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“Ocena stanu odżywienia i jego wpływu na parametry gospodarki żelazowej u pacjentów przewlekle hemodializowanych”

Rozprawa doktorska w dziedzinie nauk medycznych i nauk o zdrowiu

Dyscyplina nauki medyczne

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SPIS TREŚCI

1. WYKAZ STOSOWANYCH SKRÓTÓW

ACEI - inhibitory konwertazy angiotensyny (ang. angiotensin-converting enzyme inhibitors)

ACR - wskaźnik albumina/kreatynina (ang. albumin/creatinin ratio)

ARB - blokery receptora angiotensyny (ang. angiotensin-receptor blockers)

BMI - wskaźnik masy ciała (ang. body mass index)

CI - przedział ufności (ang. confidence interval)

ELISA - test immunoenzymatyczny (ang. enzyme-linked immunosorbent assay)

EPO - erytropoetyna (ang. erythropoietin)

ERI - wskaźnik oporności na erytropoetynę (ang. erythropoietin resistance index)

ESA - środki stymulujące erytropoezę (ang. erythropoiesis stimulating agents)

ESRD - schyłkowa choroba nerek (ang. end-stage renal disease)

FFM - masa beztłuszczowa (ang. fat-free mass)

FFMI - wskaźnik masy beztłuszczowej (ang. fat-free mass index)

FM - masa tłuszczowa (ang. fat mass)

FMI - wskaźnik masy tłuszczowej (ang. fat-mass index)

GFR - wskaźnik filtracji kłębuszkowej (ang. glomerular filtration rate)

GLM - ogólny model liniowy (ang. general linear model)

HD - hemodializa (ang. hemodialysis)

HIF - czynnik indukowany przez hipoksję (ang. hypoxia-inducible factor)

IL-18 - interleukina-18 (ang. interleukin-18)

IL-1α - interleukina-1α (ang. interleukin-1α)

IL-6 - interleukina-6 (ang. interleukin-6)

IQR - rozstęp międzykwartylowy (ang. interquartile range)

KDIGO - ang. Kidney Disease: Improving Global Outcomes

LepR - receptor dla leptyny (ang. leptin receptor)

mBCA - medyczny analizator składu ciała (ang. medical body composition analyzer)

MIA - zespół niedożywienie-zapalenie-miażdżyca (ang. malnutrition-inflammation-atherosclerosis)

MIS - skala niedożywienie-zapalenie (malnutrition-inflammation score)/zespół niedożywienie-zapalenie (malnutrition-inflammation syndrome)

MM - masa mięśniowa (ang. muscle mass)

MSC - mezenchymalne komórki macierzyste (ang. mesenchymal stem cells)

NKF-KDOQI - ang. National Kidney Foundation-Kidney Disease Outcomes Quality Initiative

NRS - skala ryzyka żywieniowego (ang. nutritional risk score)

PA - kąt fazowy (ang. phase angle)

PChN/CKD - przewlekła choroba nerek (ang. chronic kidney disease)

PEW - niedożywienie białkowo-kaloryczne (ang. protein-energy wasting)

rHuEPO - rekombinowana ludzka erytropoetyna (ang. recombinant human erythropoietin)

RRT - terapia nerkozastępcza (ang. renal replacement therapy)

SD - odchylenie standardowe (ang. standard deviation)

SGA - subiektywna globalna skala poziomu odżywienia (ang. subjective global assessment)

TBW - całkowita woda ustrojowa (ang. total body water)

TIBC - całkowita zdolność wiązania żelaza (ang. total iron binding capacity)

TNF-α - czynnik martwicy nowotworów α (ang. tumor necrosis factor α)

VAT - tkanka tłuszczowa trzewna (ang. visceral adipose tissue)

2. NOTA INFORMACYJNA

Rozprawę doktorską stanowi zbiór powiązanych tematycznie artykułów naukowych opublikowanych w czasopismach naukowych zgodnie z Art. 13.2 Ustawy o stopniach naukowych i tytule naukowym oraz o stopniach i tytule w zakresie sztuki, Dz. U. z dnia 27 września 2017 r. Poz. 1789:

1. Feret, W.; Safranow, K.; Ciechanowski, K.; Kwiatkowska, E. **How Is Body Composition and Nutrition Status Associated with Erythropoietin Response in Hemodialyzed Patients? A Single-Center Prospective Cohort Study.** J. Clin. Med. 2022, 11, 2426. https://doi.org/10.3390/jcm11092426
   Impact Factor: 4.964, **punktacja MNiSW:** 140

2. Feret, W.; Safranow, K.; Kwiatkowska, E.; Daniel, A.; Ciechanowski, K. **Malnutrition and Erythropoietin Resistance among Patients with End-Stage Kidney Disease: Where Is the Perpetrator of Disaster?** Nutrients 2022, 14, 5318. https://doi.org/10.3390/nu14245318
   Impact Factor: 6.706, **punktacja MNiSW:** 140

3. STRESZCZENIE W JĘZYKU POLSKIM

3.1. Wstęp

Przewlekła choroba nerek dotyka nawet 5 milionów Polaków, z czego około 20 tysięcy jest poddawane leczeniu nerkozastępczemu w postaci hemodializy w schyłkowym stadium choroby. U pacjentów przewlekle hemodializowanych jedną z najczęściej obserwowanych nieprawidłowości jest niedokrwistość, nierzadko wymagająca leczenia preparatami rekombinowanej erytropoetyny. Uważa się, że anemia w tej grupie pacjentów wynika głównie z niedostatecznej produkcji endogennej erytropoetyny, jednak nie sposób pominąć innych czynników, takich jak: niedobory żywieniowe wynikające z restrykcyjnej diety zalecanej pacjentom z ESRD (schyłkowa choroba nerek, ang. end-stage kidney disease), przewlekły proces zapalny i związany z nim stan kataboliczny prowadzący m.in. do niedożywienia białkowo-kalorycznego (PEW – protein-energy wasting) i nieprawidłowej hematopoezy, a także zmienne związane z samą techniką dializy. Pewien odsetek pacjentów dializowanych, mimo leczenia niedokrwistości adekwatnymi dawkami erytropoetyny oraz żelaza, nie osiąga docelowych wartości hemoglobiny i wymaga powtarzalnego przetaczania preparatów krwi. Można wówczas mówić o pojęciu oporności na erytropoetynę, a w literaturze proponuje się wskaźnik ERI (ang. erythropoietin resistance index) jako miernik nasilenia tego zjawiska. Niniejsze badanie miało na celu znalezienie czynników związanych ze wzrostem oporności na EPO u chorych dializowanych. Pod uwagę brano zmierzone za pomocą bioimpedancji parametry składu ciała, wynik w skali MIS (malnutrition-inflammation score), wybrane parametry laboratoryjne (w tym IL-6, IL-18, IL-1α, TNF-α, hepcydyna oraz leptyna) oraz długość i częstotliwość leczenia nerkozastępczego.

3.2. Materiał i metodyka

Projekt badania został zaakceptowany przez Komisję Bioetyczną Pomorskiego Uniwersytetu Medycznego (KB-0012/88/03/19). Do badania, po uzyskaniu ich wcześniejszej zgody, włączono 78 pacjentów hemodializowanych przez co najmniej 6 miesięcy w Stacji Dializ przy Klinice Nefrologii, Transplantologii i Chorób Wewnętrznych Samodzielnego Publicznego Szpitala Klinicznego nr 2 w Szczecinie. Każda z osób po zakończonej dializie została poddana pomiarowi składu ciała za pomocą profesjonalnego medycznego analizatora SECA mBCA. Kwestionariusz MIS został wypełniony dla każdego pacjenta indywidualnie zgodnie z kalkulatorem dostępnym na

stronie http://www.touchcalc.com/calculators/mis. Próbki krwi do badań laboratoryjnych zostały pobrane w czasie rutynowych badań miesięcznych w Stacji Dializ, a poza standardowymi comiesięcznymi oznaczeniami morfologii i parametrów gospodarki żelazowej, na potrzeby pracy rozszerzono panel badań o stężenia leptyny, hepcydyny, IL-6, IL-18, IL-1α oraz TNF-α. Stworzoną bazę danych poddano dalszej analizie statystycznej.

Pacjenci biorący udział w badaniu byli traktowani zgodnie z Deklaracją Helsińską i Deklaracją Stambulską.

3.3. Wyniki

Niskie BMI, niska masa tłuszczowa, mała objętość tłuszczu trzewnego, wysoka zawartość wody w organizmie (TBW - total body water [%]), niska masa beztłuszczowa i niski kąt fazowy były czynnikami związanymi ze zwiększeniem oporności na EPO. Procentowa zawartość tkanki mięśniowej nie miała związku z ERI w tej grupie badawczej. Całkowity wynik w skali MIS korelował pozytywnie z wartością ERI. Ocena składu ciała za pomocą analizy bioimpedancji lepiej nadawała się do przewidywania wartości ERI niż sam wskaźnik BMI. Stężenie IL-6 korelowało pozytywnie z wartością ERI i było jej niezależną determinantą w analizie wieloczynnikowej. Wykazano dodatni związek stężenia IL-6 z długością dializoterapii w miesiącach oraz ilością sesji HD w tygodniu. Wyższe stężenia IL-6 wiązały się z niższą wartością kąta fazowego, będącego wykładnikiem stabilności błon komórkowych. W badanej grupie stężenie IL-6 nie korelowało z wynikiem w skali MIS. Leptyna wykazała ujemną korelację z wartością ERI oraz wynikiem w skali MIS, była też istotnie dodatnio skorelowana z ilością tkanki tłuszczowej, zarówno całkowitej, jak i trzewnej. Nie wykazano korelacji stężenia TNF-α z wartością ERI lub wynikiem w skali MIS, jednak stężenie tej cytokiny było dodatnio skorelowane m.in. z wiekiem i masą tłuszczową. IL-18 oraz IL-1α nie miały związku z wartością ERI ani ze stopniem niedożywienia-zapalenia ocenianego na podstawie skali MIS.

3.4. Wnioski

Stopień oporności na erytropoetynę wyrażony za pomocą ERI jest silnie związany ze stopniem odżywienia u pacjentów hemodializowanych. Szczególnie tkanka tłuszczowa, a wraz z nią leptyna, zdaje się mieć ochronny wpływ na rozwój oporności na erytropoetynę. Pacjenci dializowani z wysokim wynikiem w skali MIS, czyli z nasilonym

niedożywieniem i w stanie prozapalnym, mieli wysoki wskaźnik ERI. Wysokie stężenia krążącej IL-6 wiążą się z gorszą odpowiedzią na EPO.

Kluczowe wydaje się zapobieganie utracie tkanki tłuszczowej i hamowanie stanu zapalnego jako element holistycznego podejścia do leczenia anemii u pacjentów dializowanych. Poza interwencjami żywieniowymi, modulacja odpowiedzi zapalnej związanej z leptyną i IL-6 może być obiecującym celem dalszych badań nad leczeniem zespołu niedożywienie-zapalenie (malnutrition-inflammation syndrome) w chorobie nerek, a tym samym uzyskiwania lepszych efektów leczenia niedokrwistości i poprawy rokowania chorych.

3.5. Słowa kluczowe

niedokrwistość, cytokiny, zapalenie, niedożywienie, skład ciała, tkanka tłuszczowa, erytropoetyna, hemodializa

4. STRESZCZENIE W JĘZYKU ANGIELSKIM

4.1. Introduction

Chronic kidney disease affects up to 5 million Poles. About 20.000 of them receive renal replacement therapy with hemodialysis in end-stage kidney disease. In chronic hemodialysis patients, anemia is a frequent finding and it often requires treatment with recombinant human erythropoietin preparations. In this specific group of patients anemia is thought to be caused mainly by insufficient production of endogenous erythropoietin in the kidneys. Nevertheless, other causal factors such as: restrictive dietary recommendations in ESRD (end-stage renal disease) leading to nutritional deficiencies, chronic inflammatory process together with pro-catabolic state resulting in protein-energy wasting (PEW) and impaired hematopoiesis, as well as aspects associated with dialysis technique, can not be omitted. A certain percentage of patients does not respond adequately to erythropoietin (EPO) treatment, not being able to reach desirable hemoglobin levels despite being treated with large-dose EPO and intravenous/oral iron and require repetitive blood transfusions. Because of this, the concept of erythropoietin resistance in hemodialyzed arises, and the ERI value (erythropoietin resistance index) is seen in the literature as a measure of the intensity of this phenomenon. This study aimed to investigate the factors associated with EPO resistance in dialysis patients. Research was based on bioimpedance body composition measurement, malnutrition-inflammation score (MIS) assessment, selected laboratory parameters (including IL-6, IL-18, IL-1α, TNF-, hepcidin and leptin) as well as dialysis vintage and frequency data.

4.2. Materials and methods

The study design was approved by the Bioethics Committee of the Pomeranian Medical University (KB-0012/88/03/19). The study group included 78 hemodialysis patients undergoing RRT at the Department of Nephrology, Transplantology and Internal Diseases of the Independent Public Clinical Hospital No. 2 in Szczecin. Individuals with at least 6-month dialysis vintage were included after obtaining their prior consent. After the dialysis session, each patient underwent body composition measurement using a professional medical analyzer SECA mBCA. The MIS questionnaire was completed for each patient individually according to the calculator available at http://www.touchcalc.com/calculators/mis. Blood samples for laboratory tests were drawn during routine monthly workup at the Dialysis Station. In addition to standard monthly

determinations of morphology and iron metabolism parameters, the test panel was extended to include leptin, hepcidin, IL-6, IL-18, IL-1α and TNF-α concentrations for the purposes of this study. The final database created was subjected to further statistical analysis.

Patients in the study were treated in accordance with the Declaration of Helsinki and the Istanbul Declaration.

4.3. Results

Low BMI, low fat mass, low visceral fat volume, high total body water (TBW), low fat free mass and low phase angle were factors associated with increased EPO resistance. Muscle percentage was unrelated to ERI in this study group. The MIS total score correlated positively with the ERI value. Assessment of body composition using bioimpedance analysis was superior at predicting ERI to BMI alone. IL-6 concentration correlated positively with ERI value and was its independent determinant in multivariate analysis. A positive relationship between the concentration of IL-6 and dialysis vintage as well as the number of HD sessions per week was demonstrated. Higher concentrations of IL-6 were associated with a lower value of the phase angle, which is an exponent of the cell membrane stability. In the study group, the concentration of IL-6 did not correlate with the MIS score. Leptin showed a negative correlation with the ERI value and MIS score, and was significantly positively correlated with the amount of adipose tissue, both total and visceral. There was no correlation between the concentration of TNF-α and the ERI value nor the MIS score, however, the concentration of aforementioned cytokine was positively correlated with e.g. age and fat mass. IL-18 and IL-1α were not associated with the ERI value nor the degree of malnutrition-inflammation assessed by the MIS scale.

4.4. Conclusions

The degree of erythropoietin resistance as expressed by the ERI value is strongly related to the nutritional status in hemodialysis patients. Adipose tissue in particular, together with leptin, seem to have a protective effect on the development of EPO resistance. Dialysis patients with a high MIS score, i.e. with severe malnutrition and in a pro-inflammatory state, had a high ERI index. High concentrations of circulating IL-6 are associated with a poorer response to EPO.

It seems crucial to prevent fat loss and inhibit inflammation as part of a holistic approach to anemia treatment in dialysis patients. In addition to nutritional interventions,

modulation of the inflammatory response associated with leptin and IL-6 may be a promising target for further research into the treatment of malnutrition-inflammation syndrome in kidney disease, and thus obtaining better results in the treatment of anemia and improving patients' outcomes.

