Uniwersytet Warmińsko-Mazurski
w Olsztynie
Collegium Medicum
Wydział Lekarski

BIAŁKA USP18 I DKK3 W CHOROBACH NEREK I UKŁADU SERCOWO-NACZYNIOWEGO

Paulina Dziamałek-Macioszczyk

ROZPRAWA NA STOPIEŃ DOKTORA NAUK MEDYCZNYCH

Promotor: Prof. dr hab. n. med. Tomasz Stompór
Katedra Chorób Wewnętrznych

Olsztyn 2023

SPIS TREŚCI

WYKAZ PRAC WCHODZĄCYCH W SKŁAD ROZPRAWY 2

WPROWADZENIE 3

CELE PRACY 6

STRESZCZENIE PREZENTOWANYCH PRAC 7

WNOSKI Z PREZENTOWANYCH PRAC 9

WYKAZ CYTOWANEGO PIŚMIENNICTWA 10

PODZIĘKOWANIA 12

THE LIST OF PAPERS FOR PhD DISSERTATION 13

INTRODUCTION 14

STUDY OBJECTIVE 16

SUMMARY OF PhD DISSERTATION'S RESEARCH ARTICLES 17

CONSLUSIONS OF PRESENTED PAPERS 19

REFERENCES 20

OŚWIADCZENIA WSPÓŁAUTORÓW PUBLIKACJI 22

WYKAZ PRAC WCHODZĄCYCH W SKŁAD ROZPRAWY

1. **Paulina Dziamałek-Macioszczyk**, Joanna M. Harazny, Norbert Kwella, Paweł Wojtacha, Susanne Jung, Thomas Dienemann, Roland E. Schmieder, Tomasz Stompór: **Relationship Between Ubiquitin-Specific Peptidase 18 and Hypertension in Polish Adult Male Subjects: A Cross-Sectional Pilot Study**. Med Sci Monit 2020; 26: e921919.
   DOI: 10.12659/MSM.921919
   - praca doświadczalna –

Czasopismo umieszczone na Liście Filadelfijskiej, wskaźnik IF ISI: **2.649**
Punktacja MNiSW: **140.000**

2. **Paulina Dziamałek-Macioszczyk**, Agata Winiarska, Anna Pawłowska, Paweł Wojtacha, Tomasz Stompór: **Patterns of Dickkopf-3 Serum and Urine Levels at Different Stages of Chronic Kidney Disease**. J. Clin. Med 2023; 12: 4750.
   DOI: 10.3390/jcm12144705
   - praca doświadczalna –

Czasopismo umieszczone na Liście Filadelfijskiej, wskaźnik IF ISI: **3.900**
Punktacja MNiSW: **140.000**

3. **Paulina Dziamałek-Macioszczyk**, Joanna M. Harazny, Tomasz Stompór: **Versatility of USP18 in physiology and pathophysiology**. Acta Biochim Pol 2019; 66: 389–392.
   DOI: 10.18388/abp.2019_2844
   - praca przeglądowa –

Czasopismo umieszczone na Liście Filadelfijskiej, wskaźnik IF ISI: **1.420**
Punktacja MNiSW: **70.000**

Łączny IF prezentowanych prac: 7.969

WPROWADZENIE

Przewlekła choroba nerek (PChN) dotyczy 11.7-15.1% światowej populacji i z biegiem lat odsetek ten systematycznie rośnie. Do najpoważniejszych w skutkach powikłań PChN należą te obejmujące układ sercowo-naczyniowy [1]. Przeprowadzono wiele badań nad patogenezą powikłań sercowo-naczyniowych w PChN, lecz mechanizmy te wciąż nie są do końca poznane. W ostatnich latach zdefiniowano nowe grupy białek biorących udział w procesach przewlekłego uszkodzenia nerek i mięśnia sercowego. Należą do nich białka z rodzin USP oraz Dkk.

Rodzina peptydaz specyficznych dla ubikwityny (*ubiquitin-specific peptidases* - USPs) to grupa białek odpowiedzialnych za odłączanie ubikwityny i podobnych do niej cząstek (*ubiquitin-like molecules* - UbLs) od znakowanych substratów. Peptydazy te regulują m.in.: cykl komórkowy, reakcję na czynniki stresowe, dokomórkowe przekazywanie sygnałów oraz odpowiedź immunologiczną [2]. Jednym z białek należących do tej grupy jest USP18 (*ubiquitin-specific peptidase 18*).

USP18 to białko wewnątrzkomórkowe, którego ekspresja jest największa w komórkach wątroby, śledziony i grasicy. Jego synteza jest pobudzana przez interferony, zakażenia wirusowe, lipopolisacharydy ściany bakteryjnej, TNF-α oraz stres genotoksyczny [3]. Złożona rola USP18 wynika z jego podwójnej natury. Z jednej strony spełnia ono funkcję enzymatyczną, polegającą na usuwaniu cząstki ubikwityno-podobnej ISG15 (*interferon-stimulated gene 15*) ze znakowanych nią białek w procesie zwanym „deISGlacją" (*deISGylation*) oraz samej ubikwityny w procesie deubikwitynacji. Z drugiej strony, pełni rolę nieenzymatyczną, związaną z hamowaniem aktywności interferonów poprzez oddziaływanie z receptorem IFNAR2 i blokowaniem aktywacji szlaku sygnałowego JAK-STAT [4].

Opisano rolę USP18 w patogenezie wielu stanów chorobowych. Należą do nich: odpowiedź na infekcje wirusowe, proces nowotworzenia czy rozwój chorób z autoagresji (m.in. cukrzycy typu 1, tocznia rumieniowatego układowego czy stwardnienia rozsianego) [5, 6, 7]. Natomiast w przypadku defektów (mutacji) w obrębie genu kodującego to białko dochodzi do rozwoju chorób genetycznych, takich jak zespół Di George'a oraz pseudo-TORCH [8].

Ponadto udowodniono udział USP18 w rozwoju niewydolności serca oraz w procesach związanych z remodelingiem mięśnia sercowego. Doświadczenia przeprowadzone u myszy transgenicznych wykazały, że zwiększona ekspresja USP18 w komórkach mięśnia sercowego w sytuacji zwiększonego obciążenia następczego ograniczała przerost kardiomiocytów, patologiczne włóknienie oraz opóźniała rozwój niewydolności serca. Z kolei *knock-out* genu USP18 wiązał się nasileniem patologicznej przebudowy serca. USP18 okazało się modulatorem szlaku sygnałowego TAK1 – p38/JNK1/2 [4]. Czyni to z niego potencjalny czynnik ochronny dla kardiomiocytów, mogący w przyszłości odegrać rolę w terapii niewydolności serca wynikającej ze zwiększonego *afterload*.

Kolejna interesująca grupa białek to rodzina glikoprotein sekrecyjnych *Dickkopf* (Dkk). Są one regulatorami szlaku sygnałowego Wnt. Jednym z jej przedstawicieli jest *Dickkopf-3* (Dkk3), białko, które aktywuje bądź hamuje kanoniczny szlak Wnt/β-katenina w zależności od kontekstu fizjologicznego lub patofizjologicznego i rodzaju tkanki [9]. Ekspresja Dkk3 zachodzi m. in. w oku, sercu, wątrobie, macicy, łożysku, komórkach β trzustki [10].

Do supresji syntezy Dkk3 dochodzi w różnych typach nowotworów, natomiast jego nasilona ekspresja związana jest hamowaniem rozwoju guza i apoptozą komórek nowotworowych [11]. Ponadto Dkk3 odgrywa rolę w procesie aterogenezy oraz w rozwoju chorób metabolicznych i sercowo-naczyniowych [12]. Zmniejszoną ekspresję Dkk3 wykazano w sercu myszy z przerostem *myocardium* wywołanym zwiększonym obciążeniem następczym oraz u pacjentów z zaawansowaną niewydolnością serca i po zawale. U myszy bez genu Dkk3 dochodziło do przerostu kardiomiocytów, natomiast u myszy ze zwiększoną jego ekspresją - do zahamowania tego procesu w sytuacji zwiększonego obciążenia. Mechanizmem działania białka jest hamowanie szlaku ASK1–JNK/p38, co wskazuje podobieństwo do USP18 [13].

Inny intrygujący aspekt dotyczący Dkk3 to jego rola w patogenezie chorób nerek. Okazało się bowiem, że jest wydzielaną przez cewki nerkowe glikoproteiną, której synteza pobudzana jest przez różne czynniki uszkadzające. Poprzez aktywację szlaku Wnt/β-katenina Dkk3 nasila włóknienie tego narządu. Badania dowiodły, że stężenie Dkk3 w moczu koreluje z nasileniem zaniku cewek i włóknieniem śródmiąższu nerek [14, 15]. Ponadto, opisano Dkk3 jako czynnik prognostyczny w przewidywaniu krótkoterminowego spadku szacowanego wskaźnika filtracji kłębuszkowej (*estimated*

glomerular filtration rate – eGFR) oraz odległej progresji PChN niezależnie od przyczyny [14, 16, 17]. Daje to nadzieję na wykorzystanie tego białka jako wskaźnika stopnia zwłóknienia nerek w ich przewlekłym uszkodzeniu oraz potencjalny biomarker w monitorowaniu odpowiedzi na terapię chorób nerek.

Przeprowadzenie badań w zakresie roli USP18 i Dkk3 w procesach uszkadzających nerki oraz układ sercowo-naczyniowy umożliwi lepsze poznanie podstaw patofizjologicznych PChN i chorób układu sercowo-naczyniowego towarzyszących PChN. Badania także mogą stać się ważnym krokiem w ocenie znaczenia USP18 i Dkk3 jako nowych biomarkerów w PChN czy niewydolności serca.

CELE PRACY

1. Analiza zależności między stężeniem USP18 w surowicy a występowaniem nadciśnienia tętniczego u nieleczonych pacjentów oraz ocena związku USP18 z czynnikami ryzyka sercowo-naczyniowego.
2. Ocena zależności między stężeniem Dkk3 w surowicy i moczu pacjentów w różnych stadiach PChN a klinicznymi, laboratoryjnymi i histopatologicznymi wskaźnikami aktywności chorób nerek.
3. Ocena białek USP18 i Dkk3 jako potencjalnych biomarkerów uszkodzenia nerek i serca.

STRESZCZENIE PREZENTOWANYCH PRAC

W pracy: "*Relationship Between Ubiquitin-Specific Peptidase 18 and Hypertension in Polish Adult Male Subjects: A Cross-Sectional Pilot Study,* Med Sci Monit 2020, 26: e921919" przedstawiono analizę wartości stężeń USP18 w surowicy u 79 mężczyzn w wieku 51 ± 11 lat (średnia ± odchylenie standardowe) podzielonych na trzy grupy na podstawie wartości ciśnienia tętniczego. Grupa badana była częścią grupy mężczyzn uczestniczących w projekcie ProM, prowadzonym przez Klinikę Nefrologii, Hipertensjologii i Chorób Wewnętrznych oraz Katedrę Patofizjologii Uniwersytetu Warmińsko-Mazurskiego w Olsztynie w latach 2014-2017. Wydzielono grupę bez nadciśnienia tętniczego (n=29), z izolowanym nadciśnieniem rozkurczowym (n=20) oraz nadciśnieniem tętniczym skurczowo-rozkurczowym (n=30). Wybór takiego kryterium podziału podyktowany był faktem, że nadciśnienie tętnicze jest jednym z głównych czynników zwiększających obciążenie następcze serca i długoterminowo przyczynia się do rozwoju niewydolności serca. Uczestnicy badania nie mieli stwierdzonych chorób przewlekłych oraz nie przyjmowali żadnych leków. Poza oceną stężenia USP18 w surowicy metodą ELISA, wykonano podstawowe badania biochemiczne moczu i surowicy.

Stwierdzono istotne różnice w stężeniu USP18 między grupami. W grupie bez nadciśnienia tętniczego średnie stężenie USP18 oraz 95% przedział ufności wyniosły 69.3 (22.1-116.5) pg/ml, w grupie z nadciśnieniem rozkurczowym 90.1 (29.0-151.3) pg/ml, natomiast w grupie z nadciśnieniem skurczowo-rozkurczowym 426.7 (163.1-690.3) pg/ml. Średnia wartość w grupie z nadciśnieniem tętniczym skurczowo-rozkurczowym była 6.2 razy większa niż w grupie bez nadciśnienia (p=0.03) oraz 4.7 razy większa niż w grupie z izolowanym nadciśnieniem rozkurczowym (p=0.19). Po uwzględnieniu wpływu typowych czynników ryzyka sercowo-naczyniowego (wiek, BMI, cholesterol całkowity, trójglicerydy, glukoza) wykazano istotną niezależną korelację między stężeniem USP18 a skurczowym ciśnieniem tętniczym (r=0.31, p=0.008), ciśnieniem tętna (r=0.37, p=0.002) oraz częstością rytmu serca (r=0.24, p=0.04). Są to parametry świadczące o zwiększonej aktywności układu współczulnego (skurczowe ciśnienie tętnicze i częstości rytmu serca) oraz pośrednio o zwiększonej sztywności naczyń krwionośnych (ciśnienie tętna). W obu przypadkach są to czynniki negatywnie wpływające na pracę mięśnia sercowego. Nie znaleziono korelacji między USP18 a wskaźnikiem albumina/kreatynina w moczu (UACR), czyli badaniem

powszechnie stosowanym w ocenie stopnia uszkodzenia nerek oraz dysfunkcji śródbłonka. We wszystkich grupach wartości UACR pozostawały w normie.

Na podstawie powyższych wyników postawiono hipotezę, że do wzrostu stężenia USP18 dochodzi w przypadku zwiększenia obciążenia lewej komory serca oraz subklinicznego uszkodzenia kardiomiocytów.

W badaniu przedstawionym w artykule: ”*Patterns of Dickkopf-3 Serum and Urine Levels at Different Stages of Chronic Kidney Disease*, J. Clin. Med. 2023, 12, 4750” oceniono stężenie białka Dkk3 w surowicy i moczu u 100 pacjentów w wieku 53 ± 17 lat. Grupę podzielono na 5 podgrup (w każdej n=20) na podstawie stopnia zaawansowania PChN (46% stanowiły kobiety): z prawidłową funkcją nerek (kontrola), z potwierdzonym biopsyjnie kłębuszkowym zapaleniem nerek z białkomoczem lub aktywnym osadem moczu z zachowaną filtracją kłębuszkową, ze stabilną PChN z obniżoną filtracją kłębuszkową, grupę pacjentów dializowanych oraz chorych po przeszczepieniu nerki w okresie stabilnej funkcji graftu. Wykonano podstawowe badania biochemiczne surowicy i moczu, ocenę biochemiczną parametrów sercowych, oznaczenie stężeń Dkk3 w surowicy i moczu metodą ELISA oraz ocenę prędkości fali tętna.

Najistotniejszą obserwacją w badaniu było najwyższe stężenie Dkk3 w moczu odniesione do stężenia kreatyniny w moczu u pacjentów ze stabilną PChN (7050 pg/mg), które znacznie przewyższało wartości w innych grupach. Ponadto, stężenie Dkk3 w moczu w grupie po przeszczepieniu nerki (5710 pg/mg) było dwukrotnie wyższe niż w populacji bez chorób nerek (2650 pg/mg) oraz grupie z kłębuszkowym zapaleniem nerek (2470 pg/mg), pomimo tego że we wszystkich tych grupach filtracja kłębuszkowa nie była upośledzona. Innym ważnym spostrzeżeniem była korelacja między Dkk3 w moczu a stężeniem kreatyniny w surowicy jedynie w grupach pacjentów ze stabilną PChN ($r=0.41$, $p=0.009$) oraz po przeszczepieniu nerki ($r=0.39$, $p=0.043$), zatem w grupach z najbardziej zaawansowaną chorobą nerek (w grupie dializowanych z uwagi na anurię moczu nie badano).

W pracy przeglądowej: ”*Versatility of USP18 in physiology and pathophysiology*, Acta Biochim Pol 2019; 66: 389–392” skupiono się na omówieniu budowy, mechanizmów działania oraz roli białka USP18 w odpowiedzi na czynniki infekcyjne, w procesie karcynogenezy, w rozwoju chorób z autoagresji oraz remodelingu mięśnia sercowego.

WNOSKI Z PREZENTOWANYCH PRAC

1. Wzrost stężenia USP18 w surowicy prawdopodobnie wynika bezpośrednio z przeciążenia lewej komory serca i może odzwierciedlać zainicjowane nim procesy naprawcze na poziomie komórkowym.
2. USP18 może być bardzo czułym i wczesnym biomarkerem uszkodzenia *myocardium*. Sugeruje to wzrost stężenia USP18 w surowicy wyprzedzający zmiany w wydalaniu albuminy z moczem.
3. Stężenie Dkk3 w moczu koreluje ze stopniem uszkodzenia nerek.
4. Wysokie stężenie Dkk3 w grupie ze stabilną PChN może wynikać z nasilonego uszkodzenia cewek oraz zaawansowanego stopnia włóknienia narządu. W grupie po przeszczepieniu mechanizm prawdopodobnie jest inny (eGFR w tej grupie wynosił >60 ml/min) i być może odzwierciedla ryzyko wystąpienia ostrego uszkodzenia nerek lub progresji PChN w przyszłości.
5. Wykonane badania potwierdzają potencjalną rolę białek USP18 i Dkk3 jako biomarkerów uszkodzenia odpowiednio serca i nerek.
6. Kontynuacja badań pozwoli ocenić rolę USP18 w uszkodzeniu nerek, a Dkk3 w uszkodzeniu mięśnia sercowego i porównać je ze sobą. Będzie to kolejny krok w poznaniu patofizjologii schorzeń obu tych narządów, który może utorować drogę do korzystania z powyższych biomarkerów w codziennej praktyce.

WYKAZ CYTOWANEGO PIŚMIENNICTWA

1. Lv JC, Zhang LX. Prevalence and Disease Burden of Chronic Kidney Disease. Adv Exp Med Biol 2019;1165: 3-15.

2. Catic A, Fiebiger E, Korbel GA, Blom D, Galardy PJ, Ploegh HL. Screen for ISG15-crossreactive deubiquitinases. PLoS One 2007; 2(7): e679.

3. Honke N, Shaabani N, Zhang DE, Hardt C, Lang KS. Multiple functions of USP18. Cell Death Dis 2016; 7(11): e2444.

4. Ying X, Zhao Y, Yao T, Yuan A, Xu L, Gao L, Ding S, Ding H, Pu J, He B. Novel protective role for ubiquitin-specific protease 18 in pathological cardiac remodeling. Hypertension 2016; 68: 1160-1170.

5. Makki MS, Ruteshouser EC, Huff V. Ubiquitin specific protease 18 (Usp18) is a WT1 transcriptional target. Exp Cell Res 2013; 319(5): 612-622.

6. Coit P, Jeffries M, Altorok N, Dozmorov MG, Koelsch KA, Wren JD, Merrill JT, McCune WJ, Sawalha AH. Genome-wide DNA methylation study suggests epigenetic accessibility and transcriptional poising of interferon-regulated genes in naive CD4+ T cells from lupus patients. J Autoimmun 2013; 43: 78-84.

7. Honke N, Shaabani N, Zhang DE, Iliakis G, Xu HC, Häussinger D, Recher M, Löhning M, Lang PA, Lang KS. Usp18 driven enforced viral replication in dendritic cells contributes to break of immunological tolerance in autoimmune diabetes. PLoS Pathog 2013; 9(10): e1003650.

8. Meuwissen ME, Schot R, Buta S, Oudesluijs G, Tinschert S, Speer SD, Li Z, van Unen L, Heijsman D, Goldmann T, Lequin MH, Kros JM, Stam W, Hermann M, Willemsen R, Brouwer RW, Van IJcken WF, Martin-Fernandez M, de Coo I, Dudink J, de Vries FA, Bertoli Avella A, Prinz M, Crow YJ, Verheijen FW, Pellegrini S, Bogunovic D, Mancini GM. Human USP18 deficiency underlies type 1 interferonopathy leading to severe pseudo-TORCH syndrome. J Exp Med 2016; 213(7): 1163-1174.

9. Schunk SJ, Floege J, Fliser D, Speer T. WNT–β-catenin signalling - a versatile player in kidney injury and repair. Nature 2021; 17: 172-184.

10. Ludwig J, Federico G, Prokosch S, Küblbeck G, Schmitt S, Klevenz A, Gröne HJ, Nitschke L, Arnold B. Dickkopf-3 acts as a modulator of B cell fate and function. J Immunol 2015; 194(6):2624-34.

11. Niehrs, C. Function and biological roles of the Dickkopf family of Wnt modulators. Oncogene 2006; 25: 7469-7481.

12. Cheng WL, Yang Y, Zhang XJ, Guo J, Gong J, Gong FH, She Z K, Huang Z, Xia H, Li H. Dickkopf-3 Ablation Attenuates the Development of Atherosclerosis in ApoE-Deficient Mice. J Am Heart Assoc 2017; 6(2): e004690.

13. Zhang Y, Liu Y, Zhu XH, Zhang XD, Jiang DS, Bian ZY, Zhang XF, Chen K, Wei X, Gao L, Zhu LH, Yang Q, Fan GC, Lau WB, Ma X, Li H. Dickkopf-3 attenuates pressure overload-induced cardiac remodelling. Cardiovas Res 2014; 102.1: 35-45.

14. Schunk SJ, Speer T, Petrakis I, Fliser D. Dickkopf 3—a novel biomarker of the 'kidney injury continuum'. Nephrol Dial Transplant 2021; 36: 761-767.

15. Federico G, Meister M, Mathow D, Heine GH, Moldenhauer G, Popovic ZV, Nordström V, Kopp-Schneider A, Hielscher T, Nelson PJ, Schaefer F, Porubsky S, Fliser D, Arnold B, Gröne HJ. Dickkopf-3 promotes the development of renal atrophy and fibrosis. JCI Insight 2016; 1(1): e84916.

16. Zewinger S, Rauen T, Rudnicki M, Federico G, Wagner M. Dickkopf-3 (DKK3) in Urine Identifies Patients with Short-Term Risk of eGFR Loss. J Am Soc Nephrol 2018; 29: 2722-2733.

PODZIĘKOWANIA

Składam serdeczne podziękowania:

Mojemu promotorowi, **prof. dr hab. n. med. Tomaszowi Stomporowi** za inspirację, motywację oraz nieocenioną pomoc na każdym etapie realizacji niniejszej pracy;

Dr hab. n. med. Joannie Harazny za umożliwienie wzięcia udziału w Projekcie ProM oraz pomoc przy przygotowaniu publikacji;

Koleżankom z Oddziału Klinicznego Nefrologicznego, Hipertensjologii i Chorób Wewnętrznych WSS w Olsztynie za pomoc przy rekrutacji pacjentów;

Specjalne podziękowania kieruję do mojego męża – **dr. inż. Jana Macioszczyka,** za pomoc przy opracowywaniu wyników badań, za stworzenie ilustrujących je grafik, a przede wszystkim za motywację i wsparcie, za które dziękuję również Rodzicom i Babci.

THE LIST OF PAPERS FOR PhD DISSERTATION

1. **Paulina Dziamałek-Macioszczyk**, Joanna M. Harazny, Norbert Kwella, Paweł Wojtacha, Susanne Jung, Thomas Dienemann, Roland E. Schmieder, Tomasz Stompór: **Relationship Between Ubiquitin-Specific Peptidase 18 and Hypertension in Polish Adult Male Subjects: A Cross-Sectional Pilot Study**. Med Sci Monit 2020; 26: e921919.
   DOI: 10.12659/MSM.921919
   – research article –

ISI Master Journal List, IF: **2.649**

MNiSW: **140.000**

2. **Paulina Dziamałek-Macioszczyk**, Agata Winiarska, Anna Pawłowska, Paweł Wojtacha, Tomasz Stompór: **Patterns of Dickkopf-3 Serum and Urine Levels at Different Stages of Chronic Kidney Disease**. J. Clin. Med 2023; 12: 4750.
   DOI: 10.3390/jcm12144705
   – research article –

ISI Master Journal List, IF: **3.900**

MNiSW: **140.000**

3. **Paulina Dziamałek-Macioszczyk**, Joanna M. Harazny, Tomasz Stompór: **Versatility of USP18 in physiology and pathophysiology**. Acta Biochim Pol 2019; 66: 389–392.
   DOI: 10.18388/abp.2019_2844
   – review article –

ISI Master Journal List, IF: **1.420**

MNiSW: 70.000

Total IF of PhD dissertation: 7.969

INTRODUCTION

Chronic kidney disease (CKD) affects 11.7-15.1% of the world's population and over the years this number is consistently growing. The most serious CKD complications include those affecting cardiovascular system [1]. Many studies have been conducted on the pathogenesis of cardiovascular complications in CKD, but these mechanisms are still not fully understood. New groups of proteins involved in the kidney and heart damage have been recently identified. These include proteins from USP and Dkk families.

Ubiquitin-specific peptidases (USPs) family is a group of proteins which cleave ubiquitin and particles similar to ubiquitin (ubiquitin-like molecules - UbLs) from their substrates. These peptidases regulate, among others: a cell cycle, response to stress factors, transmission of intracellular signals and immune response [2]. One of the members of this group is USP18 (ubiquitin-specific peptidase 18).

USP18 is an intracellular protein highly expressed in the liver, spleen and thymus. Its synthesis is stimulated by interferons (IFNs), viral infections, bacterial wall lipopolysaccharides, TNF-α and genotoxic stress [3]. Complex role of USP18 results from its dual nature. On the one hand, it executes an enzymatic function by removing the ubiquitin-like particle ISG15 (interferon-stimulated gene 15) from tagged proteins in a process called "deISGylation" and ubiquitin itself in the deubiquitination process. On the other hand, it plays a non-enzymatic roles, related to IFNs inhibition by interacting with the IFNAR2 receptor and blocking the activation of the JAK-STAT signalling pathway [4].

USP18 is involved in the development of many pathologies, such as: response to viral infections, carcinogenesis and development of autoimmune diseases (including type 1 diabetes, systemic lupus erythematosus or multiple sclerosis) [5, 6, 7]. Defects (mutations) of the gene encoding USP18 lead to genetic disorders such as Di George and pseudo-TORCH syndromes [8].

Interestingly, another role of USP18 is its contribution to the cardiac muscle remodelling process and development of heart failure. Experiments performed in transgenic mice demonstrated that increased USP18 expression in cardiomyocytes in upon increased afterload attenuated cardiomyocyte hypertrophy and myocardial fibrosis, and delayed the development of heart failure. Knock-out of the USP18 gene was associated with increased pathological remodelling of the heart. USP18 turned out to be a modulator of the TAK1–p38/JNK1/2 signalling pathway [4]. It makes USP18 a potential protective factor for

cardiomyocytes, which may play a role in therapy of heart failure resulting from increased afterload.

Another interesting group of proteins is the Dickkopf (Dkk) family. Dkks are secreted glycoproteins which regulate the Wnt signalling pathway. One of their representatives includes Dickkopf-3 (Dkk3), which activates or inhibits the canonical Wnt/β-catenin pathway depending on the tissue context [9]. Dkk3 is expressed, among others, in the eye, heart, liver, uterus, placenta, and pancreatic β cells [10].

Dkk3 synthesis is suppressed in various types of cancers, while its increased expression is associated with inhibition of carcinogenesis and apoptosis of cancer cells [11]. Moreover, Dkk3 is involved in atherogenesis and development of metabolic and cardiovascular diseases [12]. Decreased Dkk3 expression was demonstrated in the hearts of mice with myocardial hypertrophy induced by increased afterload and in patients with advanced heart failure and after myocardial infarction. In mice exposed to increased afterload cardiomyocyte hypertrophy developed following the Dkk3 gene knock-out, while in mice with increased Dkk3 expression this process was inhibited. Such an inhibition resulted from the ASK1–JNK/p38 pathway inhibition, which suggests similarity to USP18 [13].