4.5. Keywords

anemia, cytokines, inflammation, malnutrition, body composition, adipose tissue, erythropoietin, hemodialysis

5. WSTĘP

5.1. Przewlekła choroba nerek i leczenie nerkozastępcze

Przewlekłą chorobę nerek (PChN, CKD – ang. chronic kidney disease) wg aktualnej definicji KDIGO definiuje się jako utrzymujące się co najmniej 3 miesiące zaburzenia czynności lub budowy nerek, które mają znaczenie dla zdrowia. Poza kryterium czasowym, do rozpoznania PChN konieczne jest spełnienie jednego lub więcej z następujących: (1) spadku przesączania kłębuszkowego (GFR – ang. glomerular filtration rate) poniżej 60 ml/min/1.73m^2, (2) obecności albuminurii wyrażonej jako utrata białka z moczem ≥30 mg/d lub wskaźnik albumina/kreatynina (ACR – ang. albumin/creatinin ratio) ≥30 mg/g, (3) obecności nieprawidłowego osadu moczu, (4) nieprawidłowości strukturalnych nerek w badaniach obrazowych, (5) nieprawidłowości w badaniach histopatologicznych, (6) zaburzenia czynności cewek nerkowych, (7) stan po przeszczepieniu nerki. Zaawansowanie PChN klasyfikuje się na podstawie wartości GFR w stadia: G1 (GFR ≥90 ml/min/1.73m^2), G2 (GFR 60-89 ml/min/1.73m^2), G3a (GFR 45-59 ml/min/1.73m^2), G3b (GFR 30-44 ml/min/1.73m^2), G4 (GFR 15-29 ml/min/1.73m^2) i G5 (GFR ≤ 15 ml/min/1.73m2) oraz nasilenia albuminurii (A1 - ACR<30 mg/g, A2 – ACR 30-300 mg/g oraz A3 – ACR>300 mg/g). Wyższa klasyfikacja w kategoriach G oraz A wiąże się z pogorszeniem rokowania chorych. [1]

Przewlekła choroba nerek dotyka nawet pięciu milionów Polaków, co stanowi 13% populacji kraju. Najczęstszą przyczyną postępującej utraty czynności nerek jest cukrzyca, z innych częstych przyczyn wyróżnić można nadciśnienie tętnicze oraz glomerulopatie pierwotne i wtórne. PChN uważa się w związku z tym za chorobę cywilizacyjną. Mimo tak częstego występowania, możliwości wczesnego wykrywania i podejmowania adekwatnego leczenia są niewystarczające, a postęp choroby prowadzi do rozwoju przewlekłej niewydolności nerek (stopień 5 wg KDIGO) wymagającej wdrożenia leczenia nerkozastępczego. Możliwymi formami leczenia nerkozastępczego są: hemodializa, dializa otrzewnowa oraz przeszczepienie nerki. Metodą z wyboru, jako poprawiająca jakość życia pacjenta i pozwalająca na istotne wydłużenie przeżycia, jest przeszczepienie nerki - choć z oczywistych względów nie jest ona dostępna dla wszystkich chorych. Obecnie w Polsce leczeniu nerkozastępczemu w formie hemodializy poddawane jest około 20 tysięcy chorych, z czego większość stanowią mężczyźni w

wieku 65-75 lat. Hemodializa jest najczęściej stosowaną w Polsce metodą RRT (ang. renal replacement therapy) [2].

5.2. Niedokrwistość jako następstwo przewlekłej choroby nerek

U chorych z PChN jednym z najczęściej obserwowanych odchyleń w badaniach biochemicznych jest niedokrwistość charakterystyczna dla chorób przewlekłych, a więc normocytowa i normobarwliwa. Rozpoznać ją można, gdy stężenie hemoglobiny wynosi odpowiednio <13 g/dl u mężczyzn i <12 g/dl u kobiet. Jako główny czynnik rozwoju niedokrwistości w chorobie nerek wskazuje się niedobór natywnej erytropoetyny, jednak w literaturze coraz częściej mówi się o etiologii mieszanej [3,4]. Inne opisywane przyczyny niedokrwistości w PChN obejmują: upośledzone wchłanianie żelaza w przewodzie pokarmowym, przewlekłą aktywację procesów zapalnych, a tym samym nadekspresję hepcydyny regulującej uwalnianie żelaza z makrofagów – względny niedobór żelaza; bezpośredni wpływ toksyn mocznicowych na hematopoezę w szpiku, niedobory żywieniowe – w tym witamin z grupy B i folianów niezbędnych do efektywnej erytropoezy, jak również u osób dializowanych przewlekła utrata niewielkich objętości krwi podczas każdego z zabiegu hemodializ. Nie bez znaczenia jest też wpływ przyjmowanych przez pacjenta leków – immunosupresyjnych, ACEI (ang. angiotensin-converting enzyme inhibitors) czy ARB (ang. angiotensin-receptor blockers).

5.3. Leczenie niedokrwistości a oporność na erytropoetynę

Identyfikacja zmniejszonej produkcji natywnej EPO jako głównej przyczyny rozwoju niedokrwistości w przewlekłej chorobie nerek skłoniła badaczy do poszukiwania drogi dostarczenia brakującego hormonu chorym w jego egzogennej formie. Pierwsza rekombinowana ludzka erytropoetyna (rHuEPO – ang. recombinant human erythropoietin), epoetyna alfa, pojawiła się w 1989 r. w Stanach Zjednoczonych [5]. Od tamtej pory jest z powodzeniem stosowana w leczeniu niedokrwistości w przewlekłej niewydolności nerek. Obecnie na rynku poza epoetyną alfa dostępne są również inne preparaty stymulujące erytropoezę (ESA – ang. erythropoiesis stimulating agents): epoetyna beta, darbepoetyna alfa oraz glikol metoksypolietylenowy epoetyny β. Nowym lekiem jest roksadustat, stymulujący produkcję erytropoetyny endogennej inhibitor hydroksylazy prolilowej HIF (ang. hypoxia-inducible factor). Mimo pojawienia się nowych leków, to preparaty erytropoetyny w połączeniu z dożylnymi preparatami żelaza są wciąż najczęściej stosowane w grupie chorych dializowanych z powodu stosunkowo

niskich kosztów i wysokiej dostępności. KDIGO zaleca włączenie leczenia EPO u chorych dializowanych po uprzedniej identyfikacji i usunięciu możliwych innych przyczyn niedokrwistości, w tym bezwzględnego niedoboru żelaza, przy wyjściowym stężeniu hemoglobiny mieszczącym się w zakresie 9.0-10.0 mg/dl. [6] Mimo relatywnie dużej skuteczności leczenia EPO, nie należy zapominać o działaniach niepożądanych wysokich dawek leku, m.in. gorszej kontroli ciśnienia tętniczego, nadkrzepliwości czy wytworzeniu przeciwciał przeciwko erytropoetynie (sporadycznie). Nie zaleca się tym samym dążenia do uzyskania stężenia hemoglobiny jak w populacji zdrowych osób (>13 g/dl) z uwagi na wzrost częstości powikłań sercowo-naczyniowych i ryzyka zgonu. KDIGO wskazuje 11.5 g/dl jako maksymalne docelowe stężenie Hgb u chorych poddawanych hemodializie [6]. W pierwszych 6 miesiącach leczenia ESA przeciętne dawkowanie preparatów żelaza i.v. to 25-150 mg/tydz. U części chorych (ok. 10%) mimo stosowania odpowiednich dawek preparatów żelaza i skalkulowanych do masy ciała dawek EPO (dawka na kg m.c. zależna od preparatu) nie udaje się osiągnąć docelowych stężeń hemoglobiny [7,8]. Nie ma konsensusu co do jednoznacznej definicji oporności na erytropoetynę. Według roboczej definicji NKF-KDOQI (National Kidney Foundation-Kidney Disease Outcomes Quality Initiative) o oporności na erytropoetynę można mówić, gdy nie udaje się uzyskać docelowych wartości stężenia hemoglobiny lub hematokrytu (30-36%) przy stosowaniu dawek EPO większych niż 300 jednostek/kg/tydz. s.c. lub 450 jednostek/kg/tydz. i.v. [9]. KDIGO definiuje oporność na ESA jako niemożność uzyskania docelowego stężenia hemoglobiny po miesiącu adekwatnego do masy ciała dawkowania EPO [6]. W literaturze pojawia się wskaźnik oporności na erytropoetynę, ERI (ang. erythropoietin resistance index) jako proponowany miernik nasilenia tego zjawiska. Określany jest jako średnia tygodniowa dawka EPO na kilogram suchej masy ciała, podzielona przez średni poziom hemoglobiny (g/dl) z ostatnich 6 miesięcy [10, 11]. Na potrzeby niniejszej rozprawy korzystano z tego wskaźnika.

5.4. Problem niedożywienia u chorych hemodializowanych

Niedożywienie jest znaczącym, powszechnym problemem u chorych leczonych hemodializą i dotyczy dużej części (23-60%) tej populacji [12-14]. Zjawisko to wpływa istotnie nie tylko na jakość ich życia, ale także wiąże się ze zwiększoną śmiertelnością [15,16]. Pacjenci dializowani muszą przestrzegać określonych restrykcji żywieniowych, przez co ich dieta często staje się monotonna i predysponuje do niedoborów zarówno makro-, jak i mikroelementów. Zaleca się, aby w schyłkowej niewydolności nerek podaż

energii zawierała się w zakresie 30-35 kcal/kg suchej masy ciała, a dowóz białka wynosił ok. 1-1,2 g/kg suchej masy ciała [17,18]. Chorym zaleca się ograniczenie podaży płynów oraz unikanie produktów bogatych w sód, potas i fosforany, co przy wszechobecności produktów wysoko przetworzonych na sklepowych półkach może być trudne do implementacji. Ponadto, w tej grupie chorych często występują zaburzenia nastroju, co może wiązać się z utratą apetytu. Depresję notuje się nawet u 50% pacjentów poddanych HD [19]. Powyższe czynniki powodują, że dzienna podaż energii często wynosi mniej niż wymagane zapotrzebowanie kaloryczne, a bilans azotowy takiej diety jest ujemny. Nie należy zapominać, że podczas każdego zabiegu hemodializy następuje utrata żelaza, białka i aminokwasów, związana z przewlekłą utratą niewielkiej ilości krwi w ramach interwencji przy dojściu dializacyjnym, czy też zależna od techniki dializy i rodzaju błon dializacyjnych. Szacowana utrata żelaza podczas jednego zabiegu HD to ok. 1000 ug, białka – 7-8 gramów, a aminokwasów od 6 do 12 gramów [20-22]. Do oceny stanu odżywienia u chorych poddawanych hemodializie można stosować różne skale i wskaźniki. Najprostszym i najbardziej powszechnym jest BMI (ang. body mass index), wyrażony jako masa ciała [kg]/wzrost $[m]^2$. Przyjmuje się, że wartość BMI mieszcząca się w zakresie 18,5-24,9 jest normą. Wskaźnik poniżej 18,5 wskazuje na niedowagę, 25-29,9 nadwagę, a powyżej 30 – otyłość. Mimo łatwości zastosowania, jest to wskaźnik mający wiele ograniczeń, nie biorący pod uwagę różnic w kompozycji ciała osoby badanej. Pełniejszy obraz stanu odżywienia może dać nam analiza składu ciała oparta na zjawisku bioimpedancji, czyli badaniu oporności elektrycznej tkanek za pomocą prądu o niskim natężeniu. Profesjonalne analizatory medyczne są w stanie z dużą dokładnością oszacować zawartość wody (zarówno całkowitej, jak i w poszczególnych kompartmentach ciała), tkanki tłuszczowej całkowitej i wisceralnej, masy beztłuszczowej, masy mięśniowej, a także – w niektórych modelach – obliczyć kąt fazowy, czyli wskaźnik mówiący o stabilności błon komórkowych [23-25]. Istnieją też różnego rodzaju skale, służące do oceny stanu odżywienia. Najpowszechniej w praktyce klinicznej używane są skale NRS 2002 (ang. Nutritional Risk Score) oraz SGA (ang. Subjective Global Assessment). Na potrzeby tego badania posłużono się skalą MIS (ang. Malnutrition Inflammation Score) opracowaną przez Kamyara Kalantar-Zadeh [26]. Skala ta składa się z czterech części – wywiadu medycznego (zmiana masy ciała, zmiana apetytu, raportowanych przez chorego objawów ze strony układu pokarmowego, oceny sprawności w życiu codziennym oraz chorób współistniejących), badania fizykalnego z oceną zasobów tkanki tłuszczowej i mięśniowej w poszczególnych partiach ciała, oceny

wskaźnika BMI oraz badaniach biochemicznych (poziomu albuminy i transferryny, alternatywnie oznaczenia TIBC – ang. total iron binding capacity). W skali MIS maksymalnie można uzyskać 30 punktów, a wyższy wynik wiąże się z gorszym stanem odżywienia.

5.5. Przewlekła reakcja zapalna, niedożywienie, niedokrwistość

Przewlekła reakcja zapalna jest znana jako czynnik sprawczy i warunkujący progresję wielu chorób, a PChN nie jest tu wyjątkiem [27]. U wielu pacjentów hemodializowanych można zaobserwować zjawisko znane jako zespół niedożywienie-zapalenie (MIS – malnutrition – inflammation syndrome) lub niedożywienie-zapalenie-miażdżyca (MIA – malnutrition – inflammation – atherosclerosis). Wystąpienie MIS/MIA poprzez nasilenie stresu oksydacyjnego i uszkodzenia śródbłonka jest związane ze spadkiem jakości życia i wzrostem śmiertelności z przyczyn sercowo-naczyniowych w tej grupie chorych [28-30]. Niedożywienie oraz niedokrwistość w schyłkowej chorobie nerek mają wspólne podłoże, jakim jest przewlekła reakcja zapalna. Inicjatorem odpowiedzi zapalnej u hemodializowanych mogą być: nagromadzenie toksyn mocznicowych [31], zakażenia związane z dojściem naczyniowym (większe ryzyko infekcji wiąże się z HD z użyciem cewnika do żyły centralnej, niż przetoki tętniczo-żylnej) [32], niepełna kompatybilność błony dializacyjnej [33] czy też choroby współistniejące. Przewlekłe zapalenie promuje u chorych dializowanych stan kataboliczny, co ma odbicie m.in. w stężeniu albuminy i transferryny, które są tzw. odwrotnymi białkami fazy ostrej – ich stężenie w stanie zapalnym maleje, albuminy powszechniej niż transferryny używa się też jako wykładnika niedożywienia. Oba te wskaźniki z resztą pojawiają się we wspomnianej w poprzednim ustępie skali MIS. W nowych badaniach wykazano, że w populacji osób dializowanych podobnie jak albumina i transferryna zachowuje się leptyna – niezmiernie ciekawa cząsteczka, będąca adipokiną wydzielaną w tkance tłuszczowej i mającą za zadanie regulację wydatku energetycznego i apetytu [34]. U osób zdrowych odpowiada za supresję apetytu, dlatego zwana jest „hormonem sytości”. Wykazano, że leptyna może również wpływać na erytropoezę i metabolizm kostny poprzez receptory LepR w szpiku [35]. Nadmierna ekspresja cytokin, głównie IL-6, prowadzi do nadprodukcji hepcydyny, białka odpowiedzialnego za zahamowanie uwalniania żelaza z makrofagów poprzez degradację ferroportyny [36]. Toksyny mocznicowe oddziałują również bezpośrednio na szpik, hamując proliferację i metabolizm mezenchymalnych komórek macierzystych (MSC – ang. mesenchymal stem cells) [37,38]. Inne cytokiny, które wiązane są z

rozwojem niedożywienia i wzrostem śmiertelności u dializowanych to m.in. TNF-α [39,40], IL-18 [41], IL-10 [42] czy IL-1 [43].