Another intriguing aspect of Dkk3 is its role in pathogenesis of kidney disease. Dkk3 turned out to be a glycoprotein secreted by renal tubules upon exposure to several injuring factors, which induces renal fibrosis by the Wnt/β-catenin pathway activation. Various studies have shown that urine Dkk3 concentration correlates with severity of tubular atrophy and fibrosis of renal interstitium [14, 15]. Moreover, Dkk3 has been described as a prognostic factor in a short-term estimated glomerular filtration rate (eGFR) loss and long-term CKD progression regardless of the underlying cause [14, 16, 17]. It makes urinary Dkk3 an early indicator of fibrosis in chronic kidney damage and a potential biomarker in assessment of response to therapy.

Research on roles of USP18 and Dkk3 in pathologies involving kidneys and cardiovascular system will ensure better understanding of the underlying pathophysiological mechanisms leading to CKD and cardiovascular complications of CKD. This research may also become a significant step in assessing importance of USP18 and Dkk3 as new biomarkers in CKD and heart failure.

STUDY OBJECTIVE

1. Analysis of the relationship between serum USP18 concentration and incidence of arterial hypertension in patients which are naïve to treatment. Assessment relationship between serum USP18 and risk factors of cardiovascular disease.
2. Analysis of the relationship between Dkk3 concentration in serum and urine of patients at different stages of CKD and clinical, laboratory and histopathological indicators of kidney disease activity.
3. Assessment of USP18 and Dkk3 proteins as potential biomarkers of kidney and heart damage.

SUMMARY OF PhD DISSERTATION'S RESEARCH ARTICLES

The paper: "Relationship Between Ubiquitin-Specific Peptidase 18 and Hypertension in Polish Adult Male Subjects: A Cross-Sectional Pilot Study, Med Sci Monit 2020, 26: e921919" contains an analysis of serum USP18 concentration in 79 men aged 51 ± 11 years (mean ± standard deviation) divided into three groups based on their blood pressure values. The study group was part of the male population which participated in the ProM project, managed by the Department of Nephrology, Hypertension and Internal Medicine and the Department of Pathophysiology at the University of Warmia and Mazury in Olsztyn in 2014-2017. As arterial hypertension is one of the main factors increasing afterload and contributes to the development of heart failure in a long term, groups without hypertension (n=29), with isolated diastolic hypertension (n=20) and systolic-diastolic hypertension (n=30) were selected. Participants were not previously diagnosed with any chronic disease and were not treated with any drugs. Standard biochemical serum and urine tests and assessment of serum USP18 concentration using ELISA were performed.

We found significant differences in USP18 concentration between the groups. In the group without hypertension mean and 95% confidence interval were 69.3 (22.1-116.5) pg/ml, in the group with diastolic hypertension 90.1 (29.0-151.3) pg/ml, and in the group with systolic-diastolic hypertension 426.7 (163.1-690.3) pg/ml. Mean value in the group with systolic-diastolic hypertension was approximately 6 times higher than in the group without hypertension (p=0.03) and 4.7 times higher than in the group with isolated diastolic hypertension (p=0.19). Significant correlations adjusted to classical risk factors ofhypertension and atherosclerosis (age, BMI, total cholesterol, triglycerides, glucose) were found between serum USP18 and systolic blood pressure (r=0.31, p=0.008), pulse pressure (r=0.37, p=0.002), and heart rate (r=0.24, p=0.04). These parameters indicate sympathetic hyperactivity (systolic blood pressure and heart rate) and, indirectly, increased stiffness of blood vessels (pulse pressure). In both cases, these are factors which are harmful to the heart. No correlation was found between USP18 and urinary albumin-to-creatinine ratio (UACR), a test commonly used to assess the degree of kidney damage and endothelial dysfunction. In all groups, UACR values were within normal range. Based on the abovementioned results, we hypothesized that an elevation in USP18 concentration results from increased afterload for the left ventricle and subclinical myocardial damage.

In the study presented in the article: "Patterns of Dickkopf-3 Serum and Urine Levels at Different Stages of Chronic Kidney Disease, J. Clin. Med. 2023, 12, 4750" Dkk3 serum and urine concentration was assessed in 100 patients aged 53 ± 17 years. Study population was divided into 5 subgroups (n=20 each), based on the stage of CKD (46% were women): individuals with normal renal function (control group), with biopsy-proven glomerulonephritis with proteinuria and/or active urine sediment but with preserved glomerular filtration rate, with stable CKD with reduced glomerular filtration rate, a group of patients on dialysis and patients after kidney transplantation with normal graft function.
Standard biochemical serum and urine tests, including cardiac biomarkers, Dkk3 concentrations in serum and urine using ELISA and pulse wave velocity were assessed in a study.

The most important observation in the study was the highest urinary Dkk3 concentration adjusted to urinary creatinine in patients with stable CKD (7050 pg/mg), which was significantly higher than in other groups. Moreover, the concentration of urinary Dkk3 in the kidney transplant group (5710 pg/mg) was twice as high as in the control group (2650 pg/mg) and in the glomerulonephritis group (2470 pg/mg), although glomerular filtration rate was normal in all these groups. Another important observation was the correlation between urinary Dkk3 and serum creatinine only in groups of patients with stable CKD ($r=0.41$, $p=0.009$) and after kidney transplantation ($r=0.39$, $p=0.043$), i.e. in groups with more advanced kidney disease (urine was not tested in the dialysis group because of anuria).

In the review entitled "Versatility of USP18 in physiology and pathophysiology, Acta Biochim Pol 2019; 66: 389–392" we focused on discussing USP18 structure and mechanisms of action, as well as its role in response to infectious factors, in carcinogenesis, in the development of autoimmune diseases and in cardiac muscle remodelling.

CONSLUSIONS OF PRESENTED PAPERS

1. Increase in serum USP18 seems to directly result from left ventricle overload and may reflect ongoing counterregulatory processes at the cellular level.
2. USP18 may be a sensitive and early biomarker of myocardial damage. It is suggested by an increase in serum USP18 concentration, which precedes alterations in urinary albumin excretion.
3. Urinary Dkk3 concentration correlates with the degree of kidney damage.
4. Elevated urinary Dkk3 concentration in the group with stable CKD may result from the severe tubular damage and advanced renal fibrosis. In the transplant patients mechanism is probably different (eGFR in this group was >60 ml/min) and may reflect the future risk of acute kidney injury or CKD progression.
5. This research confirms the potential role of USP18 and Dkk3 as a biomarkers of heart and kidney damage.
6. Future research is necessary to assess the role of USP18 in kidney diseases and Dkk3 in heart pathologies and compare them with each other. This may be another step forward to better understanding of kidney and heart disease's pathophysiology, which will enable to use this biomarkers in everyday practice.

REFERENCES

1. Lv JC, Zhang LX. Prevalence and Disease Burden of Chronic Kidney Disease. Adv Exp Med Biol 2019;1165: 3-15.

2. Catic A, Fiebiger E, Korbel GA, Blom D, Galardy PJ, Ploegh HL. Screen for ISG15-crossreactive deubiquitinases. PLoS One 2007; 2(7): e679.

3. Honke N, Shaabani N, Zhang DE, Hardt C, Lang KS. Multiple functions of USP18. Cell Death Dis 2016; 7(11): e2444.

4. Ying X, Zhao Y, Yao T, Yuan A, Xu L, Gao L, Ding S, Ding H, Pu J, He B. Novel protective role for ubiquitin-specific protease 18 in pathological cardiac remodeling. Hypertension 2016; 68: 1160-1170.

5. Makki MS, Ruteshouser EC, Huff V. Ubiquitin specific protease 18 (Usp18) is a WT1 transcriptional target. Exp Cell Res 2013; 319(5): 612-622.

6. Coit P, Jeffries M, Altorok N, Dozmorov MG, Koelsch KA, Wren JD, Merrill JT, McCune WJ, Sawalha AH. Genome-wide DNA methylation study suggests epigenetic accessibility and transcriptional poising of interferon-regulated genes in naive CD4+ T cells from lupus patients. J Autoimmun 2013; 43: 78-84.

7. Honke N, Shaabani N, Zhang DE, Iliakis G, Xu HC, Häussinger D, Recher M, Löhning M, Lang PA, Lang KS. Usp18 driven enforced viral replication in dendritic cells contributes to break of immunological tolerance in autoimmune diabetes. PLoS Pathog 2013; 9(10): e1003650.

8. Meuwissen ME, Schot R, Buta S, Oudesluijs G, Tinschert S, Speer SD, Li Z, van Unen L, Heijsman D, Goldmann T, Lequin MH, Kros JM, Stam W, Hermann M, Willemsen R, Brouwer RW, Van IJcken WF, Martin-Fernandez M, de Coo I, Dudink J, de Vries FA, Bertoli Avella A, Prinz M, Crow YJ, Verheijen FW, Pellegrini S, Bogunovic D, Mancini GM. Human USP18 deficiency underlies type 1 interferonopathy leading to severe pseudo-TORCH syndrome. J Exp Med 2016; 213(7): 1163-1174.

9. Schunk SJ, Floege J, Fliser D, Speer T. WNT–β-catenin signalling - a versatile player in kidney injury and repair. Nature 2021; 17: 172-184.

10. Ludwig J, Federico G, Prokosch S, Küblbeck G, Schmitt S, Klevenz A, Gröne HJ, Nitschke L, Arnold B. Dickkopf-3 acts as a modulator of B cell fate and function. J Immunol 2015; 194(6):2624-34.

11. Niehrs C. Function and biological roles of the Dickkopf family of Wnt modulators. Oncogene 2006; 25: 7469-7481.

12. Cheng WL, Yang Y, Zhang XJ, Guo J, Gong J, Gong FH, She Z K, Huang Z, Xia H, Li H. Dickkopf-3 Ablation Attenuates the Development of Atherosclerosis in ApoE-Deficient Mice. J Am Heart Assoc 2017; 6(2): e004690.

13. Zhang Y, Liu Y, Zhu XH, Zhang XD, Jiang DS, Bian ZY, Zhang XF, Chen K, Wei X, Gao L, Zhu LH, Yang Q, Fan GC, Lau WB, Ma X, Li H. Dickkopf-3 attenuates pressure overload-induced cardiac remodelling. Cardiovas Res 2014; 102.1: 35-45.

14. Schunk SJ, Speer T, Petrakis I, Fliser D. Dickkopf 3—a novel biomarker of the 'kidney injury continuum'. Nephrol Dial Transplant 2021; 36: 761-767.

15. Federico G, Meister M, Mathow D, Heine GH, Moldenhauer G, Popovic ZV, Nordström V, Kopp-Schneider A, Hielscher T, Nelson PJ, Schaefer F, Porubsky S, Fliser D, Arnold B, Gröne HJ. Dickkopf-3 promotes the development of renal atrophy and fibrosis. JCI Insight 2016; 1(1): e84916.

16. Zewinger S, Rauen T, Rudnicki M, Federico G, Wagner M. Dickkopf-3 (DKK3) in Urine Identifies Patients with Short-Term Risk of eGFR Loss. J Am Soc Nephrol 2018; 29: 2722-2733.

OŚWIADCZENIA WSPÓŁAUTORÓW PUBLIKACJI

Statement

We would like to declare that the paper:

Dziamałek-Macioszczyk, P.; Harazny, J.M.; Kwella, N.; Wojtacha, P.; Jung, S.; Dienemann, T.; Schmieder, R.E; Stompór, T. ***Relationship Between Ubiquitin-Specific Peptidase 18 and Hypertension in Polish Adult Male Subjects: A Cross-Sectional Pilot Study*** *Med. Sci. Monit.* **2020**, 26, 921919. DOI: 10.12659/MSM.921919

was written and published thanks to the following co-authors' contribution:

Paulina Dziamałek-Macioszczyk 60% – conceptualization, methodology, investigation, data curation, original draft preparation, review and editing

Dziamałek-Macioszczyk ..... Olsztyn, 25.07.23 r.

Joanna M. Harazny 10% - conceptualization, methodology, formal analysis, resources, data curation, review and editing, visualization, supervision, project administration, funding acquisition

Joanna Harazny ..... Olsztyn, 28.07.23

Norbert Kwella 5% - resources, supervision, project administration

Norbert Kwella ..... Olsztyn, 07.08.2023

Paweł Wojtacha 5% - investigation

Wojtacha Paweł ..... Olsztyn, 16.08.2023

Susanne Jung 5% - review and editing

..... Olsztyn, ...

Thomas Dienemann 5% - review and editing

…… Olsztyn, ……..

Roland E. Schmieder 5% - review and editing

…….. Olsztyn, ……..

Tomasz Stompór 5% - conceptualization, methodology, formal analysis, review and editing, supervision, funding acquisition

…… Olsztyn, 07/08/2023

Statement

We would like to declare that the paper:

Dziamałek-Macioszczyk, P.; Harazny, J.M.; Kwella, N.; Wojtacha, P.; Jung, S.; Dienemann, T.; Schmieder, R.E; Stompór, T. ***Relationship Between Ubiquitin-Specific Peptidase 18 and Hypertension in Polish Adult Male Subjects: A Cross-Sectional Pilot Study*** *Med. Sci. Monit.* **2020**, 26, 921919. DOI: 10.12659/MSM.921919

was written and published thanks to the following co-authors' contribution:

Paulina Dziamałek-Macioszczyk 60% – conceptualization, methodology, investigation, data curation, original draft preparation, review and editing

…….. Olsztyn, ……..

Joanna M. Harazny 10% - conceptualization, methodology, formal analysis, resources, data curation, review and editing, visualization, supervision, project administration, funding acquisition

…….. Olsztyn, ……..

Norbert Kwella 5% - resources, supervision, project administration

…….. Olsztyn, ……..

Paweł Wojtacha 5% - investigation

…… Olsztyn, ……..

Susanne Jung 5% - review and editing

…… Olsztyn, ……..
Erlangen, 28.07.23

Thomas Dienemann 5% - review and editing

…… Olsztyn, ……..

Roland E. Schmieder 5% - review and editing

…….. Olsztyn, ……..

Tomasz Stompór 5% - conceptualization, methodology, formal analysis, review and editing, supervision, funding acquisition

…… Olsztyn, ……..

Statement

We would like to declare that the paper:

Dziamałek-Macioszczyk, P.; Harazny, J.M.; Kwella, N.; Wojtacha, P.; Jung, S.; Dienemann, T.; Schmieder, R.E; Stompór, T. ***Relationship Between Ubiquitin-Specific Peptidase 18 and Hypertension in Polish Adult Male Subjects: A Cross-Sectional Pilot Study*** *Med. Sci. Monit.* **2020**, 26, 921919. DOI: 10.12659/MSM.921919

was written and published thanks to the following co-authors' contribution:

Paulina Dziamałek-Macioszczyk 60% – conceptualization, methodology, investigation, data curation, original draft preparation, review and editing

…….. Olsztyn, ……..

Joanna M. Harazny 10% - conceptualization, methodology, formal analysis, resources, data curation, review and editing, visualization, supervision, project administration, funding acquisition

…….. Olsztyn, ……..

Norbert Kwella 5% - resources, supervision, project administration

…….. Olsztyn, ……..

Paweł Wojtacha 5% - investigation

…… Olsztyn, ……..

Susanne Jung 5% - review and editing

…… Olsztyn, ……..

Thomas Dienemann 5% - review and editing



…….. Olsztyn, 17.08.2023

Roland E. Schmieder 5% - review and editing

…….. Olsztyn, ……..

Tomasz Stompór 5% - conceptualization, methodology, formal analysis, review and editing, supervision, funding acquisition

……. Olsztyn, ……..

Statement

We would like to declare that the paper:

Dziamałek-Macioszczyk, P.; Harazny, J.M.; Kwella, N.; Wojtacha, P.; Jung, S.; Dienemann, T.; Schmieder, R.E; Stompór, T. ***Relationship Between Ubiquitin-Specific Peptidase 18 and Hypertension in Polish Adult Male Subjects: A Cross-Sectional Pilot Study*** *Med. Sci. Monit.* **2020**, 26, 921919. DOI: 10.12659/MSM.921919

was written and published thanks to the following co-authors' contribution:

Paulina Dziamałek-Macioszczyk 60% – conceptualization, methodology, investigation, data curation, original draft preparation, review and editing

…….. Olsztyn, ……..

Joanna M. Harazny 10% - conceptualization, methodology, formal analysis, resources, data curation, review and editing, visualization, supervision, project administration, funding acquisition

…….. Olsztyn, ……..

Norbert Kwella 5% - resources, supervision, project administration

…….. Olsztyn, ……..

Paweł Wojtacha 5% - investigation

…… Olsztyn, ……..

Susanne Jung 5% - review and editing

…… Olsztyn, ……..

Thomas Dienemann 5% - review and editing

…… Olsztyn, ……..

Roland E. Schmieder 5% - review and editing

…… Olsztyn, 16 Aug 2022

Prof. Dr. Roland E. Schmieder
Leiter der Klinischen Forschungsstation CRC
Universitätsklinikum Erlangen
Medizinische Klinik 4
Ulmenweg 18, D-91054 Erlangen

Tomasz Stompór 5% - conceptualization, methodology, formal analysis, review and editing, supervision, funding acquisition

…… Olsztyn, ……..

Oświadczenie

Niniejszym oświadczamy, że publikacja:

Dziamałek-Macioszczyk, P.; Winiarska, A.; Pawłowska, A., Wojtacha, P.; Stompór, T. ***Patterns of Dickkopf-3 Serum and Urine Levels at Different Stages of Chronic Kidney Disease.*** *J. Clin. Med.* **2023**, 12, 4750. DOI: 10.3390/jcm12144705

powstała w wyniku poniżej określonego, indywidualnego wkładu pracy współautorów:

Paulina Dziamałek-Macioszczyk 80% – współudział w: opracowaniu koncepcji, metodologii, wyborze grupy badanej, przeprowadzeniu badań, analizie wyników, napisaniu artykułu, przechowywaniu danych

Dziamałek-Macioszczyk ..... Olsztyn, 25.07.2023

Agata Winiarska 5% - współudział w przeprowadzeniu badań

Agata Winiarska ..... Olsztyn, 08/08/2023

Anna Pawłowska 5% - współudział w wyborze grupy badanej

Anna Pawłowska ..... Olsztyn, 08/08/2023

Paweł Wojtacha 5% - współudział w przeprowadzeniu badań

Wojtacha Paweł ..... Olsztyn, 16.08.2023

Tomasz Stompór 5% - współudział w: opracowaniu koncepcji, metodologii, pozyskaniu środków finansowych, wybraniu grupy badanej, analizie wyników, napisaniu artykułu

..... Olsztyn, 07/08/2023

Oświadczenie

Niniejszym oświadczamy, że publikacja:

Dziamałek-Macioszczyk, P.; Harazny, J.M.; Stompór, T. ***Versatility of USP18 in physiology and pathophysiology.*** *Acta Biochim. Pol.* **2019**, 66, 389-392. DOI: 10.18388/abp.2019_2844

powstała w wyniku poniżej określonego, indywidualnego wkładu pracy współautorów:

Paulina Dziamałek-Macioszczyk 90% – współudział w: opracowaniu koncepcji, analizie piśmiennictwa, napisaniu artykułu

Dziamałek-Macioszczyk ..... Olsztyn, 25.07.23.

Joanna M. Harazny 5% - współudział w opracowaniu koncepcji

..... Olsztyn, 28.07.23

Tomasz Stompór 5% - współudział w: opracowaniu koncepcji, napisaniu artykułu

..... Olsztyn, 07/08/2023

MEDICAL SCIENCE MONITOR

CLINICAL RESEARCH

e-ISSN 1643-3750
© Med Sci Monit, 2020; 26: e921919
DOI: 10.12659/MSM.921919

Received: 2019.12.04
Accepted: 2020.02.25
Available online: 2020.04.14
Published: 2020.06.12

Relationship Between Ubiquitin-Specific Peptidase 18 and Hypertension in Polish Adult Male Subjects: A Cross-Sectional Pilot Study

Authors' Contribution:
Study Design A
Data Collection B
Statistical Analysis C
Data Interpretation D
Manuscript Preparation E
Literature Search F
Funds Collection G

BDEF 1 Paulina Dziamałek-Macioszczyk
ABCDG 2,3 Joanna M. Harazny
ABD 1 Norbert Kwella
CD 4 Paweł Wojtacha
E 3 Susanne Jung
E 3 Thomas Dienemann
D 3 Roland E. Schmieder
ACDEG 1 Tomasz Stompór

1 Department of Nephrology, Hypertension and Internal Medicine, University of Warmia and Mazury in Olsztyn, Olsztyn, Poland
2 Department of Human Physiology and Pathophysiology, University of Warmia and Mazury in Olsztyn, Olsztyn, Poland
3 Clinical Research Centre, Department of Nephrology and Hypertension, Friedrich-Alexander University Erlangen-Nürnberg, Erlangen, Germany
4 Department of Industrial and Food Microbiology, University of Warmia and Mazury in Olsztyn, Olsztyn, Poland

Corresponding Author: Paulina Dziamałek-Macioszczyk, e-mail: p.dziamalek@gmail.com

Source of support: Serum and urine clinical laboratory tests, blood pressure measuring devices, containers for serum and urine samples, and printed information and documentation were funded by Polpharma SA (Poland). USP18 measurements were supported by a statutory grant from the University of Warmia and Mazury in Olsztyn (Poland; no. 61.610.001-300) to Joanna M. Harazny

Background: Arterial hypertension (HT) is a leading cause of cardiac hypertrophy and heart failure. Ubiquitin-specific peptidase 18 (USP18) has been recently described as a factor that prevents myocardial dysfunction. The present study measured serum USP18 levels in normotensive (*n*=29), isolated diastolic hypertensive (*n*=20), and systolic-diastolic hypertensive (*n*=30) male participants and correlated these results with biochemical parameters that are included in routine assessments of patients with hypertension.

Material/Methods: Seventy-nine men, aged 24 to 82 years (mean=50.8±11.4 years), were included in the study. None of the participants had ever been treated for HT. Blood and urine parameters were assessed using routine techniques. Serum USP18 levels were measured by enzyme-linked immunosorbent assay.

Results: The means and 95% confidence intervals (CIs) of USP18 levels in the HT(–), iDHT(+), and HT(+) groups were 69.3 (22.1–116.5) pg/ml, 90.1 (29.0–151.3) pg/ml, and 426.7 (163.1–690.3) pg/ml, respectively. In the HT(+) group, the mean serum USP18 level was 6.2-times higher than in the HT(–) group (p=0.014) and 4.7-times higher than in the iDHT(+) group (p=0.19). The partial correlation analysis that was adjusted for risk factors of arteriosclerosis indicated that USP18 levels were correlated with systolic blood pressure, pulse pressure, and heart rate.

Conclusions: This preliminary study found that serum USP18 levels were significantly higher in drug-naive male participants with arterial hypertension compared with normotensive controls. USP18 exerts cardiovascular-protective effects. Elevations of USP18 levels may indicate a counterregulatory process that is engaged during increases in pressure in the left ventricle.

MeSH Keywords: Heart Failure • Hypertension • Hypertrophy, Left Ventricular • Ventricular Remodeling

Full-text PDF: https://www.medscimonit.com/abstract/index/idArt/921919

2629 6 1 33

This work is licensed under Creative Common Attribution-NonCommercial-NoDerivatives 4.0 International (CC BY-NC-ND 4.0)

Indexed in: [Current Contents/Clinical Medicine] [SCI Expanded] [ISI Alerting System] [ISI Journals Master List] [Index Medicus/MEDLINE] [EMBASE/Excerpta Medica] [Chemical Abstracts/CAS]

Background

Arterial hypertension (HT) is a condition that is diagnosed in 30–45% of the global adult population, and this number is still growing [1]. Hypertension can lead to arterial wall remodeling and organ damage, such as kidney disease, myocardial infarction, and stroke, all of which are associated with high rates of morbidity and mortality [2]. The onset of organ damage may be asymptomatic for many years, and thus HT may go untreated.

One of the early markers of HT-related complications is the urinary albumin-to-creatinine ratio (UACR) [3]. The UACR has been suggested to indicate not only renal vascular damage but also systemic vascular damage, particularly an increase in vascular permeability and endothelial dysfunction [3–5]. Heart failure is a common complication in later stages of HT, which occurs as a result of progressive myocardial hypertrophy and remodeling [6,7].

Ubiquitin-specific peptidase 18 (USP18) is a recently identified member of the ubiquitin-specific peptidase (USP) family and considered a novel factor that is involved in HT [8]. Ubiquitin-specific peptidases have been reported to remove ubiquitin-like molecules from their substrates and regulate such cellular processes as the cell cycle, cell signaling, and the stress response. Ubiquitin-specific peptidases have also been reported to contribute to immune response regulation [9–11]. USP18 synthesis is stimulated by type I and type III interferons (IFNs), viral infection, bacterial lipopolysaccharides, tumor necrosis factor α (TNFα), and genotoxic stress [10].

USP18 exerts its enzymatic function by cleaving interferon-stimulated gene 15 (ISG15) and ubiquitin from their target proteins [8,12,13]. USP18 is involved in the response to viral infection and development of several autoimmune diseases. It also contributes to the pathogenesis of some genetic diseases and has antitumor properties [14–21]. Mouse studies have shown that USP18 is also crucial for survival. The lifespan of USP18 knockout mice was significantly shorter compared with wildtype mice. Mice that lacked the USP18 gene and protein were hypersensitive to IFNs and exhibited advanced damage of the central nervous system [11].

Ying et al. reported that USP18 inhibited cardiac remodeling and protected against the development of heart failure. USP18 expression was significantly higher in hearts of patients with dilated cardiomyopathy than in hearts from healthy donors [8]. In murine hearts that were exposed to an increase in afterload, the elevation of USP18 expression in cardiomyocytes was related to a decrease in left ventricular wall hypertrophy, less pronounced myocardial fibrosis, and a delayed onset of heart failure [8]. In contrast, USP18-deficient mice exhibited advanced cardiac remodeling. Ying et al. concluded that this process was a consequence of inhibition of the transforming growth factor beta-activated kinase 1 (TAK1)-p38-c-Jun N-terminal kinase 1/2 (JNK1/2) signaling pathway [8]. To our knowledge, no other clinical or epidemiological studies have investigated USP18 as a putative biomarker or predictive factor in cardiovascular disease. Thus, the aim of the present study was to compare serum USP18 levels in normotensive male subjects, male patients with isolated diastolic HT (iDHT), and male patients with systolic-diastolic HT and assess the association between USP18 levels and basic clinical parameters in early hypertension.

Material and Methods

Study population

Seventy-nine adult men (mean age=50.8±11.4 years; range=24–82 years) who participated in the ProM project (*www.clinicaltrials.gov*; NCT03559608) were included in the study. Patients with a history or signs of acute or chronic infectious or non-infectious inflammatory disease were excluded. None of the subjects suffered from diabetes or any other clinically overt disorder that could be associated with a greater risk of cardiovascular disease. None of the patients were ever treated with blood pressure (BP)-lowering medications, statins, antidiabetic agents, or acetylsalicylic acid. This provided a unique opportunity to analyze groups of hypertensive patients who were likely naive to treatment and otherwise healthy.