5.6. Podsumowanie wstępu

Problem postępującego wyniszczenia i jego następstw u osób hemodializowanych jest powszechny, ale do tej pory nie opracowano wystarczająco skutecznych metod, aby sobie z nim radzić. W wielu ośrodkach nie prowadzi się okresowego monitoringu stanu odżywienia chorych, mimo, że nie wymaga to zaawansowanych narzędzi i pozwalałoby na wcześniejsze podjęcie odpowiednich interwencji klinicznych. Niniejsza rozprawa stanowi zbiór dwóch oryginalnych publikacji naukowych zgłębiających problem wpływu zespołu niedożywienie-zapalenie na rozwój oporności na erytropoetynę w tej szczególnej grupie chorych. Analizowano wpływ komponentów składu ciała oraz wybranych cytokin na ten proces i zaproponowano możliwe interwencje, które mogłyby potencjalnie poprawić wyniki leczenia i rokowanie chorych.

6. CELE PRACY

1. Ocena stanu odżywienia chorych dializowanych za pomocą zaawansowanych metod antropometrycznych (pomiar bioimpedancji) oraz dedykowanej skali MIS (ang. Malnutrition Inflammation Score). Ocena związku stanu odżywienia pacjentów hemodializowanych z ich parametrami gospodarki żelazowej i opornością na erytropoetynę;

2. Próba określenia wpływu wybranych cytokin prozapalnych (IL-18, IL-6, TNF-α, IL1-α) oraz leptyny na nasilenie oporności na erytropoetynę, wyrażanej wskaźnikiem ERI oraz niedożywienie określone za pomocą skali MIS;

3. Zaproponowanie potencjalnych interwencji mających na celu optymalizację leczenia erytropoetyną oraz zmniejszenie skali niedożywienia w grupie chorych dializowanych.

7. MATERIAŁ I METODYKA

7.1. Materiał

Grupa badawcza, składająca się finalnie z 78 chorych została wyodrębniona spośród pacjentów Stacji Dializ przy Klinice Nefrologii, Transplantologii i Chorób Wewnętrznych Pomorskiego Uniwersytetu Medycznego w Szczecinie. Okres rekrutacji chorych trwał od marca do czerwca 2020 r. Kryteria wyłączenia z badania były następujące: (1) brak świadomej pisemnej zgody pacjenta, (2) okres RRT krótszy niż 6 miesięcy, (3) obecność wszczepionego stymulatora, kardiowertera-defibrylatora lub urządzenia resynchronizującego, (4) krwawienie z przewodu pokarmowego wymagające hospitalizacji w ciągu 6 miesięcy poprzedzających włączenie, (5) aktualne leczenie immunosupresyjne lub cytostatyczne, (6) brak suplementacji EPO. Stosowane preparaty EPO wśród osób włączonych do badania to Aranesp (INN-darbepoetyna alfa, Amgen) oraz NeoRecormon (INN-epoetyna beta, Roche Pharmaceuticals).

7.2. Metodyka

Projekt badania został zaakceptowany przez Komisję Bioetyczną Pomorskiego Uniwersytetu Medycznego (KB-0012/88/03/19). Każda z partycypujących w badaniu osób została poddana analizie składu ciała za pomocą bioimpedancji na profesjonalnym medycznym analizatorze firmy SECA. Badanie przeprowadzono zgodnie z instrukcją obsługi producenta, po zakończonym zabiegu hemodializy. Analizator zebrał następujące dane: BMI, FFM (ang. fat-free mass), FFMI (ang. fat-free mass index), FM (ang. fat mass), FMI (ang. fat-mass index), TBW (ang. total body water), PA (ang. phase angle), MM (ang. muscle mass) oraz VAT (ang. visceral adipose tissue). Na podstawie tych danych analizator porządkował pacjentów do 4 grup: otyłość, otyłość sarkopeniczna, chudość, rosnąca masa mięśniowa. W dniu badania na analizatorze pacjenci byli również oceniani przez lekarza za pomocą skali MIS dostępnej pod adresem: http://www.touchcalc.com/calculators/mis. Krew na badania laboratoryjne była pobierana podczas rutynowych badań miesięcznych w stacji dializ, a standardowy panel badań poszerzono o oznaczenie leptyny, hepcydyny, TNF-α, IL-6, IL-1α, oraz IL-18. Próbki krwi zostały odwirowane z prędkością 4000 rpm. Czas pomiędzy pobraniem a odwirowaniem nie przekraczał 15 minut. Uzyskane osocze podzielono na dwie (lub więcej, jeśli było to możliwe) probówki typu Eppendorf i zamrożono w temp. -70 ˚C. Jedna z probówek posłużyła oznaczeniu stężenia leptyny i hepcydyny metodą ELISA za

pomocą zestawu odpowiednio EuroImmun oraz Gentaur, druga – oznaczeniu cytokin za pomocą kitów Luminex Biotechne. Współczynnik oporności na erytropoetynę ERI był kalkulowany jako średnia tygodniowa dawka darbepoetyny alfa na kilogram suchej masy ciała, podzielona przez średnie stężenie hemoglobiny (g/dl) z ostatnich 6 miesięcy. Na potrzeby kalkulacji ERI, dawki epoetyny beta zostały przeliczone na równoważne dawki darbepoetyny alfa. Po 18 miesiącach obserwacji dokonano analizy śmiertelności i porównano grupy ocalałych i zmarłych pod względem składu ciała i parametrów biochemicznych.

7.3. Analiza statystyczna

Analizę statystyczną wykonano za pomocą programu Statistica 13 (StatSoft, Tulsa, OK, USA). Do badania rozkładów zastosowano test Shapiro-Wilka. Użyto nieparametrycznego testu Manna-Whitneya w celu porównania grup. Korelacje badano metodą współczynnika korelacji rang Spearmana. Dane z rozkładem normalnym opisano jako średnią z odchyleniem standardowym (SD – ang. standard deviation), przy rozkładzie innym od normalnego jako medianę, podając rozstęp międzykwartylowy (IQR – ang. interquartile range). Wartości p były uznawane za statystycznie istotne, gdy osiągały wartość $<0,05$. Do analizy wieloczynnikowej użyto ogólnego modelu liniowego (GLM – ang. general linear model). Dla każdej zmiennej niezależnej przedstawiono standaryzowany współczynnik beta i jego 95% przedział ufności (95% CI – ang. confidence interval).

8. WYNIKI

(1) Wartość ERI nie różniła się istotnie w grupach chorych skategoryzowanych wg BMI (norma vs otyłość, nadwaga vs otyłość, nadwaga vs otyłość), mimo iż wykazano istotną statystycznie odwrotną korelację wartości ERI z BMI ($R= -0.33$, $p = 0.03$). Grupy chorych skategoryzowane na podstawie składu ciała z analizatora bioimpedancji różniły się istotnie pod względem wartości ERI (podano tylko wyniki z $p<0.05$) – otyłość sarkopeniczna vs chudość ($p=0.02$) oraz otyłość vs chudość ($p=0.02$). Wartości ERI w tych grupach wynosiły odpowiednio (mediana, IQR): otyłość sarkopeniczna - 2.8, 4.2; chudość - 6.01, 8.03; otyłość - 2.9, 6.7. Wskazuje to na większą dokładność analizy składu ciała nad BMI oraz sugeruje istotność tkanki tłuszczowej jako potencjalnie ochronnej w rozwoju oporności na EPO.

(2) Wykazano istotną statystycznie ($p<0.05$) korelację poniższych parametrów składu ciała z wartością ERI: FFM ($R = 0.25$, $p = 0.035$), VAT ($R = -0.29$, $p = 0.018$), FMI ($R = -0.25$, $p = 0.037$), PA ($R = -0.33$, $p = 0.006$).

(3) Wyższy wynik w skali MIS wiązał się z wyższą wartością ERI ($R = 0.41$, $p = 0.00041$)

(4) Mediana wartości ERI w grupie badawczej wynosiła 4.885. Grupy chorych podzielone względem tej mediany różniły się istotnie pod względem BSA, FM, TBW, stężenia hepcydyny, transferryny i ferrytyny. W grupie chorych z ERI powyżej mediany odnotowano niższe BSA, niższe FM, wyższe TBW, wyższe stężenia hepcydyny i ferrytyny, a niższe transferryny.

(5) W analizie wieloczynnikowej wykazano, że niskie BMI i niska objętość tkanki tłuszczowej trzewnej oraz wysokie stężenie IL-6 były niezależnymi czynnikami wpływającymi na wzrost wartości ERI.

(6) Wartość ERI nie miała związku ze śmiertelnością w tej grupie badawczej ($p = 0.92$). W grupie chorych która zmarła w 18-miesięcznej obserwacji z jakiejkolwiek przyczyny wykazano istotną różnicę pod względem wyniku w skali MIS (wyższy niż u ocalałych, $p = 0.00087$), TBW oraz poziomu albuminy (niższe niż u ocalałych, odpowiednio $p = 0.029$ oraz $p= 0.00034$).

(7) Wykazano istotną statystycznie dodatnią korelację poziomu leptyny z masą ciała, BMI, BSA, FM, FMI, VAT oraz poziomem żelaza ($p<0.05$, R odpowiednio: 0.497, 0.652, 0.358, 0.518, 0.540, 0.561, 0.262). Stężenie leptyny korelowało ujemnie z wartością ERI, wynikiem

w skali MIS, FFM, MM i TBW ($p<0.05$. R odpowiednio: -0.31, -0.271, -0.518, -0.425, -0.548).

(8) W analizie wieloczynnikowej wykazano, że niezależnymi determinantami poziomu leptyny była płeć (wyższy poziom u kobiet), BMI, VAT i MM.

(9) Poziom TNF-α nie korelował z wartością ERI ani wynikiem w skali MIS. Wykazano istotną statystycznie dodatnią korelację poziomu tej cytokiny z wiekiem, FM, FMI oraz IL-1α ($p<0.05$, R odpowiednio: 0.238, 0.244, 0.246, 0.233). Stężenie TNF-α korelowało ujemnie z FFM ($p<0.05$, $R=0.244$).

(10) Poziom IL-6 istotnie korelował z wartością ERI, ilością dializ w tygodniu, czasem dializy w miesiącach oraz stężeniem IL-1α ($p<0.05$, R odpowiednio: 0.229, 0.282, 0.229, 0.359). Wartość kąta fazowego korelowała ujemnie z poziomem IL-6 ($p<0.05$, $R=-0.261$).

(11) Nie wykazano związku poziomu IL-1α oraz IL-18 z wynikiem w skali MIS oraz wielkością ERI. IL-1α nie była powiązana z parametrami składu ciała ani częstotliwością i długością dializy. IL-18 nie korelowała istotnie z żadnym z oznaczanych w niniejszym badaniu parametrów.

9. WNIOSKI

Należy aktywnie szukać powodów niepowodzenia leczenia EPO u osoby hemodializowanej. Pogłębiona ocena może być wykonana przy użyciu prostych narzędzi, jak np. analiza składu ciała i/lub kategoryzacja za pomocą skali MIS. Pacjenci niedożywieni są bardziej podatni na rozwój oporności na EPO. Najbardziej kluczowym elementem składowym ciała w zapobieganiu rozwoju oporności na ESA wydaje się być tkanka tłuszczowa, jako czynna hormonalnie, a wraz z nią adipokina - leptyna. Przewodnienie sprzyja rozwojowi oporności na erytropoetynę. Kąt fazowy może być przydatnym wykładnikiem dobrostanu organizmu, a jego wartość maleje przy wysokiej ekspresji IL-6. Nasilona reakcja zapalna wyrażona głównie przez zwiększone stężenia IL-6 wyższy wynik w skali MIS, wiąże się z gorszą reakcją na EPO.

Możliwe interwencje mające na celu tłumienie progresji zespołu niedożywienie-zapalenie mogą obejmować: (1) modulację sygnalizacji związaną z adipokinami i cytokinami, (2) poprawę jakości dializy poprzez wybór biokompatybilnych membran, umożliwiających skuteczniejsze usuwanie toksyn mocznicowych oraz (3) stałe poradnictwo żywieniowe. Zapobieganie niedożywieniu powinno być elementem holistycznego podejścia do leczenia anemii u pacjentów dializowanych, ponieważ nie służy jedynie leczeniu niedokrwistości per se, ale poprawia rokowanie i jakość życia chorych. Lekarze zajmujący się pacjentami poddawanymi RRT powinni pracować w zespołach multidyscyplinarnych wraz z dietetykami i fizjoterapeutami w celu zapewnienia odpowiedniej podaży makro- i mikroelementów w diecie, zmniejszenia stresu oksydacyjnego, zapobiegania utracie tkanki tłuszczowej oraz przeciążenia płynami, a także zwiększaniu codziennej sprawności chorych.

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11. PUBLIKACJE STANOWIĄCE ROZPRAWĘ DOKTORSKĄ

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How Is Body Composition and Nutrition Status Associated with Erythropoietin Response in Hemodialyzed Patients? A Single-Center Prospective Cohort Study

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Abstract: Background: Anemia is the most common finding in patients with end-stage kidney disease undergoing renal replacement therapy. A certain percentage of patients does not respond adequately to erythropoietin (EPO) treatment, not being able to reach desirable hemoglobin levels even when treated with large-dose EPO and intravenous/oral iron. In our study, we wanted to further investigate how nutritional status is associated with erythropoietin responsiveness. To quantify EPO response, we used the Erythropoietin Resistance Index (ERI), which is defined as the weekly weight-adjusted dose of EPO divided by the hemoglobin level. Patients and methods: Seventy-eight patients undergoing hemodialysis were included. All of them were measured by a SECA mBCA body composition analyzer and evaluated by Kalantar-Zadeh's MIS score. Routine biochemical tests were also taken into account. The Shapiro-Wilk test was used to study the distributions of quantitative variables, which were significantly different from normal ($p < 0.05$). We used nonparametric Mann-Whitney U-test to compare groups. Correlations were studied by means of Spearman's rank correlation coefficient. Bonferroni correction for multiple testing was performed. To find independent determinants of ERI, we additionally performed multivariate analysis using the General Linear Model (GLM). Results: In terms of body composition, factors that are associated with high ERI are low BMI, low fat mass, low visceral fat volume, high total body water percentage, low phase angle and low fat-free mass. In addition to body composition parameters, total MIS score and IL-6 serum levels correlated positively with ERI value. IL-6 was an independent determinant of ERI value, based on multivariate analysis. After correction for multiple analysis, BMI and eGFR both remained significant factors associated with EPO response. Conclusions: It seems crucial to prevent inflammatory malnutrition as a part of a holistic approach to anemia treatment in dialysis patients.