ProM project

This cross-sectional study was performed between December 2014 and December 2017 in the Department of Nephrology, Hypertension, and Internal Medicine and Department of Pathophysiology at the University of Warmia and Mazury in Olsztyn (Poland). The primary aim of the study was to analyze the prevalence of HT and risk factors for the development of HT in an unselected group of Polish men from the region of Warmia and Mazury, which is considered one of the most underprivileged regions in Poland in terms of social and employment status. The study protocol was approved by the Ethics Committee of the Regional Chamber of Physicians, District of Warmia and Mazury, Poland. The study was conducted according to the Declaration of Helsinki and Good Clinical Practice guidelines. Each participant signed a written informed consent.

The decision to study only male individuals was based on data from the World Health Organization (*http://www.euro.who.int/en/countries/poland/data-and-statistics*; accessed May 16, 2018). These data indicate that the mean life expectancy in Poland is 8 years shorter in males (74 years) than in females (82 years). In Western Europe, this difference does not exceed

This work is licensed under Creative Common Attribution-NonCommercial-NoDerivatives 4.0 International (CC BY-NC-ND 4.0)

Indexed in: [Current Contents/Clinical Medicine] [SCI Expanded] [ISI Alerting System] [ISI Journals Master List] [Index Medicus/MEDLINE] [EMBASE/Excerpta Medica] [Chemical Abstracts/CAS]

5 years (*https://www.statista.com/statistics/274514/life-expectancy-in-europe/*; accessed May 16, 2018). Moreover, the risk of cardiovascular disease in European countries, assessed by Systematic COronary Risk Evaluation (SCORE) risk charts, indicates a higher risk for cardiovascular disease and associated complications in Polish men compared with other high-risk nations in Europe [22]. Arterial HT is a key risk factor for lower life expectancy in the Polish male population. The aim of the ProM project is to investigate the prevalence of HT and promulgate interest in prevention and treatment in male inhabitants of the Warmia and Mazury region in Poland.

Arterial BP was measured according to 2013 European Society of Cardiology/European Society of Hypertension (ESC/ESH) Guidelines in 631 randomly selected subjects [2]. All of the participants completed a coded and standardized health questionnaire and underwent blood and urine tests that were performed in a certified clinical laboratory (ISO 9001: 2018). The biochemical analyses were performed using a Cobas 6000 modular analyzer (Roche, Basel, Switzerland). Serum was tested for glucose, creatinine, and lipid profile. Microalbuminuria and creatinine were measured in urine, and the UACR was calculated. The estimated glomerular filtration rate (eGFR) was calculated according to the MDRD formula.

Blood pressure measurement

Office blood pressure (BP) and heart rate (HR) were measured by pulse detection of both arms using an Omron M3 device (Kyoto, Japan). After 10 min of rest in a sitting position, we repeated office BP measurements on the arm with the higher systolic blood pressure (SBP) value twice, and the mean of 2 measurements was used for further analyses. Mean arterial pressure (MAP) was calculated as the sum of two thirds of diastolic blood pressure (DBP) and one third of SBP. Pulse pressure (PP) was calculated as the difference between SBP and DBP. Three groups could be distinguished among the study participants according to 2013 ESH/ECS Guidelines: HT(–) (normotensive men, SBP <140 mmHg, DBP <90 mmHg, *n*=29 [36.7%]), iDHT(+) (isolated diastolic hypertensive men, SBP <140 mmHg, DBP ≥90 mmHg, *n*=20 [25.3%]), HT(+) (systolic-diastolic hypertensive men, SBP ≥140 mmHg, DBP ≥90 mmHg, *n*=30 [38.0%]).

USP18 measurement

Blood samples were collected by qualified medical staff. Approximately 2 ml of each serum sample was frozen at –80°C. The samples were then thawed at –20°C for 24 h, 6°C for the next 24 h, and then at room temperature for 30 min immediately before mixing by a vortex shaker. Serum USP18 levels were assayed using an enzyme-linked immunosorbent assay (ELISA) kit for human USP18 (Wuhan Fine Biotech Co., Ltd., Wuhan, Hubei, China) by a technician with more than 10 years of experience in ELISA procedures. The technician was blinded to the clinical data. To verify reliability of the ELISA method, USP18 measurements were repeated twice for each sample with a coefficient of variation (CV) of 0.84%. The CV was calculated as the ratio of the standard deviation to the mean multiplied by 100.

Statistical analysis

The data distribution was first checked using the Kolmogorov-Smirnov test. The data were then analyzed using SPSS 23 software (IBM, New York, USA). Two-tailed values of $p<0.05$ were considered statistically significant. To compare differences between 2 groups, unpaired Student's *t*-test with Bonferroni correction was used for data with a normal distribution, the results of which are expressed as means±standard deviations. For data with a non-normal distribution, the nonparametric Mann-Whitney U test for unpaired samples was used, the results of which are presented as medians and interquartile ranges. To compare differences between 3 groups, one-way analysis of variance was used for data with a normal distribution, and the nonparametric Kruskal-Wallis test for unpaired samples was used for data with a non-normal distribution. *p* values were not calculated for the BP parameters, which were used to define the groups. Systolic blood pressure in the iDHT(+) and HT(–) groups did not contribute to this division; in this case, *p* values were calculated.

To determine associations between clinical parameters and USP18 levels, Pearson and Spearman correlation coefficients and partial correlations that were adjusted for risk factors of arteriosclerosis (i.e., age, Body Mass Index [BMI], total cholesterol, triglycerides, and glucose) were calculated. Analysis of covariance was performed using univariate and multivariate linear analyses. Age, BMI, total cholesterol, triglycerides, and glucose were used as confounding variables.

Non-adjusted and adjusted *p* values are presented in the Results section, Tables 1–5, and Figure 1. The graphical representation of data variability is illustrated by error bars as a 95% confidence interval (CI) of the measured values.

Results

Demographic, anthropometric, and biochemical parameters in the study groups are summarized in Table 6 (*n*=30 for HT[+] group, *n*=20 for iDHT[+] group, *n*=29 for HT[–] group). Table 1 shows the comparisons between groups in terms of the clinical parameters. Of the 79 participants, only 7 (8.86%) declared awareness of their having HT that remained untreated, and 72 (91.14%) had never been diagnosed with HT.

This work is licensed under Creative Common Attribution-NonCommercial-NoDerivatives 4.0 International (CC BY-NC-ND 4.0)

Indexed in: [Current Contents/Clinical Medicine] [SCI Expanded] [ISI Alerting System] [ISI Journals Master List] [Index Medicus/MEDLINE] [EMBASE/Excerpta Medica] [Chemical Abstracts/CAS]

Table 1. Comparison of anthropometric and biochemical parameters between the study groups*.

	Mean±SD Median (IQR)			p^1			p^2
	HT(–) n=29	iDHT(+) n=20	HT(+) n=30	HT(+) *vs.* HT(–)	iDHT(+) *vs.* HT(–)	HT(+) *vs.* iDHT(+)	
Age [years]	49.3±12.4 48.5 (40.3–63.8)	50.25±9.6 53.0 (45.5–57.0)	52.9±11.3 51.0 (43.5–57.5)	>0.99	>0.99	>0.99	0.93
BMI [kg/m^2]	25.4±3.1 25.5 (24.3–28.5)	28. 0±3.0 29.2 (24.8–31.0)	28.3±3.8 28.7 (25.1–31.2)	0.0093	>0.99	0.061	0.0060
Triglycerides [mg/dl]	122±78 103 (72–176)	147±115 112.0 (93–208)	145±92 128 (73–165)	0.53	0.26	0.61	0.52
Total cholesterol [mg/dl]	207.3±41.1 216 (184–241)	207.3±36.2 201 (173–233)	211.0±42.4 215 (189–250)	0.81	0.46	0.42	0.68
Glucose [mg/dl]	101.7±17.2 100 (92–106)	96.8±11.1 91 (87–101)	100.1±17.0 98 (92–106)	0.90	0.074	0.076	0.14
Creatinine [mg/dl]	0.986±0.14 1.0 (0.90–1.10)	0.945±0.16 0.90 (0.80–1.00)	0.987±0.14 1.0 (0.90–1.10)	0.75	0.051	0.077	0.11
eGFR [ml/min]	83.5±13.1 82.3 (71.6–93.1)	87.5 ±16.4 87.0 (77.1–103.5)	81.7±13.1 80.1 (74.4–91.5)	>0.99	0.18	0.41	0.16
UACR [mg/g]	3.07±3.72 1.84 (1.19–2.51)	2.34±1.34 2.05 (0.97–3.67)	3.41±8.24 1.94 (1.20–3.08)	0.70	0.80	0.88	0.92
SBP [mmHg]	124.8±7.4 125.5 (119–132)	134.3±4.0 132.3 (128–137)	156.2±14.2 153 (145–162)	–	0.0073	–	–
DBP [mmHg]	80.9±5.9 82.3 (76–85)	93.4±2.4 93.3 (92.3–95.1)	98.5±10.5 97.5 (94–104)	–	–	–	–
MAP [mmHg]	95.5±5.3 96 (94–100)	107.0±2.0 106 (104–108)	117.7±10.7 115 (112–121)	–	–	–	–
PP [mmHg]	43.9±7.8 42.8 (37.5–49.8)	40.9±5.0 37.8 (35.0–41.1)	57.8±10.7 53.0 (48.0–62.0)	<0.0012	0.0051	<0.0011	<0.0010
HR [beats/min]	69.8±9.9 71 (63–77)	76.6±10.8 76 (71–80)	76.7±12.8 74 (65–87)	0.16	0.071	>0.99	0.039
USP18 [pg/ml]	69.3±124.0 22.3 (0.0–77.0)	90.1±130.6 44.6 (0.0–120.6)	426.7±706 80.4 (20.3–317)	0.030	0.58	0.19	0.088

* Respecting the values distribution (Table 6), p^1 values were calculated by the Mann-Whitney U test for data with a non-normal distribution or by unpaired Student's t-test with Bonferroni correction for data with a normal distribution; p^2 values were calculated for 3 independent groups of participants by non-parametric Kruskal Wallis test for data with a non-normal distribution or by one-way analysis of variance for data with a normal distribution. *p* values were not calculated for the blood pressure parameters, which defined the groups. Systolic blood pressure in iDHT(+) and HT(–) did not contribute to this division; in this case, *p* value was calculated; n – patients number; SD – standard deviation; IQR – interquartile range.

This work is licensed under Creative Common Attribution-NonCommercial-NoDerivatives 4.0 International (CC BY-NC-ND 4.0)

Indexed in: [Current Contents/Clinical Medicine] [SCI Expanded] [ISI Alerting System] [ISI Journals Master List] [Index Medicus/MEDLINE] [EMBASE/Excerpta Medica] [Chemical Abstracts/CAS]

Table 2. Simple correlation analysis between USP18 and BMI, UACR, serum and blood pressure parameters.

	USP18	
	r	*p*
BMI*	0.31	0.0061
Triglyceride**	0.42	0.0018
Total cholesterol	–0.36	0.0013
Creatinine	–0.053	0.65
eGFR	0.0060	0.96
UACR	–0.072	0.53
SBP	0.27	0.016
DBP	0.26	0.023

* Pearson's correlation analysis; ** Spearman's correlation analysis.

In the HT(+) group, mean serum USP18 levels were significantly higher than in the HT(–) group (Figure 1, Table 1). The difference between median USP18 levels in the HT(–) and HT(+) groups was also statistically significant (22.3 pg/ml in HT[–] group and 80.4 pg/ml in HT[+] group). Systolic blood pressure in the iDHT(+) group was <140 mmHg. Systolic blood pressure was significantly higher in the iDHT(+) group than in the HT(–) group ($p<0.001$). Pulse pressure was significantly higher in the HT(+) group than in both the HT(–) and iDHT(+) groups (Table 1). The UACR did not differ between groups. In each group, the UACR was below the threshold for microalbuminuria (<30 mg/g; Table 1). No correlation was found between USP18 levels and the UACR ($r=–0.072$, $p=0.53$), serum creatinine ($r=–0.053$, $p=0.65$), and eGFR ($r=0.006$, $p=0.96$). USP18 levels were significantly correlated with BMI ($r=0.309$, $p=0.006$), triglycerides ($r=0.423$, $p<0.001$), total cholesterol ($r=–0.356$, $p=0.001$), SBP ($r=0.269$, $p=0.016$), and DBP ($r=0.255$, $p=0.023$; Table 2). No other significant correlations were found between USP18 levels and the clinical parameters.

Table 3. Correlation between USP18 and blood pressure parameters.

	Pearson correlation		Partial correlation*	
	r	*p*	*r*	*p*
SBP	0.33	0.0040	0.314	0.0081
DBP	0.11	0.38	0.14	0.24
MAP	0.23	0.057	0.234	0.051
PP	0.39	0.0013	0.37	0.0023
HR	0.25	0.035	0.24	0.043

* Partial correlations were adjusted by risk factors of arteriosclerosis (age, BMI, serum total cholesterol, triglyceride and glucose).

Table 4. Univariate linear analysis between USP18 and BMI, UACR, serum and blood pressure parameters.

	USP18		
	R beta	R^2	*p*
Age	0.049	0.0022	0.67
BMI	0.0022	<0.0014	0.99
Triglyceride	0.234	0.055	0.038
Total cholesterol	0.19	0.034	0.10
Glucose	-0.013	<0.0011	0.91
Creatinine	-0.032	0.0012	0.78
eGFR	0.00	<0.0012	1.0
UACR	0.069	0.0054	0.55
SBP	0.36	0.13	0.0013
DBP	0.17	0.028	0.14
MAP	0.27	0.073	0.016
PP	0.39	0.16	<0.0012
HR	0.32	0.11	0.0044

This work is licensed under Creative Common Attribution-NonCommercial-NoDerivatives 4.0 International (CC BY-NC-ND 4.0)

Indexed in: [Current Contents/Clinical Medicine] [SCI Expanded] [ISI Alerting System] [ISI Journals Master List] [Index Medicus/MEDLINE] [EMBASE/Excerpta Medica] [Chemical Abstracts/CAS]

Table 5. Multivariate analysis between USP18 and selected parameters*.

	USP18			
	CI 95% lower limit	CI 95% upper limit	*R* beta standardized	*p*
Age	–8.95	9.73	0.0094	0.93
Total cholesterol	–1.58	3.73	0.090	0.42
Creatinine	–752.63	628.83	–0.019	0.86
UACR	–17.17	21.96	0.028	0.81
PP	5.33	23.50	0.34	0.0022
HR	1.30	19.018	0.25	0.025

* CI 95% – 95% confidence interval.

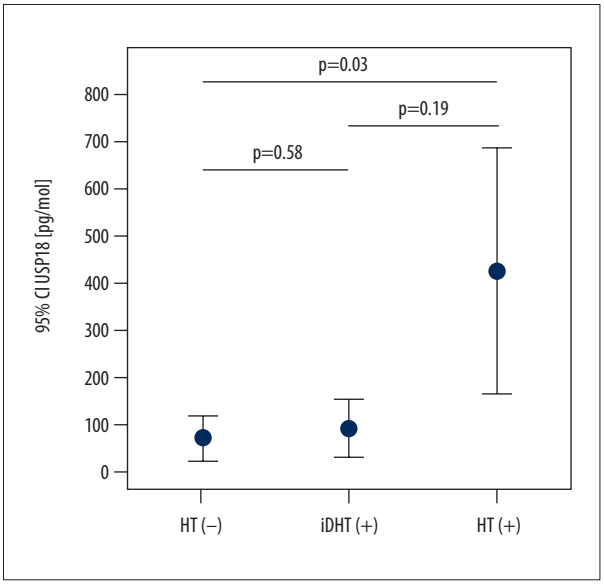


Figure 1. Comparison of 95% confidence intervals (CI) of USP18 levels in the normotensive (HT[–]) group, isolated diastolic hypertensive (iDHT[+]) group, and systolic-diastolic hypertensive (HT[+]) group. The *p* values were calculated using the Mann-Whitney U test.

After adjusting for risk factors for atherosclerosis (i.e., age, BMI, total cholesterol, triglycerides, and glucose), USP18 levels were significantly correlated with SBP (p=0.008), PP (p=0.002), and HR (p=0.043) in the whole study cohort (Table 3). Univariate and multivariate analyses confirmed associations between parameters (Table 4). The *p* values and lower and upper limits of 95% CIs in the multivariate analysis indicated that HR and PP were independently related predictors of high USP18 levels in hypertensive men (Table 5).

Discussion

Arterial HT increases afterload and leads to myocardial hypertrophy. If left untreated, it ultimately leads to congestive heart failure [6,7]. In the long term, pressure overload induces the mitogen-activated protein kinase (MAPK)-dependent signaling pathway, modifies the structure of contractile proteins, and causes a pattern of concentric myocardial hypertrophy. Concentric hypertrophy and heart failure with a preserved ejection fraction may lead to eccentric hypertrophy and progressive cardiomyocyte damage, resulting in cardiac dilation and heart failure with a reduced ejection fraction [23].

Ying et al. reported that USP18 is a novel factor that is involved in the development of cardiac hypertrophy. Under conditions of chronic increases in afterload, USP18 inhibited the development of congestive heart failure in both an experimental mouse model and humans by blocking MAPKs within the TAK1-p38-JNK1/2 axis [8]. Based on these data, we hypothesized that high USP18 levels may reflect an ongoing counterregulatory process in response to an increase in afterload in an effort to maintain proper cardiomyocyte structure and compensate for their contractile function. Therefore, an increase in serum USP18 levels may be an early marker of cardiac remodeling.

The present results suggested that an increase in USP18 levels in the HT(+) group may be a biochemical signature of early cardiomyocyte remodeling. The HT(+) group was characterized by significantly higher serum USP18 levels compared with the HT(–) group. The 3 groups in the present study did not differ in age or other demographic, anthropometric, or biochemical parameters, with the exception of BMI. Serum USP18 levels were not associated with BMI (Table 4). Only the difference in BP emerged as a determinant of higher USP18 levels in the HT(+) group compared with the HT(–) and iDHT(+) groups. This implies that iDHT at this stage of the disease does not evoke cardiac remodeling. This is consistent with previous studies that found that cardiac hypertrophy was more prevalent in individuals

This work is licensed under Creative Common Attribution-NonCommercial-NoDerivatives 4.0 International (CC BY-NC-ND 4.0)

Indexed in: [Current Contents/Clinical Medicine] [SCI Expanded] [ISI Alerting System] [ISI Journals Master List] [Index Medicus/MEDLINE] [EMBASE/Excerpta Medica] [Chemical Abstracts/CAS]

Table 6. Anthropometric and biochemical parameters of the whole study population*.

	Distribution Kolmogorov-Smirnov test	All participants n=79	
		Mean±SD	Median (IQR)
Age [years]	Normal	50.9±11.3	50 (43; 58)
BMI [kg/m^2]	Normal	27.1±3.6	27.4 (24.6; 30.1)
Triglyceride [mg/dl]	Non-normal	137.0±93.2	109 (78; 161)
Total cholesterol [mg/dl]	Non-normal	209.0±40.0	212 (183; 241)
Glucose [mg/dl]	Non-normal	99.8±15.7	99.00 (90.75; 105.25)
Creatinine [mg/dl]	Non-normal	0.98±0.15	1.00 (0.90; 1.025)
eGFR [ml/min]	Normal	83.7±14.02	84.6 (74.9; 92.4)
UACR [mg/g]	Non-normal	3.37±5.6	1.89 (1.18; 3.19)
SBP [mmHg]	Non-normal	139.1±93.2	136.5 (128.5; 149.5)
DBP [mmHg]	Normal	90.7±10.7	91.0 (83.0; 95.5)
MAP [mmHg]	Non-normal	106.9±12.1	106.5 (97.8; 113.5)
PP [mmHg]	Non-normal	48.4±11.3	46 (41; 54)
HR [beats/min]	Normal	74.1±11.6	74 (65; 80)
USP18 [pg/ml]	Non-normal	210.3±473.3	37.72 (0; 163.22)

* n – patients number; SD – standard deviation; IQR – interquartile range.

with systolic hypertension than in individuals with diastolic hypertension [24–26]. Nevertheless, iDHT patients are up to 23 times more likely than normotensive individuals to develop systolic-diastolic hypertension during the next 10 years [27,28]. The UACR is generally accepted as a marker of early vascular damage, particularly endothelial damage, that is not necessarily limited to the kidneys. The UACR in the present study was within the normal range and not different between any of the groups [3,4]. Unlike USP18 levels, the UACR was not correlated with BP parameters. Such a trend was detectable only for PP (r=0.20, p=0.077). This observation suggests that elevations of USP18 levels may occur earlier than increases in the UACR.

Clinical studies of the relationship between cardiovascular and BP parameters and USP18 levels in humans are lacking. Associations between PP (which indirectly reflects vascular stiffness) and different cytokines or markers of cardiac damage have been previously reported. For example, Yamada et al. described an independent correlation between PP and TNFα in elderly hypertensive patients. Sasaki et al. reported an association between PP and N-terminal pro-B-type natriuretic peptide (NT-pro-BNP) [29,30]. Several BP parameters (including PP) were correlated with NT-pro-BNP in patients with peripheral vascular disease [31]. Such an observation was also recently confirmed in the large, population-based Atherosclerosis Risks in Communities study [32]. Sympathetic overactivity, reflected by an increase in heart rate, is one of the most important determinants of cardiac injury, reflected by NT-pro-BNP or cardiac troponins [33].

One limitation of the present study was that we did not measure markers of inflammation; thus, we were unable to analyze possible links between markers of inflammation and USP18 levels. Such an association, however, is unlikely because of our very careful patient selection and clinical assessments. One advantage of the present study was that none of the subjects had ever received antihypertensive medications, and the subjects did not have immunological or cardiovascular disease.

Conclusions

In summary, serum USP18 levels were significantly higher in patients with early-stage systolic-diastolic arterial HT than in normotensive subjects and were also higher than in patients with iDHT. Our findings suggest that elevations of USP18 levels may represent subclinical cardiomyocyte remodeling. Given the correlations we found between USP18 levels and HR and PP, further studies should assess possible relationships between USP18 levels and other markers of cardiomyocyte damage, echocardiographic parameters, common carotid artery intima-media thickness, and pulse wave velocity (i.e., parameters that reflect the degree of atherosclerosis and arterial stiffness).

Acknowledgements

We thank the students from the Students' Scientific Association of Nephrology and Students' Scientific Association of Pathophysiology at the University of Warmia and Mazury

This work is licensed under Creative Common Attribution-NonCommercial-NoDerivatives 4.0 International (CC BY-NC-ND 4.0)

Indexed in: [Current Contents/Clinical Medicine] [SCI Expanded] [ISI Alerting System] [ISI Journals Master List] [Index Medicus/MEDLINE] [EMBASE/Excerpta Medica] [Chemical Abstracts/CAS]

in Olsztyn. We thank Adam Ossowski for his valuable contribution to the ProM project, Monika Olchowska-Sobczak for excellent administrative support, and Polpharma SA (Poland) for financial support.

References:

1. Lawes CMM, Vander Hoorn S, Rodgers A: Global burden of blood-pressure-related disease, 2001. Lancet, 2008; 371(9623): 1513–18
2. Mancia G, Fagard R, Narkiewicz K et al: 2013 ESH/ESC guidelines for the management of arterial hypertension. J Hypertension, 2013; 31(7): 1281–57
3. Wang TJ, Evans JC, Meigs JB et al: Low-grade albuminuria and the risks of hypertension and blood pressure progression. Circulation, 2005; 111(11): 1370–76
4. Deckert T, Feldt-Rasmussen B, Borch-Johnsen K et al: Albuminuria reflects widespread vascular damage: The Steno hypothesis. Diabetologia, 1989; 32(4): 219–26
5. Murai S, Tanaka S, Dohi Y et al: The prevalence, characteristics, and clinical significance of abnormal albuminuria in patients with hypertension. Sci Rep, 2014; 4: 3884
6. Díez J: Arterial hypertension in patients with heart failure. Heart Fail Clin, 2014; 10(2): 233–42
7. Kannan A, Janardhanan R: Hypertension as a risk factor for heart failure. Curr Hypertens Rep, 2014; 16(7): 447
8. Ying X, Zhao Y, Yao T et al: Novel protective role for ubiquitin-specific protease 18 in pathological cardiac remodeling. Hypertension, 2016; 68: 1160–70
9. Catic A, Fiebiger E, Korbel GA et al: Screen for ISG15-crossreactive deubiquitinases. PLoS One, 2007; 2(7): e679
10. Honke N, Shaabani N, Zhang DE et al: Multiple functions of USP18. Cell Death Dis, 2016; 7(11): e2444
11. Tokarz S, Berset C, La Rue J et al: The ISG15 isopeptidase UBP43 is regulated by proteolysis via the SCFSkp2 ubiquitin ligase. J Biol Chem, 2004; 279(45): 46424–30
12. François-Newton V, Magno de Freitas Almeida G, Payelle-Brogard B et al: USP18-based negative feedback control is induced by type I and type III interferons and specifically inactivates interferon α response. PLoS One, 2011; 6(7): e22200
13. Sarasin-Filipowicz M, Wang X, Yan M et al: Alpha interferon induces long-lasting refractoriness of JAK-STAT signaling in the mouse liver through induction of USP18/UBP43. Mol Cell Biol, 2009; 29(17): 4841–51
14. Potu H, Sgorbissa A, Brancolini C: Identification of USP18 as an important regulator of the susceptibility to IFN-α and drug-induced apoptosis. Cancer Res, 2010; 70(2): 655–65
15. Santin I, Moore F, Grieco FA et al: USP18 is a key regulator of the interferon-driven gene network modulating pancreatic beta cell inflammation and apoptosis. Cell Death Dis, 2012; 3: e419
16. MacParland SA, Ma XZ, Chen L et al: Lipopolysaccharide and tumor necrosis factor alpha inhibit interferon signaling in hepatocytes by increasing ubiquitin-like protease 18 (USP18) expression. J Virol, 2016; 90(12): 5549–60
17. Meuwissen ME, Schot R, Buta S et al: Human USP18 deficiency underlies type 1 interferonopathy leading to severe pseudo-TORCH syndrome. J Exp Med, 2016; 213(7): 1163–74
18. Makki MS, Ruteshouser EC, Huff V: Ubiquitin specific protease 18 (Usp18) is a WT1 transcriptional target. Exp Cell Res, 2013; 319(5): 612–22
19. Coit P, Jeffries M, Altorok N et al: Genome-wide DNA methylation study suggests epigenetic accessibility and transcriptional poising of interferon-regulated genes in naive CD4+ T cells from lupus patients. J Autoimmun, 2013; 43: 78–84
20. Honke N, Shaabani N, Zhang DE et al: Usp18 driven enforced viral replication in dendritic cells contributes to break of immunological tolerance in autoimmune diabetes. PLoS Pathog, 2013; 9(10): e1003650
21. Schwer H, Liu LQ, Zhou L et al: Cloning and characterization of a novel human ubiquitin-specific protease, a homologue of murine UBP43 (Usp18). Genomics, 2000; 65(1): 44–52
22. Zdrojewski T, Jankowski P, Bandosz P et al: [A new version of cardiovascular risk assessment system and risk charts calibrated for Polish population.] Kardiol Pol, 2015; 73(10): 958–61 [in Polish]
23. Kehat I, Molkentin JD: Molecular pathways underlying cardiac remodeling during pathophysiological stimulation. Circulation, 2010; 122(25): 2727–35
24. Asgari S, Khalili D, Mehrabi Y et al: Incidence and risk factors of isolated systolic and diastolic hypertension: A 10-year follow-up of the Tehran Lipids and Glucose Study. Blood Press, 2016; 25(3): 177–83
25. Franklin SS, Pio JR, Wong ND et al: Predictors of new-onset diastolic and systolic hypertension: The Framingham Heart Study. Circulation, 2005; 111: 1121–27
26. Pini R, Cavallini MC, Bencini F et al: Cardiovascular remodeling is greater in isolated systolic hypertension than in diastolic hypertension in older adults: The Insufficienza Cardiaca negli Anziani Residenti (ICARE) a Dicomano Study. J Am Coll Cardiol, 2002; 40(7): 1283–89
27. Johnson HM, Bartels CM, Thorpe CT et al: Differential diagnosis and treatment rates between systolic and diastolic hypertension in young adults: A multidisciplinary observational study. J Clin Hypertens, 2015; 17(11): 885–94
28. Sheriff HM, Tsimploulis A, Valentova M et al: Isolated diastolic hypertension and incident heart failure in community-dwelling older adults: Insights from the Cardiovascular Health Study. Int J Cardiol, 2017; 238: 140–43
29. Yamada E, Takeuchi M, Kurata M et al: Association of pulse pressure with serum TNF-α and neutrophil count in the elderly. J Diabetes Res, 2014; 2014: 972431
30. Sasaki N, Yamamoto H, Ozono R et al: Association of N-terminal pro B-type natriuretic peptide with blood pressure and pulse pressure in elderly people: A cross-sectional population study. Circ J, 2018; 82(8): 2049–54
31. Svensson P, de Faire U, Niklasson U et al: Plasma NT-proBNP concentration is related to ambulatory pulse pressure in peripheral arterial disease. Blood Press, 2005; 14(2): 99–106
32. Madan N, Lee AK, Matsushita K et al: Relation of isolated systolic hypertension and pulse pressure to high-sensitivity cardiac troponin-T and N-terminal pro-B-type natriuretic peptide in older adults (from the Atherosclerosis Risk in Communities Study). Am J Cardiol, 2019; 124(2): 245–52
33. Triposkiadis F, Karayannis G, Giamouzis G et al: The sympathetic nervous system in heart failure: Physiology, pathophysiology, and clinical implications. J Am Coll Cardiol, 2009; 54(19): 1747–62

Conflicts of interest

None.