Keywords: erythropoietin resistance; body composition; hemodialysis; ERI; anemia

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1. Introduction

Chronic kidney disease affects up to five million Poles, which constitutes 13% of the general population. Despite such a high prevalence of this disease in the population, it is still believed that the possibilities of early diagnosis and therapy are insufficient. It is estimated that about 21,000 people are currently receiving hemodialysis in Poland [1]. Hemodialysis patients must adhere to certain dietary restrictions—it is recommended that they limit their fluid intake and avoid foods that are high in sodium, potassium, and phosphate. These requirements are not easy for patients to maintain and are often associated with insufficient protein and calorie intake. Moreover, in this group of patients, depression is not uncommon, causing an additional decrease in appetite. The aforementioned difficulties contribute to the development of various types of disorders in the nutritional status of patients undergoing renal replacement therapy. However, there are factors that

are beyond the patient's control, such as the loss of amino acids and iron during each hemodialysis session [2]. Finally, the hemodialysis procedure itself increases catabolism, with a mild inflammatory reaction as an underlying cause [3]. When assessing nutritional status, most clinicians use body mass index (BMI) and classify the patients as "normal", "overweight", "obese" or "underweight". Such a definition of the nutritional state may suggest that dietary intervention is sufficient to obtain clinical improvement. According to the available literature, there is another type of malnutrition, referred to as type II malnutrition: MIA syndrome (malnutrition, inflammation, atherosclerosis) or MIC (malnutrition, inflammation, cachexia); here, inflammation is a significant factor in the development of malnutrition, and the dietary intervention itself is ineffective [4,5]. Chronic inflammation can also contribute to anemia, as the expression of hepcidin increases in response to inflammation. Hepcidin is responsible for inhibiting the release of macrophage-stored iron [6]. In hemodialyzed patients, it might therefore be assumed that anemia is not only due to deficiency of erythropoietin produced and secreted in the kidneys; the mechanism is more complex. A significant proportion of patients are taking erythropoietin or other ESAs, yet the response to treatment is unsatisfactory, and ERI (erythropoietin resistance index) is notable in this group [7]. For this reason, we wanted to investigate the parameters of iron metabolism and erythropoietin response in patients undergoing hemodialysis and assess how they are associated with the nutritional status of these patients, with greater focus on body composition analysis.

2. Materials and Methods

This study obtained approval of the Bioethical Committee of Pomeranian Medical University in Szczecin (KB-0012/88/03/19).

The study included patients with end-stage chronic kidney disease, undergoing renal replacement therapy in The Independent Public Clinical Hospital No. 2 at Pomeranian Medical University in Szczecin. The registration period of enrolled patients was March–June 2020. All of the patients had given their informed consent to participate in the study. It consisted of three elements: blood sample collection, assessment of each individual by malnutrition inflammation score (MIS) questionnaire [8] and body composition analysis. The latter was conducted using a professional medical body composition analyzer, Seca mBCA 525, following the user manual [9]. The body weight and height of participants were both measured manually before body composition analysis. A brief description of the characteristics of main body composition elements that were measured using the Seca mBCA 252 can be found below (Table 1).

Table 1. List of measured body composition parameters.

Parameter	Description
BMI—body mass index [kg/m^2]	A value derived from body mass divided by the square of the body height, traditionally used to group individuals as underweight, normal, overweight or obese.
FFM—fat free mass [kg], relative to weight [%]	Calculated by subtracting body fat weight from total body weight; also referred to as "lean body mass".
FFMI—fat free mass index [kg/m^2]	Describes the amount of fat-free mass ("lean body mass") in relation to height and weight. Similar to BMI.
FM—fat mass [kg], relative to weight [%]	Total amount of fat; percentage of total bodyweight that is fat.
FMI—fat mass index [kg/m^2]	Describes the amount of fat mass in relation to height and weight. Similar to BMI.

Table 1. *Cont.*

Parameter	Description
TBW—total body water [l], relative to weight [%]	The sum of intracellular water and extracellular water volume; approx. 60% of body weight of a normovolemic individual.
Phase angle φ [°]	Calculated by reactance/resistance ratio during bioelectrical impedance measurement. Used as an indicator of cell wall stability. Helpful in health risk assessment.
VAT—visceral adipose tissue [l]	Also known as abdominal fat, describes adipose tissue that surrounds the organs in the abdominal cavity. Overdeposition of visceral fat in the abdomen is known as visceral obesity.

The testing is quick and non-invasive, based on an 8-point electrical impedance measurement on the patient's body surface. The electric current used during the test is 100 μA; thus, patients with any cardiac implantable electrical device were excluded from the study, taking the measurement method into consideration. Each patient was measured after the hemodialysis procedure. The analyzer assigned each individual into one of four subsets, based on the body composition chart: increasing sarcopenic obesity, increasing obesity, increasing thinness or increasing muscle mass [9]. The authors also divided participants into four groups based on BMI: underweight (<18.5), normal (18.5–24.9), overweight (25–29.9) or obese (30 or more). Later, we compared the erythropoietin resistance index in relation to patients' body composition chart placement and BMI as a determinant.

Body surface area (BSA) was calculated separately for each individual using the Du Bois formula ($BSA = 0.007184 \times W^{0.425} \times H^{0.725}$). Follow-up time was 18 months; after that, overall survival was calculated. Blood samples were collected at the baseline during routine monthly blood workup. Besides the MIS questionnaire and anthropometric measurements listed in Table 1, parameters included in the database for final analysis next to those needed to calculate ERI were age, dialysis vintage, IL-6, hepcidin, ferritin, transferrin, iron, TSAT%, PTH, eGFR, Kt/V and intradialytic weight gain (IDWG). The authors used the baseline laboratory parameters. Blood samples were drawn mid-week. The ESA preparations used among our patients were Aranesp (INN-darbepoetin alpha, by Amgen) and NeoRecormon (INN-epoetin beta, by Roche Pharmaceuticals). We recalculated the epoetin beta dose to darbepoetin alpha dose and used mean EPO units in further analysis. The erythropoietin resistance index in this study was calculated as an average weekly erythropoietin dose per kg body weight per average hemoglobin (g/dl), over the last 6 months. Taking this into account, we also excluded patients whose RRT duration was less than 6 months. EPO dosing in our center is calculated for clinically optimal body weight after dialysis. By "optimal", the authors mean that a patient does not have any clinical symptoms of hyper- or hypovolemia (e.g., dyspnea, edemas, hypotonia, cramps, increased thirst after HD session, etc.). The flow chart of participants' recruitment can be seen below (Figure 1).

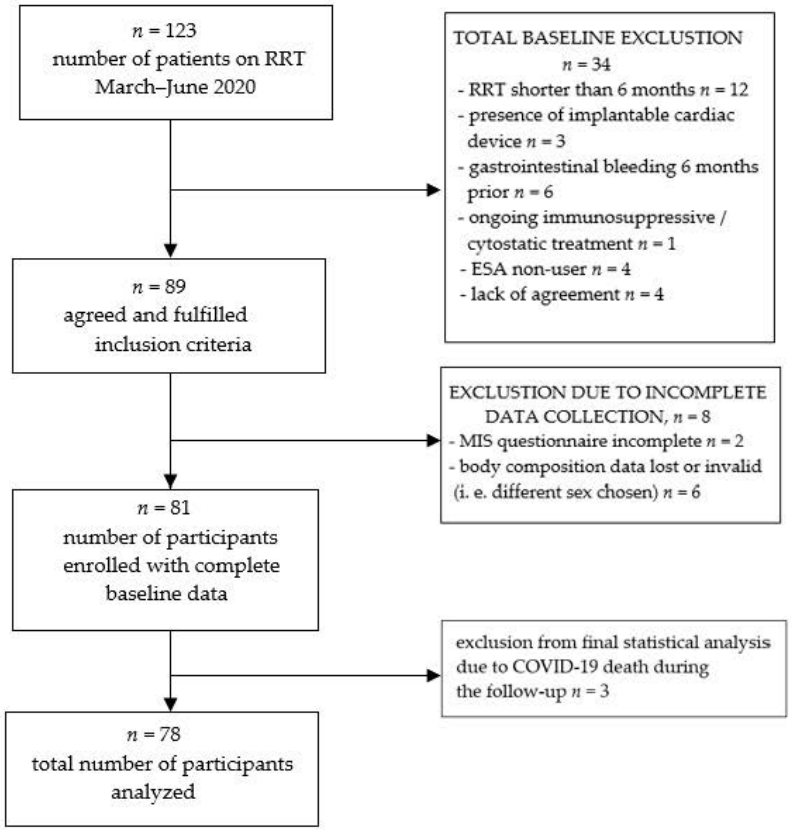

Figure 1. Study group recruitment.

The initial number of participants included was 81. Due to SARS-CoV2 spread during the time of the 18-month follow-up, three of them died. The authors excluded them from the final analyses, as little was known about the disease at that time. As it affected mortality in a sudden manner, the authors wanted to preserve the "natural", previously observed mortality pattern in our group of hemodialyzed patients. Seventy-eight participants with complete data were finally taken into account in the study, 31 of which were female and 47 male. During the follow-up, there were no drop-outs due to relocation or kidney transplantation. Detailed group characteristics are given in the results section (Table 2). In our population, according to the KDIGO working definition of ESA resistance (EPO dose greater than 300 units/kg/weekly s.c. or 450 units/kg/weekly i.v. without appropriate Hgb level), only one person fulfilled these criteria. As a result, we chose ERI as a better, in our opinion, indicator of ESA response, as it takes average hemoglobin level as one of its ingredients. Similar to other researchers on EPO hyporesponsiveness, we compared groups of patients on both sides of the median and/or within terciles, as there is no clear cut-off point of ERI that defines EPO-naïve individuals by definition.

Table 2. Group characteristics.

Overall Participants	*n* = 78	
Male	*n* = 47 (60.3%)	
Age [years]	Median: 65; IQR = 21	
Dialysis vintage [months]	Median: 28.5; IQR = 42	
Patients' nutrition by BMI [%]	underweight normal overweight obese	2.6% 26.9% 42.3% 28.2%
Patients' nutrition by SECA mBCA body composition chart [%]	increasing sarcopenic obesity: 23.2% increasing obesity: 30.4% increasing thinness: 17.4% increasing muscle mass: 29%	
ERI [IU/kg/g/dL/week]	Median: 4.9; IQR = 6.8	
IL-6 [pg/mL]	Median: 3; IQR = 2.9	
Albumin [mg/mL]	Median: 41; IQR = 5	
Transferrin [g/L]	Median: 1.7; IQR = 0.26	
Transferrin saturation [%]	Mean: 29.2 (SD 12.7)	
Hepcidin [ng/mL]	Median: 92.55; IQR = 108.8	
Ferritin [µg/L]	Median: 475; IQR = 557	
Hemoglobin [mmol/L]	Mean: 6.72 (SD 0.86)	
PTH [pg/mL]	Median: 322; IQR = 290	
Kt/V	Mean: 1.14 (SD 0.23)	
Intradialytic weight gain [% of total body weight]	Median: 2.26; IQR = 2.82	
eGFR [mL/min/1.73 m^2]	Median: 7; IQR = 4	
Total MIS score	Median: 5; IQR = 5	
Mortality rate (18-month follow-up)	Overall: *n* = 23 (29.5%) Cardiovascular reasons: *n* = 9 (11.5%)	

Abbreviations: IQR—interquartile range, BMI—body mass index, mBCA—medical body composition analyzer, ERI—erythropoietin resistance index, eGFR—estimated glomerular filtration rate, MIS—malnutrition inflammation scale.

Statistical analysis: Statistical analysis was made using Statistica 13 software (StatSoft, Tulsa, OK, USA). The Shapiro-Wilk test was used to study the distributions of quantitative variables that were significantly different from normal ($p < 0.05$). We used nonparametric Mann–Whitney U-test to compare groups. Correlations were studied by means of Spearman's rank correlation coefficient. Data were described as mean ± SD or median (interquartile range—IQR). *p*-values were significant when <0.05 without correction for multiple testing. Since 30 statistical tests were performed to analyze associations of ERI with other variables, the Bonferroni-corrected *p*-value threshold of significance was 0.05/30 = 0.0016. To find independent determinants of ERI, we additionally performed multivariate analysis using the General Linear Model (GLM). To assess survival, we calculated the total number of patients who died, and among those we extracted individuals who died due to cardiovascular events. Groups of survivors and deceased during the 18-month follow up were compared in terms of body composition and laboratory findings.

3. Results

Baseline characteristics of the study participants: Seventy-eight individuals were included in the study. The majority of them were male, with median age of 65 and median dialysis vintage of 28.5 months. Most of the participants were classified as overweight by BMI. On body composition chart, most of them were placed in the "increasing obesity" area. The median MIS score was 5. Overall 18-month mortality rate was 29.5%. Detailed laboratory findings can be found in Table 2 above.

3.1. Superiority of Body Composition Analysis over BMI Value in Predicting EPO Response

Whilst comparing ERI value between groups of patients categorized by BMI, median ERI was not significantly different between the groups (overweight vs. obese, normal vs. overweight and normal vs. obese), even though in general we found an inverse correlation between ERI and BMI in the Spearman's rank test considering the whole study group (Figure 2B, see below). Body composition analysis proved to be a more precise tool in predicting erythropoietin resistance in certain cohorts, as compared groups differed significantly in terms of ERI (Table 3). Individuals that were assigned to the "sarcopenic obesity" and "obesity" group based on the body composition chart had significantly lower ERI than those in the "thinness" group. These results may suggest that adipose tissue itself is especially noteworthy when it comes to EPO resistance. The authors believe that these findings place body composition analysis over BMI as a more accurate tool in predicting erythropoietin response, as it gave statistically significant results even in a relatively small population.

Table 3. BMI vs. BCA as predictors of ESA response (significant results in bold, $p < 0.05$).

Comparison of BMI and mBCA as Predictors of ERI Value	
BMI Group	**ERI, Median; IQR**
underweight	not included in the statistical analysis due to small sample size ($n = 2$)
normal	6.1; 4
overweight	3.5; 5.8
obese	3.2; 6.7
Comparison of BMI groups (U-Mann-Whitney Test)	
ERI overweight vs. *obese*	$p = 1$
ERI normal vs. *overweight*	$p = 0.09$
ERI in normal vs. *obese*	$p = 0.1$
BCA Group	**ERI, Median; IQR**
increasing sarcopenic obesity	2.8; 4.2
increasing obesity	2.9; 6.7
increasing thinness	6.01; 8.03
increasing muscle mass	6.5; 7.2
Comparison of BCA Groups (U-Mann-Whitney Test)	
ERI sarcopenic obesity vs. *obesity*	$p = 0.8$
ERI sarcopenic obesity vs. *thinness*	$p = 0.02$
ERI sarcopenic obesity vs. *muscle mass*	$p = 0.52$
ERI obesity vs. *thinness*	$p = 0.02$

In our study, we found statistically significant correlations of ERI value with certain body composition–related parameters ($p < 0.05$).