This work is licensed under Creative Common Attribution-NonCommercial-NoDerivatives 4.0 International (CC BY-NC-ND 4.0)

Indexed in: [Current Contents/Clinical Medicine] [SCI Expanded] [ISI Alerting System] [ISI Journals Master List] [Index Medicus/MEDLINE] [EMBASE/Excerpta Medica] [Chemical Abstracts/CAS]

Journal of
Clinical Medicine

MDPI

Article

Patterns of Dickkopf-3 Serum and Urine Levels at Different Stages of Chronic Kidney Disease

Paulina Dziamałek-Macioszczyk [1,*], Agata Winiarska [1], Anna Pawłowska [1], Paweł Wojtacha [2] and Tomasz Stompór [1]

[1] Department of Nephrology, Hypertension and Internal Medicine, University of Warmia and Mazury in Olsztyn, 10-561 Olsztyn, Poland
[2] Department of Industrial and Food Microbiology, University of Warmia and Mazury in Olsztyn, 10-726 Olsztyn, Poland
* Correspondence: p.dziamalek@gmail.com

Abstract: Dickkopf 3 (Dkk3) is a WNT/β-catenin signaling pathway regulator secreted by tubular epithelial cells upon the influence of different stressors. Recently Dkk3 was described as a biomarker of tubular cell injury and a tool that may estimate the risk of chronic kidney disease (CKD) progression. The data about Dkk3 concentrations at particular stages of CKD are lacking. The aim of this study was to measure serum and urine Dkk3 levels in patients with different 'renal status' and evaluate its role as a biomarker of renal damage. One hundred individuals, aged between 24 and 85 years (mean 53.1 ± 17.1), were enrolled in the study. Five groups of 20 subjects each were recruited based on their kidney function. Serum and urine Dkk3 levels were measured by ELISA. The highest median urinary Dkk3 normalized to urinary creatinine was found in patients with established CKD (7051 pg/mg). It was two times higher in renal transplant patients (5705 pg/mg) than in healthy individuals (2654 pg/mg) and the glomerulonephritis group (2470 pg/mg). Urinary Dkk3 was associated with serum creatinine in participants with established CKD and following transplantation. Our results confirm the potential role of Dkk3 as a biomarker of an ongoing renal injury.

Keywords: chronic kidney disease; renal fibrosis; tubular injury; epithelial-to-mesenchymal transition

check for updates

Citation: Dziamałek-Macioszczyk, P.; Winiarska, A.; Pawłowska, A.; Wojtacha, P.; Stompór, T. Patterns of Dickkopf-3 Serum and Urine Levels at Different Stages of Chronic Kidney Disease. *J. Clin. Med.* **2023**, *12*, 4705. https://doi.org/10.3390/jcm12144705

Academic Editor: Liang Ma

Received: 11 June 2023
Revised: 8 July 2023
Accepted: 13 July 2023
Published: 15 July 2023

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

1. Introduction

Fibrosis and cellular atrophy are well-recognized mechanisms leading to progressive organ failure [1,2]. If this process affects kidneys, it results in chronic kidney disease (CKD), which appears one of the most prevalent health problems worldwide and contributes to premature and excess morbidity and mortality [2–4]. Several proteins engaged in multiple signaling pathways are involved in CKD progression. One such pathway includes WNT/β-catenin signaling which contributes to: cellular proliferation and migration, expression of pro-fibrotic cytokines, and epithelial-to-mesenchymal transition (EMT) [3,5,6]. WNT/β-catenin pathway activation in the short term has a protective effect on tubular cells since it mitigates apoptosis and promotes tubular regeneration (for example, following acute kidney injury [AKI]) [5,7]. On the contrary, constant and prolonged activation of this pathway results in kidney fibrosis in the course of chronic nephropathies, including nephroangiosclerosis, sequelae of AKI (now classified as acute kidney disease and CKD following AKI), proteinuric kidney diseases and renal pathologies with cyst formation [5–9].

The role of WNT/β-catenin pathway in the pathophysiology of CKD is—among other mediators—orchestrated by members of the Dickkopf (Dkk) family proteins. One of them is Dickkopf 3 (Dkk3)—stress-induced glycoprotein secreted by renal tubular epithelial cells. Most Dkk proteins (Dkk1, Dkk2, and Dkk4) inhibit the WNT/β-catenin pathway by interaction with two receptors: lipoprotein receptor-related protein (LPR) 5 and LPR6 [5,8,10,11]. Dkk3 either activates or inhibits WNT/β-catenin signaling depending on the 'tissue context' [5,12,13]. In the kidney, it activates WNT/β-catenin signaling in tubular epithelial

cells (with their reprogramming into proinflammatory phenotype) and promotes fibrosis via lymphocyte T activation [5,12]. A study conducted by Federico et al. revealed that Dkk3 is expressed in the developing kidney, silenced in adulthood, and then re-expressed in response to various stressors [3,12]. Other studies provided evidence that genetic and antibody-mediated deletion of the Dkk3 gene resulted in preserved kidney function in various animal models of CKD [1].

Moreover, recent studies suggest that Dkk3 may serve as the clinical biomarker of renal damage and a prognostic tool for estimating the risk of CKD progression. Schunk et al. described urinary Dkk3 as an indicator of tubular injury and predictor of both short-term glomerular filtration rate (GFR) loss as well as the long-term CKD progression, independent from albuminuria or the underlying cause of CKD in humans [1,3,5]. They also demonstrated that elevated urinary Dkk3 level is associated with an increased risk for AKI and CKD following cardiac surgery [3,5]. Few studies describing Dkk3 as a kidney outcome prognostic tool in autoimmune diseases, namelylupus, and ANCA-associated vasculitis were also published [14,15].

To the best of our knowledge no studies are available that would systematically describe the patterns of serum and urine Dkk3 concentrations in patients representing the major groups of 'kidney' patients, i.e., those with proteinuria in the course of biopsy-proven glomerular pathology, established CKD (with GFR permanently reduced below 60 mL/min/1.73 m^2), kidney transplant recipients and patients with end-stage renal disease (ESRD) treated with hemodialysis. The aim of our study was to measure serum and urine Dkk3 in mentioned groups of patients and to evaluate the possible role of this protein as a biomarker of renal injury and cardiovascular risk.

2. Materials and Methods

2.1. Study Population

One hundred individuals were enrolled in our study. The mean age equaled 53.1 ± 17.1 years (range 24–85 years) and 46% were female. Five groups of subjects (20 people each) were recruited.

Group 1 (G1) of healthy individuals with normal kidney function, i.e., eGFR ≥60 mL/min/1.73 m^2 and normal urine biochemistry and sediment, served as a control group. None of them was previously diagnosed with any chronic disease, including arterial hypertension (AHT) and diabetes (DM).

Group 2 (G2) consisted of patients with preserved kidney function who were admitted to our department to perform a kidney biopsy. Enrollment criteria included eGFR ≥ 60 mL/min/1.73 m^2 and urinary albumin-to-creatinine ratio (UACR) > 0.5 g/g and/or abnormal urine sediment. All patients diagnosed with DM, a history of cardiovascular event, or prior immunosuppressive treatment were excluded (we screened 50 consecutive patients to establish the final group). Biopsy-based diagnoses were as follows: IgA nephropathy (8 patients; 40%), anti-PLA_2R antibody-positive membranous nephropathy (5 patients; 25%), primary focal/segmental glomerulosclerosis (3 patients; 15%), type IV collagen disease (2 patients; 10%), minimal change disease and C3 nephropathy with membrano-proliferative pattern of injury (1 patient i.e., 5% each).

Group 3 (G3) comprised patients with established CKD G3-5 (for those with stage 5—not yet on dialysis). Mean time since CKD diagnosis equaled 3.5 ± 7.8 years. All patients were recruited from our outpatient department. Four patients in this group (20%) had a history of a cardiovascular event, none of them suffered from diabetes nor received immunosuppressive agents at the time of assessment. Causes of CKD were as follows: CKD attributed to cardio-renal syndrome or ischemic/hypertensive nephropathy (13 patients; 60%), chronic glomerulonephritis (3 patients; 15%), nephrolithiasis, autosomal dominant polycystic kidney disease (ADPKD), CKD following chronic pyelonephritis (1 patient or 5% each).

Individuals included in group 4 (G4) were treated in our dialysis center (mean time on dialysis 4.1 ± 7.2 years). All of them were dialyzed 3 times a week for 4.0–4.5 h using High Flux biocompatible FX-class dialyzers (Fresenius, Bad Homburg, Germany). Six patients had a history of cardiovascular events, none of them was treated with immunosuppressive drugs at the time of assessment. End-stage renal disease (ESRD) resulted from glomerulonephritis in 11 patients (55%), ischemic/hypertensive nephropathy in 4 (20%), and ADPKD in 1 (5%). In the case of 4 patients (20%) origin of ESRD remained unknown.

Group 5 (G5) comprised kidney transplant recipients (KTx) with eGFR $\geq$ 60 mL/min/1.73 m^2 (mean time following KTx 3.7 ± 5.4 years and mean time on RRT before KTx 2.6 ± 1.5 years). Only one (5%) had a history of prior cardiovascular events. Immunosuppressive regimens were as follows: steroids/mycophenolate mofetil/tacrolimus (70%), steroids/mycophenolate mofetil/cyclosporine (25%), steroids/mycophenolate sodium/tacrolimus (5%).

2.2. Laboratory Measurements and Ardiovascular Status Assessment

Blood samples were collected from all participants. Morning (first void) urine samples were not collected only from G4 patients because of anuria or negligible urine volume. Biochemical parameters were analyzed at the certified laboratory using Cobas 6000 analyzer (Roche, Basel, Switzerland). Urine albumin used to calculate UACR was measured using immunoturbidimetric assay by the same analyzer. Blood pressure (BP) and heart rate (HR) were measured using the Omron M3 device (Kyoto, Japan) according to the ESH/ESC Guidelines. Pulse wave velocity (PWV) was measured with a Sphygmocor X-cell device (AtCor Medical Pty. Ltd., Sydney, Australia).

2.3. Dkk3 Measurements

Fasting blood for assessing Dkk3 and other parameters was centrifuged immediately after sampling at 4 °C with a speed of 1000× *g* for 15 to 30 min. All serum and urine samples were stored at −80 °C. Then material was thawed at −20 °C for 24 h, 6 °C for the next 24 h, and finally at room temperature for 30 min prior to mixing by a vortex shaker. Dkk3 level was assayed using Human Dickkopf 3 ELISA kit (Bioassay Technology Laboratory, Shanghai, China, Cat. No. E2065Hu). All measurements were repeated twice for each sample with a coefficient of variation (CV) of 0.84%. The CV was calculated as the ratio of the standard deviation to the mean multiplied by 100. We normalized urinary Dkk3 (uDkk3) concentrations to urinary creatinine levels to correct urine volume (uDkk3/creat.).

2.4. Definitions

Body mass index (BMI) was calculated as weight in kilograms divided by the square of height in meters. Arterial hypertension (AHT) was defined as a systolic blood pressure (SBP) $\geq$ 140 mmHg and/or diastolic blood pressure $\geq$ 90 mmHg and/or any antihypertensive therapy, according to 2019 ESH/ESC Guidelines. The estimated glomerular filtration rate (eGFR) was calculated using creatinine-based abbreviated MDRD formula. Cardiovascular event was defined as: the history of myocardial infarction and/or stroke, transient ischaemic attack and/or peripheral vascular disease, and/or diagnosis of heart failure (based on clinical data and confirmed by echocardiography and/or NT-pro-BNP). Chronic kidney disease (CKD) was diagnosed and staged according to the KDIGO criteria.

2.5. Statistical Analysis

All statistical analyses were performed using the SciPy library (version 1.7.1) in Python language (version 3.8.10, Python Software Foundation, Beaverton, OR, USA). Data distribution was checked with the Shapiro–Wilk test. To compare differences between 2 independent groups, Student's *t*-test with Bonferroni correction was applied. A comparison of differences among 5 independent groups was conducted using the Kruskal–Wallis test. Spearman's correlation coefficients were calculated to determine the association between Dkk3 and biochemical parameters. Serum and urine Dkk3 protein amounts were pre-

sented as the median and interquartile range (IQR). Values of $p < 0.05$ were considered statistically significant.