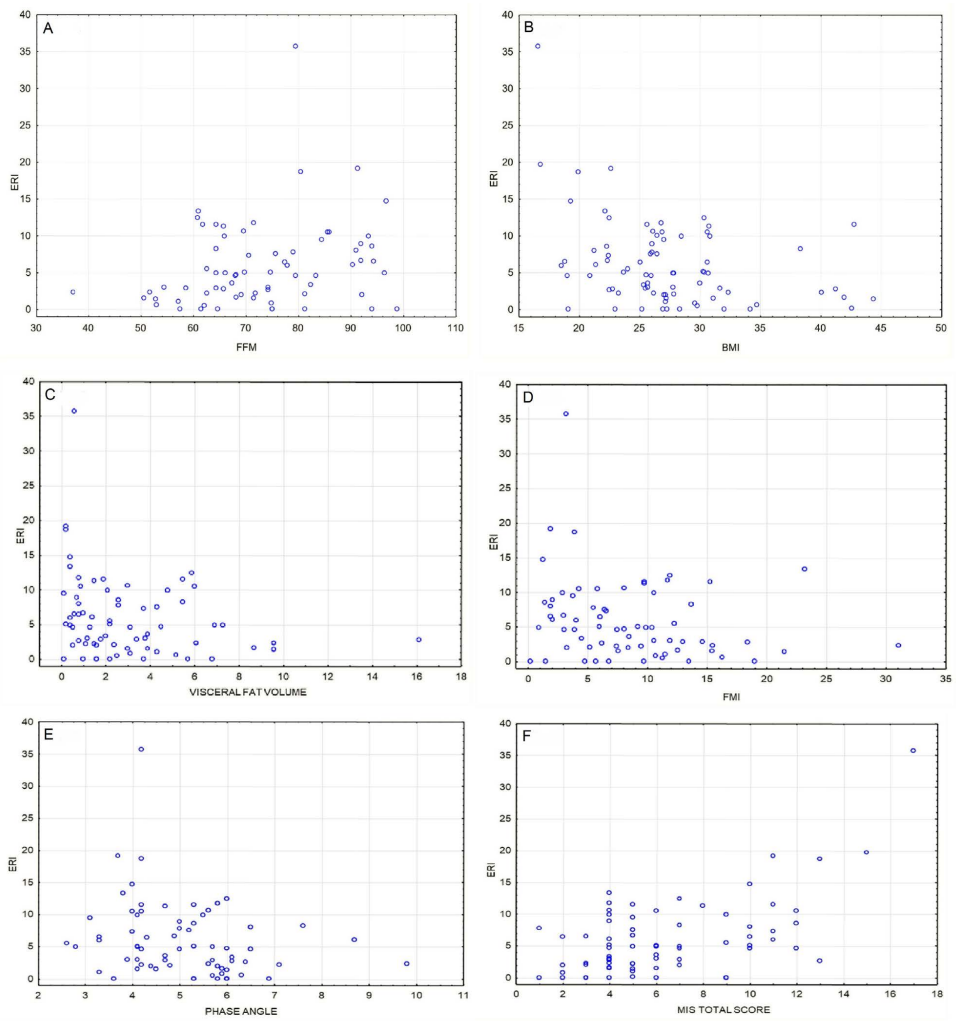

Figure 2. (**A**) Positive correlation between fat-free mass [%] and ERI value [IU/kg/g/dL/week] ($\rho = 0.25$, $p = 0.035$). (**B**) Inverse correlation between BMI [kg/m^2] and ERI value [IU/kg/g/dL/week] ($\rho = -0.33$, $p = 0.03$). (**C**) Inverse correlation between visceral fat volume [l] and ERI value [IU/kg/g/dL/week] ($\rho = -0.29$, $p = 0.018$). (**D**) Inverse correlation between fat mass index [kg/m^2] and ERI value [IU/kg/g/dL/week] ($\rho = -0.25$, $p = 0.037$). (**E**) Inverse correlation between phase angle (°) and ERI value [IU/kg/g/dL/week] ($\rho = -0.33$, $p = 0.006$). (**F**) Positive correlation between ERI [IU/kg/g/dL/week] and MIS total score ($\rho = 0.41$, $p = 0.00041$); this association remains significant after Bonferroni correction.

The median ERI in our study group was 4.885. In individuals with an ERI lower than this, the median body weight was higher than in individuals whose ERI ranged above the median. The lower-ERI group, in comparison with higher-ERI group, also had higher BMI, higher BSA (Figure 3A), lower MIS score, lower fat free mass, higher fat mass (Figure 3B) and fat mass index, higher phase angle, lower total body water (Figure 3C), lower hepcidin, higher transferrin, and lower ferritin serum level (Figure 3D–F).

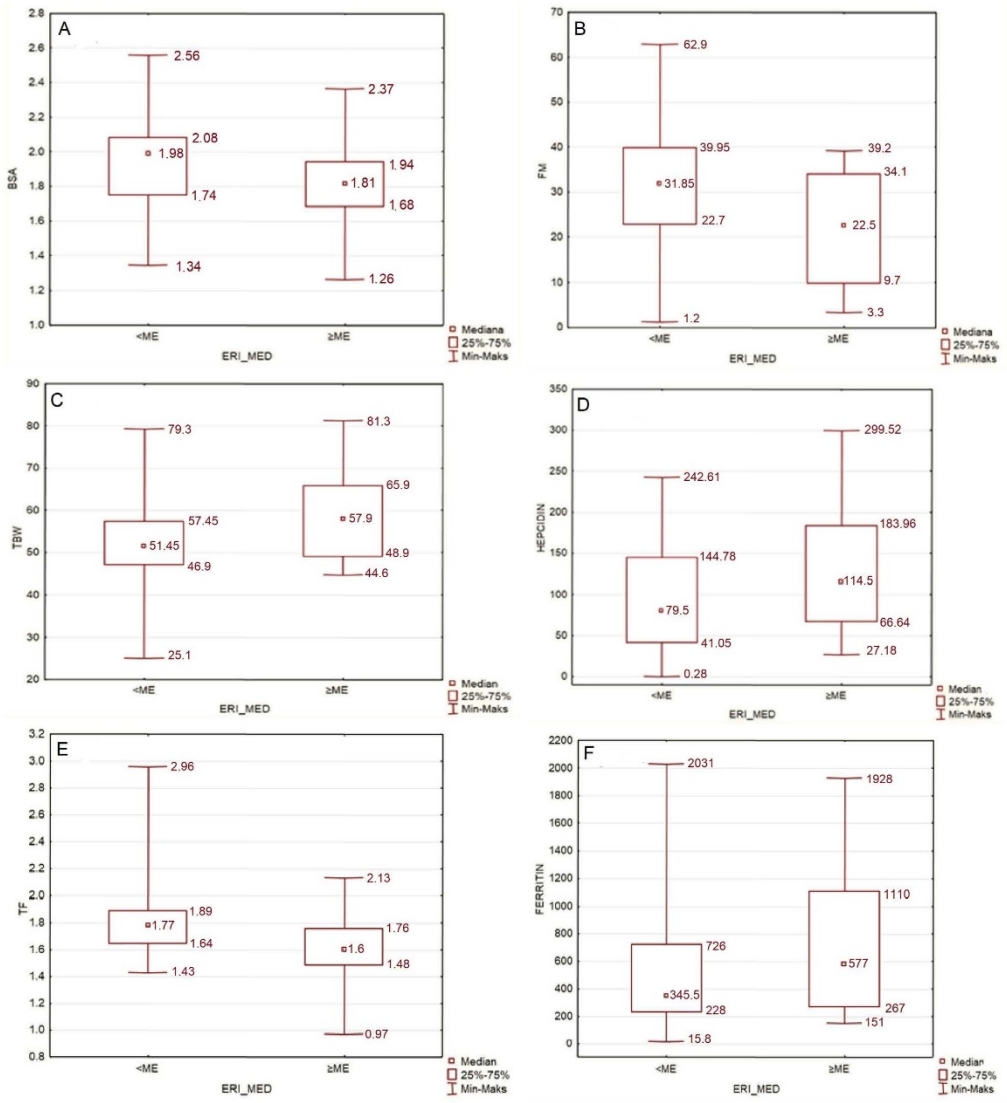

Figure 3. (**A**) Comparison of BSA in groups with ERI lower and higher than median ($p = 0.033$). (**B**) Comparison of FM in groups with ERI lower and higher than median ($p = 0.024$). (**C**) Comparison of TBW [%] in groups with ERI lower and higher than median ($p = 0.024$). (**D**–**F**) Comparison of hepcidin [ng/mL], transferrin [g/L] and ferritin serum level [µg/L] in groups with ERI lower and higher than median ($p = 0.043$; $p = 0.002$; $p = 0.041$, respectively).

There was a significant, even after Bonferroni correction, inverse correlation between eGFR and ERI ($\rho = -0{,}40$; $p = 0.0004$, plot not shown).

We compared Kt/V values between groups of different body composition and BMI (Table 4), and the results were as below.

Table 4. Kt/V and nutrition as determined by BMI and body composition analysis.

KT/V and Nutrition Comparison between Groups (U-Mann-Whitney Test)			
In Groups Divided by BMI		In Groups Divided by BCA	
Category	KT/V, Mean; SD	Category	KT/V, Mean; SD
normal	1.24; 0.24	increasing sarcopenic obesity	1.1; 0.23
overweight	1.13; 0.20	increasing obesity	1.1; 0.21
obese	1.05; 0.21	increasing thinness	1.31; 0.30
		increasing muscle mass	1.12; 0.15
normal vs. *overweight*	$p = 0.10$	*sarcopenic obesity* vs. *obesity*	$p = 0.72$
normal vs. *obese*	$p = 0.02$	*sarcopenic obesity* vs. *thinness*	$p = 0.052$
overweight vs. *obese*	$p = 0.41$	*sarcopenic obesity* vs. *muscle mass*	$p = 0.77$
		obesity vs. *thinness*	$p = 0.02$
		obesity vs. *muscle mass*	$p = 0.55$
		thinness vs. *muscle mass*	$p = 0.07$

Neither parathormone serum concentration nor intradialytic weight gain (IDWG) were significantly associated with ERI in our study ($\rho = -0.203$; $p = 0.076$ and $\rho = 0.059$; $p = 0.61$, respectively; plots not shown).

3.2. *Independent Determinants of ERI Value*

To find the independent determinants of logarithmically transformed ERI, we additionally performed a multivariate analysis in which age, gender, BMI and IL-6 were independent variables. IL-6 was used as an indicator of inflammation in our study group. In multivariate analysis, low BMI and high IL-6 are factors significantly associated with high ERI, independent of age and sex; $p = 0.003$ and $p = 0.03$, respectively (Table 5, model 1).

Table 5. General Linear Model (GLM) analysis of independent determinants of ERI value in terms of body composition and inflammatory indicators. (VFT—visceral fat tissue, IL-6—interleukin-6, BMI—body mass index).

GLM MODEL 1 ($p = 0.0069$)				
	Beta (ß)	**−95%CI Beta**	**+95%CI Beta**	***p***
SEX	−0.037	−0.26	0.18	0.73
AGE	−0.038	−0.26	0.19	0.74
BMI	−0.34	−0.56	−0.12	0.003
Log IL-6	0.25	0.026	0.47	0.03
GLM MODEL 2 ($p = 0.016$)				
	Beta (ß)	**−95%CI Beta**	**+95%CI Beta**	***p***
SEX	0.11	−0.14	0.35	0.4
AGE	−0.04	−0.28	0.21	0.76
Log VFT	−0.35	−0.6	−0.093	0.0083
Log IL-6	0.27	0.034	0.5	0.025

In another model with age, gender, IL-6 and abdominal fat, and serum IL-6 and abdominal fat volume as independent variables (Table 5, model 2) low abdominal fat volume and high IL-6 concentration significantly contributed to high ERI ($p = 0.025$ and $p = 0.0083$, respectively).

3.3. *Factors Associated with Mortality in the Study Group*

After the 18-month follow-up, we calculated the total number of patients who died ($n = 23$; 29.48%), and among those, we extracted individuals who died due to a cardiovascular event ($n = 9$; 11.5% in total). We did not find statistically significant association

between ERI value and all-cause mortality ($p = 0.92$) as well as death due to cardiovascular reasons ($p = 0.1$). This result, somewhat contrary to the available literature, may be caused by the relatively small sample size. Parameters we proved to be positively associated with all-cause mortality among our study group were age and MIS total score, while TBW% and serum albumin were negatively associated. In terms of death due to cardiovascular reasons, parameters we proved to be significantly positively associated were BMI, and FM, while FFM ($p = 0.047$) and TBW were negatively associated. Results are presented in the table below (Table 6).

Table 6. Comparison of survivors and deceased in terms of ERI, body composition, dialysis vintage and significant laboratory findings (U-Mann-Whitney test, IQR—interquartile range).

Death of Any Cause			
	Deceased ($n = 23$)	**Survivors ($n = 55$)**	***p*-Value**
ERI value (median; IQR)	4.98 (7.02)	4.88 (7.71)	$p = 0.92$
Age, years (mean)	69.7	59.6	$p = 0.0069$
MIS total score (median; IQR)	9 (6.5)	5 (3)	$p = 0.00087$
TBW, % (median; IQR)	49.3 (8)	55.9 (14.5)	$p = 0.029$
Serum albumin (median; IQR)	38.5 (4)	42 (5)	$p = 0.00034$
Dialysis vintage in months (median, IQR)	32 (37)	25 (45)	$p = 0.81$
Death Due to Cardiovascular Disease			
	Deceased ($n = 9$)	**Survivors ($n = 55$)**	***p*-Value**
ERI value (median; IQR)	1.35 (4.53)	4.96 (7.2)	$p = 0.1$
BMI, [kg/m^2] (median; IQR)	29.77 (11.44)	26.16 (7.35)	$p = 0.04$
FFM, % (median; IQR)	63.2 (14.1)	74.3 (18.7)	$p = 0.047$
FM, % (median; IQR)	36.8 (14.1)	25.7 (18.7)	$p = 0.047$
TBW, % (median; IQR)	47 (6.75)	55.4 (12.2)	$p = 0.0051$
Dialysis vintage in months (median, IQR)	28 (56)	29 (36)	$p = 0.66$

4. Discussion

4.1. Anemia and EPO Resistance as a Major Burden in Chronic Kidney Disease

Anemia is by far the most common finding in patients with end-stage kidney disease undergoing long-term renal replacement therapy. Decreased production of native erythropoietin by the kidneys is recognized as the main cause of such a state. Taking this into account, scientists first struggled to find a way to replace the deficient hormone by its exogenous form. This led to development of the first recombinant human erythropoietin (rHuEPO), epoetin alpha, in 1989 in the US [10]. It was successfully introduced as CKD-related anemia treatment in the 1980s and has been a standard of care since then. Although other erythropoiesis-stimulating agents, including biosimilars, are gaining growing attention these days, rHuEPO is the most widely used because of its cost-effectiveness. Despite this, a certain percentage of patients seem to not respond adequately to EPO treatment, not being able to reach desirable hemoglobin levels, even when treated with large-dose EPO and intravenous iron. This fact led to further research and established a belief in the multifactorial etiology of anemia in chronic kidney disease [11,12]. Besides EPO deficiency, several aspects are nowadays known to be linked to anemia development: repetitive blood loss during each HD procedure [13,14]; functional iron deficiency linked to impaired release of iron stores from the macrophages due to hepcidin overexpression [15,16]; abundance of proinflammatory cytokines in CKD, which further leads to hepcidin overproduction [6,17,18]; impaired hematopoiesis in the bone marrow as a sequel

of uremia [19]; and folate and B12 deficiency. Disturbances in all the aforementioned areas can lead not only to anemia development, but also to hemodialyzed individuals becoming EPO-naïve.

4.2. Erythropoietin Response and Nutrition in ESRD

It is well-established in the available literature that malnutrition carries greater mortality risk than obesity in patients undergoing renal replacement therapy, contrary to the general population [20,21]. This phenomenon is known as reverse epidemiology. In our study, we wanted to further investigate how nutritional status affects erythropoietin responsiveness. We found that high erythropoietin resistance index (ERI) in our group of hemodialyzed patients is generally related to poorer nutritional status.

In our study group, in terms of body composition analysis, individuals with poor EPO response had lower body weight, lower BMI, lower fat mass, lower visceral fat volume and lower phase angle.

4.2.1. ERI and Phase Angle

A phase angle is a derivative of reactance and resistance values, obtained during bioelectrical impedance measurement [22]. It depends mainly on water and lipid content in the cell membrane and ECW/ICW ratio. Higher phase angle indicates a fair supply of nutrients and higher cell-wall stability, although there is no clear cut-off point of PhA in malnutrition/sarcopenia detection [23,24]. In poorly nourished patients, a lack of ingredients to build a stable lipo-protein component of the cell wall is the reason for low phase angle. Our findings seem to prove that sharp (low) phase angle, being a marker of cell-wall instability and fluid imbalance, can predict poor EPO response (Figure 2E). Fluid overload is also directly related to phase angle, so in HD patients, a low phase angle could indicate malnutrition, fluid overload or a combination of the two.

4.2.2. ERI, Fat Mass, Fat Free Mass, Visceral Fat Volume and BMI

We found that low BMI and low fat mass in an individual were associated with higher ERI, which stands in line with the "reverse epidemiology" in CKD. Surprisingly, we also managed to find an inverse correlation between absolute visceral fat volume [l] and ERI. Whilst comparing patients assigned to one of four groups based on the body composition chart, we found that adipose tissue itself seems to be in favor of overcoming EPO resistance (Table 3). In the multivariate analysis, we found that visceral fat volume and IL-6 serum concentration are both strong predictors of EPO hyporesponsiveness (Table 2). These findings suggest that in patients with ESRD undergoing RRT, "any" kind of fatty tissue is beneficial when it comes to EPO treatment outcomes. There are many erythropoietin receptors in the adipose tissue. Although adipose tissue is known to be a source of proinflammatory cytokines, mainly IL-6, which deprives EPO sensitivity, it is also a source of leptin. Adipocyte-derived leptin has been shown to have an erythropoiesis stimulating effect. It reduces the pro-inflammatory effect of adipose tissue and enhances the anti-inflammatory effect. In an interventional study by Hung et al., high-calorie intake in HD patients leading to hyperleptinemia markedly improved hematopoiesis [25]. This is most likely why not muscle mass, but visceral fat and fat mass in general is a factor that is particularly associated with erythropoietin sensitivity [26,27].

Our research supports the results of a study by Vega et al., which showed inverse correlation between fat mass and ERI, as well as BMI and ERI [28]. Kotanko et al. studied a group of 479 African American HD patients and, similar to our research, found that higher ERI is related to low fat mass [29]. In the same study, Kotanko observed an inverse correlation between muscle mass and ERI specifically in women. In our research, higher fat-free mass was linked to higher ERI in the whole study group, independently of sex (Figures 2A–D and 3B).

4.2.3. ERI and Fluid Status: Total Body Water [%] and Intradialytic Weight Gain

We discovered that the total percentage of body water (TBW%) was positively correlated to ERI value. The authors hypothesize that in patients who are chronically fluid overloaded, greater hemoglobin and hematocrit levels due to hemoconcentration are less likely to be observed, and this may cause a bias on EPO-response assessment. Besides TBW%, we also investigated intradialytic weight gain (IDWG) as total body weight percentage and its relation to ERI, but in our group the correlation was not visible ($\rho = 0.058$; $p = 0.61$); thus, these results should be interpreted with caution. Recently, Gracia-Iguacel et al. found a link between protein-energy wasting, erythropoietin resistance and overhydration [30], concluding that patients who were overhydrated were more prone to develop protein-energy wasting. Furthermore, in the above-mentioned study, the presence of PEW was associated with higher rates of rHuEPO hyporesponsiveness. Protein-energy wasting syndrome in a hemodialyzed individual is associated with loss of muscle and fat mass due to uremia. All this stands in line with what we succeeded to show in our study: patients with low muscle and fat mass are those with higher ERI. In a small study by Hara et al., in which 14 patients on PD were enrolled, the ECW/TBW index was independently associated with ERI [31]. Although the RRT method was different in this paper, we can assume that fluid overload is linked to EPO hyporesponsiveness. Total body water (%) is linked to both fluid status and proportion of fat mass, so this association could be related to either of those factors. Fluid status and EPO response is still not well investigated and needs further studies. Nevertheless, the authors believe that insufficient control of lean body mass might play a significant role in EPO response. Taking all the above into account, repeatable body composition analysis may help achieve better clinical outcomes in patients undergoing RRT (Figure 3C).

4.2.4. ERI and Malnutrition Inflammation Score

In addition to body composition analysis, we used another nutrition assessment tool: the malnutrition–inflammation score by Kalantar-Zadeh et al. [8]. This scale focuses on several elements: patient's medical history in the last 3–6 months, physical exam regarding signs of muscle wasting and subcutaneous fat loss, BMI, and laboratory parameters such as albumin and transferrin. Total score in the MIS can range from 0 to 30 points. In our study, individuals within the highest ERI tercile also had significantly higher median MIS score than those in the lowest tercile (7 vs. 4, respectively). Our findings should be no surprise, as MIS assesses, among others, two elements that are prominent in the presence of protein-energy wasting syndrome: muscle (protein) and fat (energy) loss in an individual. As stated previously, both these factors are linked to higher ERI. Previously, some authors have proved that total MIS score correlates inversely with the severity of anemia and, on the other hand, is positively correlated with total weekly weight-adjusted dose of EPO [32–36] (Figure 2F).

4.2.5. ERI Value and Mortality Rate

In our study, we found no link between ERI value and mortality rate, neither all-cause nor due to cardiovascular events. This stands contrary to other authors' findings, as Lu et al. concluded that patients with higher ERI also had higher rates of all-cause mortality and cardiovascular death [37]. The same conclusions were made by Pan et al. [38]. Bae et al. found ERI value to be a predictor of all-cause mortality in hemodialysis patients, but not in peritoneal dialysis patients [39]. These differences might be due to relatively smaller sample size in comparison to the aforementioned papers, as well as shorter follow-up time; reassessment in larger, multi-center studies would be of immense value.

4.2.6. ERI and IL-6 Serum Concentration

Interleukin-6 is by far one of the most important proinflammatory cytokines, with a vast spectrum of biological effects. It regulates acute phase response and acts mainly in hepatocytes, bone marrow, B-cells, T-cells and fibroblasts [40]. By stimulating hepatocytes,

it promotes hepcidin production and contributes to anemia of chronic inflammation. In our study in univariate analysis, IL-6 did not correlate with the ERI value. IL-6 did not differ between terciles depending on the ERI value. As with the results of our multivariate analysis (Table 2), high IL-6 has shown to be associated with a higher ERI. In a novel clinical trial conducted by Pergola et al., lowering IL-6 level by ziltivekimab, an anti-IL-6 antibody, significantly reduced EPO requirements in HD patients [41]. Similarly, Won et al. found that hemodialyzed individuals within the highest tercile of ERI had significantly higher levels of IL-6 and concluded that IL-6 serum level is a strong predictor of poor ESA response in HD patients [42]. These findings stand in line with our study results.

4.2.7. ERI and Iron-Metabolism Biomarkers: Ferritin, Transferrin and Hepcidin

We also investigated the dynamics of iron metabolism biomarkers in our study group. Patients with an ERI higher than the median, in comparison with patients with an ERI lower than the median, had higher hepcidin and ferritin serum levels as well as lower transferrin serum level. These findings can be easily explained. Hepcidin is a small protein produced by the liver, which plays a key role in iron homeostasis. It is responsible for inhibiting iron release from its storage in the macrophages and inhibits iron absorption from the gut, so its biological effect is the lowering of iron serum levels. Thus, hepcidin is expressed in any state of iron overload, such as hemochromatosis or functional iron deficit (visible in anemia of chronic diseases). Next to ferritin, it is an acute-phase protein, so concentration of both these proteins also increases in the presence of any kind of inflammation [15]. Transferrin level decreases in infection, inflammation and cachexia. These biochemical findings support the theorem that erythropoietin-resistance in ESRD is, among other factors, inflammation-driven (Figure 3D–F).

4.2.8. ERI and Dialysis Adequacy: Kt/V and the Effect of Uremia on EPO Response

Inadequate dialysis is thought to play an important role in erythropoietin response, as the concentration of uremic toxins leads to chronic activation of the inflammatory cascade and various metabolic consequences, with blunted erythropoiesis being one of them. The most commonly used indicator of dialysis adequacy is Kt/V_{urea}, which is a dimensionless measure of urea removal during a single RRT procedure. In this ratio, K stands for dialyzer clearance and represents the volume of blood in milliliters that passes through the dialyzer per minute. K is specific for the dialyzer model. Another factor, *t*, indicates dialysis time in minutes, and V in the denominator represents the volume distribution of urea. It is believed in everyday practice that with greater values of Kt/V, dialysis is more effective. Nevertheless, we should bear in mind that urea, although best known, is not the only toxic molecule that needs to be removed during dialysis: The European Uremic Toxin Work Group described over 140 substances that have a negative impact on biological functions while not excreted by the kidneys [43]. Developing different membranes, dialysis fluids and HD techniques with greater biocompatibility allows clinicians to slightly impede the burden of chronic uremic inflammation. Still, the way to achieve high dialysis adequacy in an individual varies globally, depending on the region's income and public health policy [44]. Previously published studies proved that adequate dialysis (measured by Kt/V) allowed lower rHuEPO doses. Having found this, we further compared Kt/V indexes among groups of different body types based on BMI and BCA (Table 4). In our cohort, obese individuals had lower Kt/V than those classified as "normal" by BMI and "thin" by BCA. These results unfortunately indicate that in our dialysis center, RRT quality for obese patients is not relevant. Hruby and Nowicki suggested that for individuals with greater body weight, better effects can be achieved by increasing blood flow and dialyzer surface [45,46]. Regrettably, our dialysis center does not have access to high-flux dialyzers, so the healthcare policy in our region negatively impacts treatment results and may be a cause of such bias in this study; for patients with lower BMI, our standard low-flux dialyzer may be enough, and the dialyzer surface will also be adequate, but it is insufficient for obese patients. On the other hand, our obese group still had lower ERI despite lower Kt/V.

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Our results considering ERI and Kt/V should therefore be interpreted with caution. Many interesting reports have recently suggested that Kt/V is an obsolete indicator of dialysis adequacy, as it does not take individual patient characteristics (e.g., "other-than-average" body composition) and other uremic solutes into account [47–49]. It is undoubtable that accumulation of other uremic toxins (such as beta-2-microglobulin, IL-6, indoxyl sulfate, *p*-cresyl sulfate, etc.) and not urea alone, accounts for dysregulation of erythropoiesis and malnutrition aggravation in hemodialyzed patients through a variety of mechanisms that are beyond the range of this publication [49,50].

5. Conclusions

Screening for the possible underlying reasons of EPO hyporesponsiveness in a hemodialyzed individual does not require great effort and can be made using simple tools, such as body composition analysis and/or MIS paired with IL-6 serum level testing. Our study further established some previously observed patterns, that generally malnourished patients with chronic inflammation are more prone to develop EPO resistance. The most crucial take-home point from our study is, in our opinion, the importance of adipose tissue in overcoming erythropoietin resistance. Adipocyte-derived leptin is believed to stimulate erythropoiesis and dampen the pro-inflammatory effect of visceral obesity in terms of red blood cell production. Bearing all this in mind, it seems crucial to prevent malnutrition and frailty as a part of a holistic approach to anemia treatment in dialysis patients. Clinicians should take an individualized approach towards hemodialysis techniques for each patient and cooperate with nutritionists and physiotherapists to ensure adequate macro- and micronutrient dietary intake, prevent fatty tissue loss, control fluid overload, reduce oxidative stress and prevent sarcopenia through patient-adjusted physical activity.

6. Strengths

The study group was assessed not only with a nutritional questionnaire, but also with examination of body composition. Erythropoietin resistance was calculated over 6 months of treatment. Follow-up to assess overall mortality lasted 18 months.

7. Limitations

This study involved a relatively small sample size and was a single-center study. Only two associations of ERI (positive with and MIS total score and negative with eGFR) remained significant after Bonferroni correction for multiple testing.

Author Contributions: Conceptualization, E.K. and W.F.; methodology, K.S.; formal analysis, K.S.; resources, K.C.; data curation, K.S.; writing—original draft preparation, W.F.; writing—review and editing, E.K.; visualization, K.S.; supervision, K.C. All authors have read and agreed to the published version of the manuscript.

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

Data Availability Statement: For additional data, please contact ewakwiat@gmail.com.

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

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Załącznik 2 - Malnutrition and Erythropoietin Resistance among Patients with End-Stage Kidney Disease: Where Is the Perpetrator of Disaster? *Nutrients* **2022**, *14*, 5318.

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Malnutrition and Erythropoietin Resistance among Patients with End-Stage Kidney Disease: Where Is the Perpetrator of Disaster?

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Abstract: Background: Hemodialyzed patients with poor erythropoietin response tend to have low volume of visceral adipose tissue and score high on malnutrition-inflammation score. This study investigates in-depth the role of leptin and chosen cytokines in the development of malnutrition-inflammation syndrome (MIS) and erythropoietin resistance. Methods: Eighty-one hemodialyzed patients with erythropoietin-treated anemia were enrolled in the study. Their body composition was measured. Erythropoietin resistance index was calculated. Blood samples for leptin, IL-6, IL-18, TNF-alpha, and IL-1-alpha serum levels were drawn. Results: Leptin showed negative correlation with erythropoietin resistance index (ERI), whilst IL-6 showed the opposite. IL-6 seemed to be linked more to HD parameters and vintage, while TNF-alpha and leptin were more dependent on body composition. IL-18 and IL-1-alpha did not affect nutritional parameters nor ERI. Conclusion: Modulation of adipokine- and cytokine-related signaling is a promising target in tempering malnutrition in hemodialyzed, and thus achieving better outcomes in anemia treatment. Large clinical studies that target the inflammatory response in hemodialysis, especially regarding IL-6, TNF-alpha, and leptin, would be of great worth.

Keywords: malnutrition; dialysis; inflammation; cytokines; adipokines; erythropoietin resistance; obesity

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1. Introduction

Malnutrition is a significant and growing problem among patients treated with hemodialysis, affecting 23–60% of individuals [1–3]. It is linked not only with increased mortality, but also with substantial decrease in quality of life as well [4,5]. Despite technological development giving clinicians better tools to assess nutritional status, such as professional body composition analyzers, proper instruments and interventions to impede the burden of undernourishment in this population are still not well established. A fatal combination of inflammation, hypercatabolism, altered appetite with insufficient food intake, and dialysis-associated factors is thought to play a crucial role in development of malnutrition and its complications [6]. Protein-energy wasting (PEW) relates to the situation in which an individual progressively loses his fat stores, muscle mass and albumin levels in the course of chronic kidney disease (CKD). Malnutrition-inflammation syndrome (MIS) is another, wider expression used to describe nutritional alternations seen in PEW, but with additional impact on oxidative stress and uremic toxins upregulating the inflammatory cascade [7]. MIS is directly linked with erythropoietin hyporesponsiveness in hemodialyzed individuals and makes treatment of anemia, the most common complication of end-stage renal disease (ESRD), very challenging [8,9]. Chronic inflammation

is associated with higher levels of hepcidin, the master regulator of iron metabolism in humans. Hepcidin prevents iron release from the macrophages by degrading ferroportin and contributes to anemia development independently from other factors, such as lower endogenous EPO production in the kidneys [10,11]. We believe that erythropoietin resistance index (ERI) can be used as an additional record while screening for malnutrition. In one of our previous studies, we found that hemodialyzed individuals who responded better to EPO not only scored lower in Kalantar-Zadeh's malnutrition-inflammation score [12], which was mostly consistent with previous data, but also presented with notably higher overall fat mass and visceral fat volume, while muscle mass did not affect ERI [13]. This is why authors hypothesized that adipose tissue in the first place is crucial in preventing EPO hyporesponsiveness and decided to further investigate that. Adipose tissue is known to be hormonally active, releasing adipocytokines, such as leptin, adiponectin, and resistin, which together control energy expenditure and satiety. Nevertheless, its function goes far beyond that and is still being investigated in many fields. It is thought to play role in immune response and erythropoiesis as well [14–17]. In this single-center cohort study, authors aimed to investigate which of the chosen inflammatory markers (leptin, IL-18, IL-6, IL-1α, TNFα) may contribute to malnutrition and its consequences the most, and further reflected upon possible interventions, based on the most up-to-date literature.

2. Materials and Methods

This study obtained approval of the Bioethical Committee of Pomeranian Medical University in Szczecin (KB-0012/88/03/19).

The study focused on patients undergoing renal replacement therapy via hemodialysis in Nephrology Department of The Independent Public Hospital No. 2 in Szczecin, Poland. Time of enrollment was March–June 2020. Detailed group recruitment with baseline and further exclusion criteria is shown below (Figure 1).

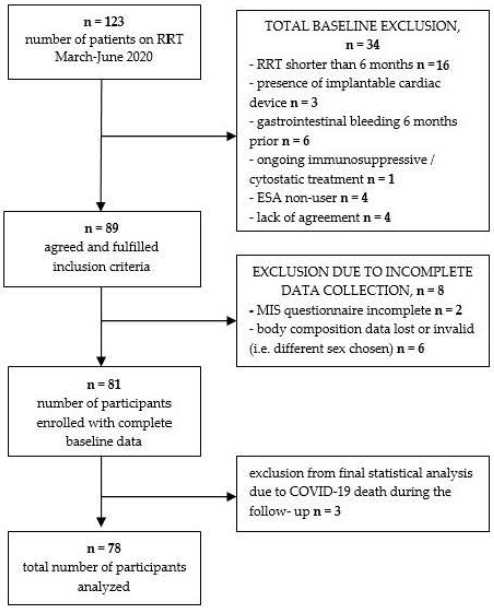

Figure 1. Study group recruitment.

Nutritional assessment was performed two-way. First, body composition analysis of each individual was performed using a professional Medical Body Composition Analyzer by SECA (Seca mBCA 525) following the producer's manual. BCA was made after dialysis session. Seca mBCA 525 uses 8-point bioimpedance to measure body composition parameters. Before each analysis, height, weight and waist circumference had to be entered manually to derive results such as: body mass index (BMI) (kg/m^2), fat free mass (FFM) (%), fat free mass index (FFMI) (kg/m^2), fat mass (FM) (%), fat mass index (FMI) (kg/m^2), total body water (TBW) (%), phase angle (φ) (°), and visceral adipose tissue (VAT) (liters). Taking all these into account, the analyzer assigned each individual into one of four body composition groups: increasing sarcopenic obesity, increasing thinness, increasing muscle mass, and increasing obesity. Then, every individual was scored with a MIS questionnaire [16] which consisted of four parts: (1) patient's related medical history of last 3–6 months (change in end-dialysis dry weight, dietary intake, gastrointestinal symptoms, functional capacity and co-morbidity including years on dialysis), (2) physical examination regarding signs of fat and muscle loss performed by a qualified physician, (3) body mass index, and (4) laboratory parameters (albumin and transferrin). Total MIS score was calculated at the end, with the maximum possible score being 30 points (http://www.touchcalc.com/calculators/mis (accessed on 7 March 2020)).

Blood samples to determine levels of leptin, TNF-alpha, IL-6, IL-1-alpha, and IL-18 were collected during the mid-week hemodialysis session, in addition to routine monthly biochemical workup performed in our Dialysis Center. Each blood sample was centrifuged, divided into two or more (if possible) Eppendorf tubes and frozen at −70 °C. One tube was used for leptin levels ELISA measurement, using a kit from Euroimmun Poland. The second tube was for measuring TNF-alpha, IL-6, IL-1-alpha, and IL-18 levels, using Luminex kits by Biotechne.

The erythropoietin resistance index (ERI) was determined as an average weekly erythropoietin dose/kg body weight/average hemoglobin (g/dL), over the last 6 months.

Mortality was assessed after 18 months of follow-up. The initial number of participants included was 81. Due to SARS-CoV2 spread during the time of the 18-month follow-up, three of the patients enrolled died. The authors excluded them from the final analyses, as little was known about the disease at that time. As it affected mortality in a sudden manner, the authors wanted to preserve the "natural", previously observed mortality pattern in our group of hemodialyzed patients.

Statistical Analysis

Statistical analysis was performed using Statistica 13 software (StatSoft, Tulsa, OK, USA). The Shapiro–Wilk test was used to check whether the distributions of quantitative variables were significantly different from normal ($p < 0.05$). We used non-parametric Mann–Whitney U-test to compare groups. Correlations were studied by means of Spearman's rank correlation coefficient (ρ). Data were described as mean ± SD or median (interquartile range—IQR). p-values were considered significant when <0.05. To find independent determinants of leptin levels, we additionally performed multivariate analysis using the general linear model (GLM) with log-transformed leptin concentration as the dependent variable. Standardized Beta coefficient and its 95% confidence interval (95%CI) was presented for each independent variable. Similar multivariate analysis was performed for log-transformed ERI as the dependent variable. To assess survival, we calculated the total number of patients who died, and among those we extracted individuals who died due to cardiovascular events. Groups of survivors and deceased during the 18-month follow up were compared in terms of body composition and laboratory findings. Power analysis was not performed in the study group.

3. Results

Seventy-eight individuals with complete data were included in the study. All of them were Caucasian, 47 were male (60.3%). Only male/female gender categories were taken into account. Detailed group characteristics can be found in Table 1.

Table 1. Group characteristics.

Overall Participants	n = 78
Male	n = 47 (60.3%)
Age	Median: 65; IQR = 21
Dialysis vintage [months]	Median: 28.5; IQR = 42
HD sessions per week	Median: 3; IQR = 0
ERI [IU/kg/g/dL/week]	Median: 4.9; IQR = 6.8
IL-6 [pg/mL]	Median: 3; IQR = 2.87
TNFα [pg/mL]	Median: 3.49; IQR = 2.17
IL-1α [pg/mL]	Median: 0.69; IQR = 0.55
IL-18 [pg/mL]	Median: 408.08; IQR = 209.03
Albumin [mg/mL]	Median: 41; IQR = 5
Transferrin [g/L]	Median: 1.7; IQR = 0.26
Ferritin [mcg/L]	Median: 475; IQR = 557
Hepcidin [ng/mL]	Median: 92.55; IQR = 108.8
Hemoglobin [mmol/L]	Mean: 6.72 (SD = 0.86)
Iron [mcg/dL]	Median: 65; IQR = 39
Leptin [ng/mL]	Median: 16.36; IQR = 51.77
Uric acid [mg/dL]	Mean: 6.24 (SD = 1.53)
Triglycerides [mg/dL]	Median: 149.5; IQR = 103
Total MIS score	Median: 5; IQR = 5
Kt/V	Mean: 1.14 (SD 0.23)
Patients' nutrition by BMI [%]	underweight 2.6%; normal 26.9%; overweight 42.3%; obese 28.2%
Patients' nutrition by SECA mBCA body composition chart [%]	increasing sarcopenic obesity: 23.2%; increasing obesity: 30.4%; increasing thinness: 17.4%; increasing muscle mass: 29%
eGFR [mL/min/1.73 m^2]	Median: 7; IQR = 4

Abbreviations: IQR—interquartile range, BMI—body mass index, mBCA—medical body composition analyzer, ERI—erythropoietin resistance index, eGFR—estimated glomerular filtration rate, MIS—malnutrition inflammation scale.

3.1. Leptin

In our study, we found statistically significant correlations between circulating leptin levels and the following parameters (Table 2):

Table 2. Association of leptin levels, nutritional parameters and biochemical data; n = 78.

	ρ (Rho)	*p*
leptin & body weight	0.497	<0.001
leptin & BMI	0.652	<0.001
leptin & BSA	0.358	0.001
leptin & MIS total score	−0.271	0.020
leptin & FFM	−0.518	<0.001
leptin & FM	0.518	<0.001
leptin & FMI	0.540	<0.001
leptin & muscle%	−0.425	<0.001
leptin & VAT	0.561	<0.001
leptin & TBW	−0.548	<0.001
leptin & ERI	−0.310	0.006
leptin & Fe	0.262	0.021
Leptin & TG	0.288	0.010
leptin & UA	0.301	0.007

Abbreviations: BMI—body mass index, BSA—body surface area, MIS—malnutrition inflammation scale, FFM—fat free mass, FM—fat mass, FMI—fat mass index, VAT—visceral adipose tissue, TBW—total body water, ERI—erythropoietin resistance index, Fe—iron, TG—triglycerides, UA—uric acid.

In this study group, leptin levels did not correlate in any way with age (ρ = 0.13, $p = 0.258$), phase angle (ρ = 0.23, $p = 0.06$), dialysis vintage (ρ = −0.023, $p = 0.81$), TNF-alpha (ρ = 0.02, $p = 0.85$), IL-6 (ρ = −0.06, $p = 0.62$), IL-1-alpha (ρ = −0.006, $p = 0.95$), IL-18 (ρ = −0.11, $p = 0.33$), hepcidin (ρ = 0.06, $p = 0.61$), eGFR (ρ = 0.03, $p = 0.79$), HD sessions per week (ρ = −0.08, $p = 0.48$), albumin (ρ = 0.17, $p = 0.15$), transferrin (ρ = 0.19, $p = 0.10$), nor ferritin levels (ρ = −0.003, $p = 0.98$) (data not shown in Table 2).

Due to the multitude of statistically significant correlations, we decided to additionally use general linear model to look for independent determinants of leptin level. Leptin level was independently associated with sex (higher in women), uric acid levels, percentage of muscle mass, visceral adipose tissue, and BMI (Table 3).

Table 3. General Linear Model (GLM) analysis of independent determinants of log-transformed leptin levels in terms of body composition and biochemical data; n = 78.

	GLM Model ($p = 0.0092$)			
	p	Beta (β)	−95.00% CI Beta	+95.00% CI Beta
Sex (M vs. F)	0.0001	−0.378	−0.557	−0.198
Age	0.75	−0.025	−0.179	0.130
Uric acid	0.001	0.262	0.110	0.415
Log muscle%	0.025	−0.207	−0.386	−0.027
Log VAT	0.008	0.317	0.087	0.546
Log BMI	0.002	0.327	0.122	0.533

Abbreviations: VAT—visceral adipose tissue, BMI—body mass index.

Authors compared leptin levels between groups of different ERI. Median ERI in our group was 4.89. Individuals with ERI values lower than median had significantly higher leptin levels than those whose ERI ranged above median (Table 4, $p = 0.017$).

Table 4. Comparison of leptin levels among groups with higher and lower erythropoietin resistance index (ERI). Median ERI in the whole study group was 4.89; n = 78.

		Mean	Median	Minimum	Maximum	Lower Quartile	Upper Quartile	IQR	SD
Leptin levels [ng/mL]	ERI lower than median	48.88	33.88	0.96	152.37	9.12	95.45	86.33	45.98
	ERI higher than median	23.47	7.36	0.50	134.68	2.70	22.50	19.80	33.73

Abbreviations: ERI—erythropoietin resistance index, IQR—interquartile range, SD—standard deviation.

According to body composition analyses, authors compared leptin levels in each subgroup. There was no statistically significant difference of leptin levels between "sarcopenic obesity" and "obesity"; "thinness" and "muscle mass" subgroups also were not different. We found differences between:

- "sarcopenic obesity" vs. "thinness", $p = 0.029$ (median: 19.63 ng/mL vs. 5.71 ng/mL)
- "obesity" vs. "muscle mass", $p = 0.0001$ (median: 56.99 ng/mL vs. 8.08 ng/mL)
- "sarcopenic obesity" vs. "muscle mass", $p = 0.026$ (median: 19.63 ng/mL vs. 8.08 ng/mL)
- "obesity" vs. "thinness", $p = 0.0008$ (median: 56.99 ng/mL vs. 5.71 ng/mL)

Median ERI in each subgroup was as follows: sarcopenic obesity—2.8, IQR = 4.215; obesity—2.9, IQR =6.69; thinness—6.01, IQR =8.03; muscle mass—6.5, IQR =7.155.

3.2. *TNF-Alpha*

In our study, we found statistically significant correlations of TNF-alpha levels and following parameters (Table 5):

Table 5. Association of TNFα levels, age, body composition and biochemical markers; n = 78.

	ρ (Rho)	*p*
TNFα & age	0.238	0.039
TNFα & FFM	−0.244	0.047
TNFα & FM	0.244	0.047
TNFα & FMI	0.246	0.045
TNFα & IL1α	0.233	0.043

Abbreviations: FFM—fat free mass, FM—fat mass, FMI—fat mass index.

In this study group, TNF-alpha levels were not associated with MIS total score ($\rho = 0.21$, $p = 0.063$), BMI ($\rho = -0.06$, $p = 0.072$), VAT ($\rho = 0.04$, $p = 0.054$), TBW ($\rho = -0.12$, $p = 0.24$), muscle% ($\rho = -0.16$, $p = 0.068$), phase angle ($\rho = -0.21$, $p = 0.11$), ERI ($\rho = 0.08$, $p = 0.51$), dialysis vintage ($\rho = 0.02$, $p = 0.61$), eGFR ($\rho = -0.19$, $p = 0.29$), HD sessions per week ($\rho = -0.01$, $p = 0.89$), IL-6 ($\rho = 0.16$, $p = 0.22$), IL-18 ($\rho = 0.01$, $p = 0.81$), leptin ($\rho = 0.02$, $p = 0.81$), hepcidin ($\rho = 0.19$, $p = 0.71$), albumin ($\rho = 0.004$, $p = 0.43$), transferrin ($\rho = -0.07$, $p = 0.32$) nor ferritin levels ($\rho = 0.10$, $p = 0.38$) (data not shown in Table 5).

TNF-alpha levels were significantly higher in deceased of any cause during 18-month follow up than in survivor group, $p = 0.019$ (median 4.8 pg/mL vs. 4.4 pg/mL). TNF-alpha levels did not differ between groups of survivors and deceased due to cardiovascular reasons.

While comparing TNF-alpha levels among groups of different body composition, the only difference was seen between "increasing sarcopenic obesity" and "increasing muscle mass" subgroups (median 4.15 pg/mL vs. 3.53 pg/mL; $p = 0.033$).

3.3. *IL-6*

In our study, we found statistically significant correlations between IL-6 levels and the following parameters (Table 6):

Table 6. Association of IL-6 levels, body composition, biochemical parameters and dialysis-related data; n = 78.

	ρ (Rho)	*p*
IL-6 & ERI	0.229	0.046
IL-6 & phase angle	−0.261	0.033
IL-6 & HD sessions/week	0.282	0.014
IL-6 & dialysis vintage	0.229	0.047
IL-6 & IL-1α	0.359	0.002

Abbreviations: ERI—erythropoietin resistance index.

IL-6 level did not correlate with any other body composition parameter besides phase angle. It was not associated with MIS total score (ρ = 0.22, $p = 0.11$), IL-18 (ρ = 0.19, $p = 0.21$), TNF-alpha (ρ = 0.16, $p = 0.22$), leptin (ρ = −0.06, $p = 0.62$), albumin (ρ = −0.15, $p = 0.32$), ferritin (ρ = 0.05, $p = 0.23$), transferrin (ρ = −0.13, $p = 0.18$), hepcidin (ρ = 0.04, $p = 0.06$), nor eGFR (ρ = −0.08, $p = 0.91$). Comparing subgroups in terms of body composition, we did not find a significant difference in IL-6 levels between any of them.

IL-6 was shown to be an independent determinant of ERI value in the general linear model, $p = 0.0069$ (Table 7).

Table 7. General Linear Model (GLM) analysis of independent determinants of log-transformed ERI value in terms of body composition and biochemical data; n = 78.

	GLM Model ($p = 0.009$)			
	p	**Beta (β)**	**−95.00% CI Beta**	**+95.00% CI Beta**
Sex (M vs. F)	0.735	0.037	−0.255	0.181
Age	0.736	0.038	−0.265	0.188
BMI	0.003	0.338	−0.559	−0.116
Log IL-6	0.029	0.248	0.026	0.470

Abbreviations: BMI—body mass index.

3.4. IL-1α and IL-18

In our study group, IL-1α levels did correlate with TNFα (rho = 0.233, $p = 0.043$) and IL-6 (rho = 0.358, $p = 0.002$) levels. IL-1α had no association with ERI (ρ = 0.12, $p = 0.27$), total MIS score (ρ = 0.08, $p = 0.43$), any of the body composition compounds, age (ρ = −0.13, $p = 0.98$), or dialysis-related data, such as dialysis vintage (ρ = 0.22, $p = 0.47$) or HD sessions per week (ρ = −0.14, $p = 0.66$). Levels of this cytokine did not differ significantly between body composition subgroups.

IL-18 did not show any significant correlation with any of the aforementioned parameters at all in our database.

3.5. Association of Studied Cytokines and Body Composition with ERI and MIS Score—Summary

Summary of correlations of studied cytokines with ERI and MIS score can be found below (Tables 8 and 9).

Table 8. Correlations of main cytokines in the study with ERI value.

	ρ	*p*-Value
ERI & TNFα	0.075	0.518
ERI & IL-6	0.229	0.046
ERI & IL-1α	0.128	0.271
ERI & IL-18	0.093	0.424
ERI & leptin	−0.310	0.006

Table 9. Correlations of main cytokines in the study with MIS score.

	ρ	*p*-Value
MIS & TNFα	0.212	0.063
MIS & IL-6	0.216	0.114
MIS & IL-1α	0.009	0.427
MIS & IL-18	0.090	0.641
MIS & leptin	−0.271	0.022

4. Discussion

Inflammation is thought to play a crucial role in the development and progression of numerous diseases, with chronic kidney disease being no exclusion [18]. In patients undergoing hemodialysis, several anomalies can be observed. Due to strict dietary requirements in ESRD and often a lack of clinical dietician support, patients fail to maintain a proper nutritional status. Their fluid balance is hard to control, as well as calorie and protein intake which might be insufficient. Avoiding certain foods, together with impaired appetite in uremic state, makes them prone to develop protein-energy wasting (PEW). The majority of hemodialyzed patients develop a syndrome called MIS (malnutrition-inflammation syndrome) or MIA (malnutrition-inflammation-atherosclerosis). The occurrence of MIS/MIA is known to reduce their quality of life and is linked to greater mortality in this population [19,20]. Inflammation in hemodialyzed may be triggered by uremic toxins [21,22], type of HD access (fistula vs. central venous catheter) [23] as well as underlying conditions. Chronic inflammation can directly affect erythropoiesis and erythropoietin responsiveness, as well as deteriorate level of nutrition [24,25]. In one of our previous studies, we found that erythropoietin resistance index (ERI) is strongly dependent on BMI, fat mass, visceral fat volume, total body water and phase angle, as well as total MIS score and IL-6 levels [13]. That is why authors decided to investigate in-depth how a wider panel of cytokines of choice: IL-6, IL-18, IL-1alpha, TNFalpha, and leptin, which is an adipokine excreted in adipose tissue, influence erythropoietin resistance and nutritional parameters in the population of hemodialyzed patients.

Based on our results, we can clearly see that the only cytokines that affected ERI were IL-6 and leptin. Levels of circulating IL-6 were positively correlated with high ERI, meaning poor erythropoietin response. Leptin levels, on the other hand, showed negative correlation with ERI value, which can indicate a possible protective function of leptin in this case. Moreover, what's worth noticing is that in our group IL-6 levels did not show correlation to MIS total score nor any of the basic body composition parameters, besides phase angle, which is an indicator of cell wall stability [26]. This stands in contrast to some of the previous data, in which IL-6 levels were positively correlated with MIS score and total body water and negatively correlated with fat tissue index and lean tissue index [27]. A two-year observational study by Beberashvili et al. showed that fat mass and phase angle correlated with IL-6 levels at baseline: every 1-pg/mL increase in IL-6 was associated with reductions in fat mass and phase angle, but changes in IL-6 serum levels over two years did not significantly correlate with changes in body composition [28].

In our study, higher concentrations of IL-6 were observed in individuals whose dialysis vintage was longer and who had more HD sessions per week. Thus, we assume that IL-6 is a cytokine which is mostly linked to RRT duration per se and does not relate to body composition parameters a lot. In available literature, a time-dependent increase in IL-6 serum levels of HD patients was also seen [28–30].

IL-6 promotes expression of hepcidin, a ferroportin-degrading molecule, and thus iron release is impeded, preventing proper hematopoiesis. In our opinion, IL-6 serum level reduction is a promising target in overcoming erythropoietin resistance. There are some interesting emerging data regarding this topic. A recent randomized controlled trial of ziltivekimab (NCT02868229), a novel anti-IL-6-ligand antibody, showed that it can significantly reduce EPO requirements in ESRD patients [31]. Another IL-6 inhibitor, tocilizumab, was used to treat inflammation-induced anemia in cancer patients with good clinical effect [32]. Other group of research on reducing IL-6 levels in hemodialyzed is based on choosing adequate dialysis technique. Donati et al. found that asymmetric cellulose acetate (ATA) dialyzers were superior when it comes to removing IL-6 over polymethylmethacrylate (PMMA) dialyzers [33]. A randomized controlled trial by Weiner et al. aimed to examine the efficacy of medium cut-off dialyzers vs. standard high-flux dialyzers in removal of uremic toxins during four- and 24-week period. The MCOD group demonstrated significantly larger reduction ratios for complement factor D, free k light chains, TNF-alpha, and b2-microglobulin ($p < 0.001$ for all), but not for IL-6 [34]. These data support the need for further research into IL-6 signaling blockade as it has potential to improve hematopoiesis. More effective means of blood purification have yet to be implemented to reduce IL-6 levels in hemodialyzed patients. Further research regarding such interventions and their influence on inflammation-driven malnutrition are certainly needed.

As we previously mentioned, in our study group, leptin was negatively correlated with ERI. This adipokine also showed a significant negative correlation with MIS total score, fat-free mass, percentage of muscle mass and total body water. Positive correlations of leptin levels were seen with body weight, BMI, BSA, fat mass, fat mass index, and visceral adipose tissue. Sex, muscle mass, visceral adipose tissue, uric acid levels, and BMI were shown to be independent determinants of leptin levels in our study group (Table 2). Comparing groups in terms of body composition chart placement, leptin levels, and ERI, it can be noted that individuals that were grouped as obese or sarcopenic obese had higher leptin levels and lower ERI than those considered thin or muscular. No correlation with any of the examined cytokines was seen. These data suggest that high leptin levels are generally seen in individuals who are well-nourished and score low on MIS scale. Women tend to have higher leptin levels, which is consistent with available data and is thought to be caused by leptin–estrogen interplay [35,36]. Leptin is an interesting adipokine with a multitude of biological actions. It not only takes part in regulating appetite and energy expenditure, but is also linked to erythropoiesis and bone-mineral metabolism [37,38]. Similar to albumin, it is a reverse-acute phase protein in the population undergoing HD [39]. In a study by Risovic et al., leptin turned out to be associated with two independent determinants of mortality in malnourished and overhydrated patients on HD, and was significantly lower in individuals who died during 12-month follow-up. In this study, patients with low leptin levels also had low BMI, high TBW, low fat tissue index, and low fat free mass index [40,41], which stands in line with our results. In a small study by Rafieian-Kopaei, higher leptin levels were associated with higher hemoglobin and lower EPO requirements in ESRD [39]. Abi et al. studied the association between leptin levels and resting energy expenditure (REE) in a CKD stage 3–5 KDIGO population without hemodialysis and found a positive correlation of leptin and REE independently of sex-energy expenditure was greater with higher leptin levels [36]. Authors of the aforementioned study hypothesized that leptin antagonist or LepR-blocker administration in this population may help ameliorate PEW and cachexia in the course of CKD, just as it did in animal studies [42,43]. In the population of hemodialyzed patients, an interesting

phenomenon of inverse epidemiology seems to relate to leptin levels as well: higher leptin means better nutrition. We suppose it might be due to central or peripheral leptin resistance, which in this case seems to play a protective role against malnutrition and appetite decrease. According to Axelsson, both visceral fat mass and leptin can be a predictor of EPO response in hemodialyzed [44]. Leptin stimulates erythropoiesis by activating bone marrow and spleen HSC niches [38]. Sturzebecher et al. administered leptin to mice with lipodystrophy. In lipodystrophy, due to the inability to store fat in adipose tissue, it is stored in the liver and consequently, causes vascular abnormalities similar to those seen in obesity. In this study, leptin reduced endothelial inflammation by inhibiting endothelial to mesenchymal transformation and reduced vascular leakage [45]. Thus, we hypothesize that it might be one of the protective mechanisms of leptin in our population, although of course animal models cannot be directly addressed to humans. It is possible that either leptin-signaling inhibitors or supraphysiological doses of leptin, together with increased energy intake and/or intradialytic parenteral nutrition [46], could be a strategy to ameliorate MIS and erythropoietin resistance in malnourished HD individuals, but this hypothesis surely needs further studies.

TNF-alpha is another important biomarker of inflammation, often described together with IL-6 as a red flag of uremic milieu. It was primarily thought to reduce appetite, promote muscle protein breakdown and endothelial dysfunction and its elevated levels were linked with age, visceral obesity and overhydration in ESRD [47]. It is still poorly understood whether high TNF-alpha is one of the reasons or rather one of the effects of uremia. Novel data on TNF-alpha and malnutrition are lacking, but in a recent study by Caldiroli et al. higher levels of TNF-alpha were seen in patients with higher MIS scores in univariate analysis [48]. Zhong et al. assessed the level of circulating mitochondrial DNA (MtDNA) and correlated it with TNFalpha and IL-6 levels in maintenance HD patients. Not only were TNFalpha levels higher in ESRD patients comparing to healthy individuals, but it correlated positively with MtDNA copies. MtDNA is released during cell death, so high levels of TNF-alpha can be somehow linked to impaired cell wall stability and necrosis [49]. In our study, we observed a positive correlation of TNF-alpha and age, IL-1alpha, as well as fat mass and fat mass index. It was negatively correlated with fat-free mass. It did not correlate with ERI or MIS score in any way. Additionally, TNF-alpha levels were higher in individuals who died during 18-month follow up. Although in our group TNF-alpha had no relation to MIS score, our findings seem to be rather consistent with the abovementioned data. Based on our results, apart from age—which is unfortunately an unmodifiable factor, TNF-alpha is mostly linked to body composition parameters. As it is negatively associated with fat-free mass, we hypothesize that higher protein intake together with resistance training can be beneficial when it comes to diminishing its systemic effects and might also reduce mortality. Of course, higher levels of TNF-alpha in deceased in our study group might also be linked to their older age (mean: 59 vs. 69 in deceased).

A very worthy, novel clinical trial by Catar et al. (Permeability Enhancement to Reduce Chronic Inflammation-II, NCT02084381) assessed the viability of high-flux vs. medium-cut off dialyzers in removing vascular endothelial growth factor (VEGF) during HD procedure. Not only did this study show the superiority of MCO dialyzers over high-flux, but also identified TNF-alpha as a catalyst for VEGF activation [50]. Thus, modifying dialysis-related parameters can also be a promising direction in repressing TNF-alpha signaling.

IL-1-alpha is also known as "alarmin", as current data support its role in acute phase reaction and cell necrosis. Its elevated levels had previously been described in acute kidney injury with tubular necrosis or ANCA vasculitis [51]. Data on IL-1-alpha utility in chronic kidney disease is limited, but authors chose to assess the levels of this cytokine in this particular study as it was previously shown to promote anorexia in rats by suppressing appetite [52]. IL-6 administration also temporarily increased leptin levels in cancer patients in a dose-dependent manner [53]. Newer data show that higher levels of IL-1-alpha can also be seen in cancer cachexia [54]. Thus, we hypothesized that it could impact nutritional status in hemodialyzed patients, but in our study IL-1-alpha levels showed positive correlation

only with TNF-alpha and IL-6 levels. None of the nutritional or erythropoiesis-related parameters we assessed had any link to IL-1-alpha. Nevertheless, it is worth noting that there are some data on the possible administration of bermekimab, a monoclonal antibody targeting IL-1alpha, in treating cancer cachexia by successfully improving lean mass [55]. The importance of IL-1a signaling in protein-energy wasting and MIS development in end-stage kidney disease still requires further studies.

Last but not least, we chose to assess IL-18 levels, as IL-18 is a part of NRLP3 inflammasome, whose role is currently gaining attention in the development of chronic kidney disease and its complications [56–59]. Bi et al. showed that higher IL-18 levels are linked to severity of PEW measured by albumin and pre-albumin levels in hemodialyzed [60]. In a study by Pourhassan et al., elevated levels of IL-18 were independently linked to decreased appetite in hospitalized patients [61]. Similar conclusions were drawn by Francesconi et al. in an animal model, where the administration of recombinant IL-18 inhibited food intake in mice for at least 6 h and had an anorexigenic effect by acting on neurons of the bed nucleus of the stria terminalis (BST) [62]. Chang stated that increase in IL-18 can be a predictor of cardiovascular events in hemodialyzed patients but emphasized that various chronic diseases can interfere with the result [56]. In some studies, elevated level of IL-18 was paired with increase in TNF-alpha in chronic kidney disease [59,63]. In our study group, IL-18 showed no significant correlation with any of the anthropometric or biochemical parameters. There are currently two substances available targeting IL-18 signaling: a monoclonal antibody GSK1070806 and a recombinant human endogenous IL-18 inhibitor, Tadekinig Alfa. Those were already investigated in delayed graft function, type 2 DM, Crohn's disease, and Still's disease [59]. Although our study did not elucidate the role of IL-18 in the development of malnutrition and erythropoietin resistance in ESRD, we are sure it might be an interesting area for our future, larger studies.

5. Conclusions

Inflammation is an important contributor to the development of malnutrition and erythropoietin resistance in hemodialyzed individuals. Due to the multitude of inflammatory markers that can exacerbate MIS, multidirectional possible interventions need to be taken into account. Those can include: (1) modulation of adipokine- and cytokine-related signaling, (2) improving dialysis quality by choosing different membranes which would be more effective in removing uremic toxins, and (3) nutritional counselling [64]. Large clinical studies that target the inflammatory response in hemodialyzed patients, especially IL-6, TNF-alpha, and leptin, would be of great worth.

6. Limitations

This study relied on a relatively small sample size, as it was undertaken in a single dialysis center. Power analysis for such sample was not performed. Authors believe that repetitive blood sampling for cytokines and leptin levels during follow up, paired with body composition analysis, could add more valuable data concerning the dynamics of MIS and erythropoietin resistance development. Unfortunately, this could not be performed due to a lack of funds.

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12. OŚWIADCZENIA WSPÓŁAUTORÓW PUBLIKACJI

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Malnutrition and Erythropoietin Resistance among Patients with End-Stage Kidney Disease: Where Is the Perpetrator of Disaster? Nutrients 2022, 14, 5318. https://doi.org/10.3390/nu14245318

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How Is Body Composition and Nutrition Status Associated with Erythropoietin Response in Hemodialyzed Patients? A Single-Center Prospective Cohort Study. J. Clin. Med. 2022, 11, 2426. https://doi.org/10.3390/ jcm11092426

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