3. Results

The distribution of serum Dkk3 (sDkk3) values was normal only in G1, i.e., healthy individuals. In other groups it was non-normal. The highest median sDkk3 concentration was found in subjects from G1 (79 ng/mL; IQR 30–278). In G2, i.e., a proteinuric group with normal renal function, it was the lowest (34 ng/mL; IQR 30–39) and 57% lower than in G1. In order to reduce the false positive results, differences between the two groups were checked by Student's *t*-test with Bonferroni correction. They were significant only for G2 vs. G4 (dialysis) ($p = 0.02$).

In case of uDkk3/creat. concentrations, data distribution was non-normal in all groups. The highest median uDkk3/creat. value was found in G3 patients (established CKD) (7050 pg/mg; IQR 5090–11,730). It was two times higher in G5 (transplant patients) (5710 pg/mg; IQR 3230–11,680) than in G1 (2650 pg/mg; IQR 1730–8940) and in G2 (2470 pg/mg; IQR 1840–4280) (Figure 1). Differences in uDkk3/creat. were significant when G2 was compared with G3 and with G5 ($p = 0.002$ and $p = 0.04$, respectively). Table 1 shows a comparison of anthropometric and biochemical parameters across all 5 groups. The differences were assessed by the Kruskal–Wallis test and were significant for both sDkk3 and uDkk3 ($p = 0.016$ and $p < 0.001$, respectively), but not for uDkk3/creat ($p = 0.38$).

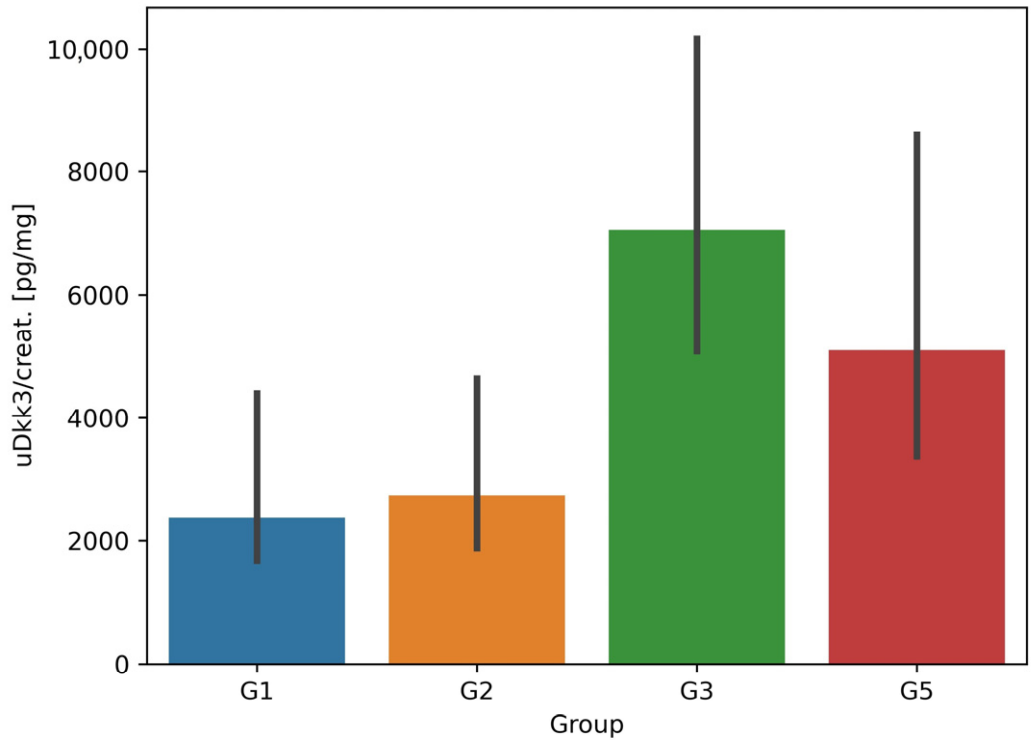


Figure 1. Differences between uDkk3/creat. values within study groups. Data are presented as median (boxes) and interquartile ranges (black vertical lines) because of non-normal distribution. Median uDkk3/creat. is the highest in G3. In G5 is two times higher than in G1 and G2. In G4 was not measured (urine absent or of negligible volume). For *p* values of the differences between respective groups—see text.

Table 1. Comparison of anthropometric and biochemical parameters across all study groups. Data with normal distribution are presented as mean ± standard deviations, and data with non-normal distribution—as median and interquartile ranges. *p*-values were calculated using the Kruskal–Wallis test; *—$p < 0.05$, #—$p < 0.01$, $—$p < 0.005$, &—$p < 0.001$.

Parameter	Unit	G1	G2	G3	G4	G5	*p*-Value
Age	[years]	30.5 (28.0; 38.0)	56.0 (39.0; 63.0)	69.1 ± 11.0	59.3 ± 14.3	50.5 ± 12.3	<0.001 &
Weight	[kg]	71.0 ± 13.6	83.7 ± 12.5	82.5 ± 15.4	75.9 ±12.7	77.4 ± 12.8	0.026
BMI	[kg/m^2]	24.3 ± 3.7	27.0 ± 4.0	28.7 (26.4; 31.2)	26.1 (23.9; 28.5)	26.9 ± 3.2	0.003 $
SBP	[mmHg]	122 ± 8	141 ± 20	144 ± 17	145 ± 20	133 ± 15	<0.001 &
DBP	[mmHg]	77 (70; 85)	86 ± 13	84 ± 12	81 ± 12	83 (80; 90)	0.068
HR	[beats/min]	75.4 ± 10.1	78.0 (70.0; 81.0)	75.7 ± 13.2	78.0 (76.0; 80.0)	76.8 ± 7.9	0.596
PWV	[m/s]	7.57 ± 1.27	8.54 ± 1.80	11.56 ± 1.77	8.30 (7.65; 9.45)	9.41 ± 2.13	<0.001 &
Total Cholesterol	[mg/dL]	179 ± 28	212 (184; 259)	169 ± 40	148 ± 29	171 ± 33	<0.001 &
LDL	[mg/dL]	104 ± 32	143 (112; 178)	102 ± 27	87 ± 29	84 ± 23	<0.001 &
HDL	[mg/dL]	64 ± 17	56 (46; 67)	52 ± 12	45 ± 16	65 ± 19	0.005 #
Triglycerides	[mg/dL]	101 ± 38	160 (94; 184)	138 (112; 191)	168 ± 71	139 (127; 169)	0.003 $
Creatinine	[mg/dL]	0.85 ± 0.11	0.90 (0.80; 1.00)	1.70 (1.30; 2.25)	8.22 ± 2.71	1.00 (0.90; 1.03)	<0.001 &
eGFR	[ml/min]	86.7 (79.0; 97.5)	85.7 ± 23.7	32.8 ± 11.3	6.3 (4.7; 8.3)	69.4 (62.6; 82.9)	<0.001 &
Urea	[mg/dL]	26.9 ± 6.2	36.2 ± 11.5	60.5 (54.3; 94.5)	118.1 ± 36.5	36.5 ± 7.5	<0.001 &
UACR	[mg/g]	2.7 (1.3; 2.7)	960.6 (369.3; 2238.1)	13.3 (3.5; 556.6)	-	3.5 (1.3; 5.3)	<0.001 &
Troponin T	[ng/mL]	0.0040 (0.0030; 0.0043)	0.0080 (0.0048; 0.014)	0.016 (0.009; 0.033)	0.053 (0.036; 0.083)	0.009 (0.007; 0.014)	<0.001 &
CK-MB	[U/L]	12.5 (11.0; 16.3)	14.0 (12.0; 17.0)	14.0 (12.0; 18.3)	11.58 ± 3.20	17.6 ± 5.6	0.002 $
NT-proBNP	[pg/mL]	40 ± 19	95 (38; 261)	457 (237; 2896)	6420 (3317; 14,187)	141 (107; 164)	<0.001 &
sDkk3	[ng/mL]	133 ± 117	34 (30; 39)	40 (30; 81)	50 (42; 98)	46 (36; 70)	0.016 *
uDkk3	[ng/mL]	3.33 ± 0.90	4.16 ± 0.66	4.57 (4.19; 5.10)	-	4.11 ± 0.44	<0.001 &
uDkk3/creat.	[pg/mg]	2650 (1730; 8940)	2470 (1840; 4280)	7050 (5090; 11,730)	-	5710 (3230; 11,680)	0.38

To verify an association between parameters, Spearman's correlation analysis was performed. We found a correlation between uDkk3 vs. troponin T (r = 0.44, $p = 0.042$) (Figure 2A) and CK-MB (r = 0.45, $p = 0.008$) (Figure 2B) in G1, uDkk3 and CK-MB in G2 subjects (r = 0.42, $p = 0.028$). In G2 patients, the group characterized by proteinuria and normal renal function, uDkk3 and uDkk3/creat. correlated inversely with UACR (r = −0.50, $p = 0.009$ and r = −0.39, $p = 0.007$ respectively) (Figure 2C). We also found an inverse correlation between uDkk3/creat. and UACR in G5 patients (r = −0.62, $p = 0.001$) (Figure 2D). Interestingly, uDkk3 was associated with serum creatinine in G3 (r = 0.41, $p = 0.009$) (Figure 2E) and G5 patients (r = 0.39, $p = 0.043$) (Figure 2F), i.e., those with more advanced CKD. These results together with the fact that the highest uDkk3/creat. values were found in G3 and G5 suggest that urinary Dkk3 may indicate ongoing renal injury and can reflect the advancement of kidney damage. Only in G2 uDkk3/creat. correlated with age (r = −0.42, $p = 0.004$).

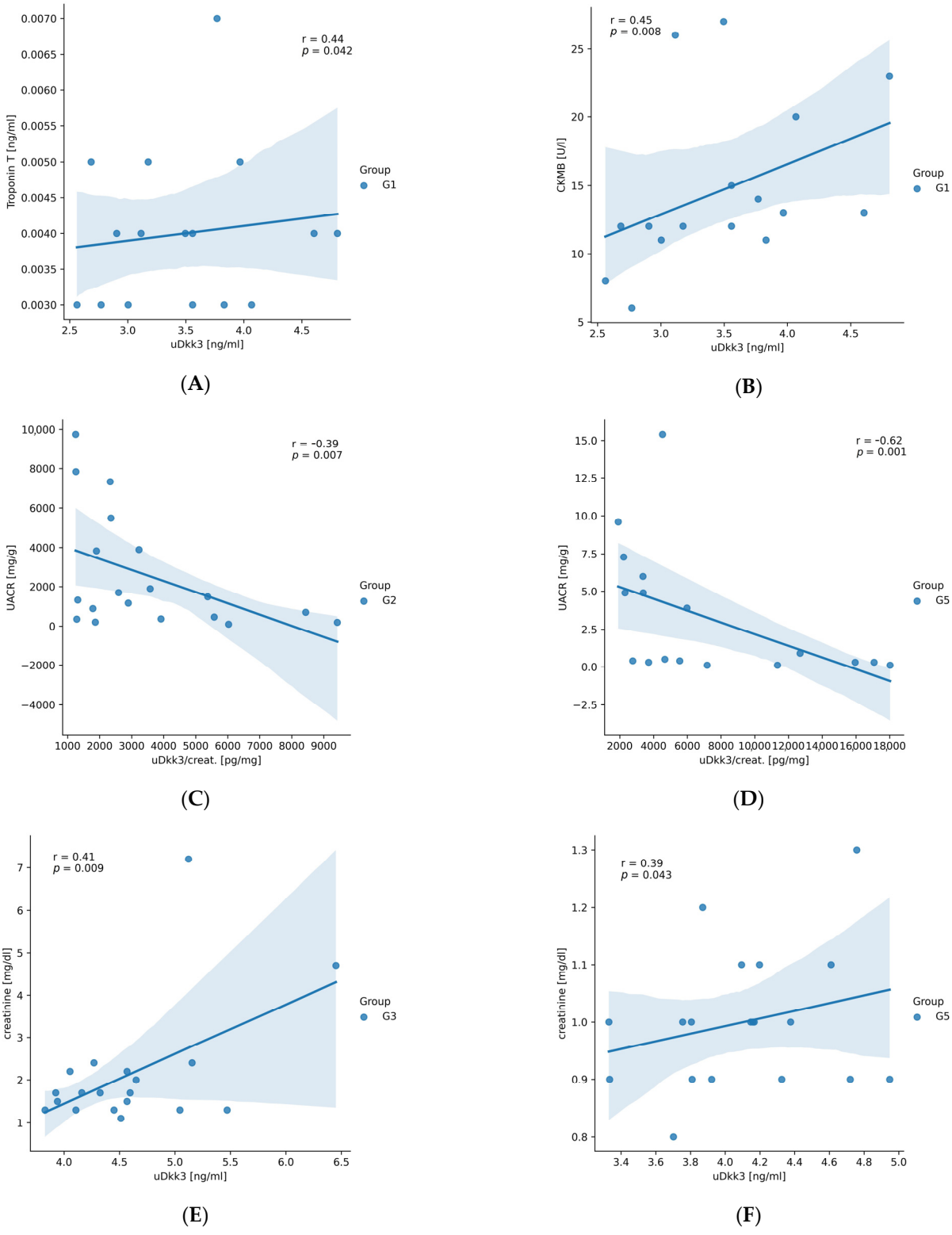


Figure 2. Spearman's correlation analysis between uDkk3 and different parameters: (**A**) correlation between uDkk3 and troponin T in group 1, (**B**) correlation between uDkk3 and CK-MB in group 1, (**C**) correlation between uDkk3/creat. and UACR in group 2, (**D**) correlation between uDkk3/creat. and UACR in group 5, (**E**) correlation between uDkk3 and creatinine in group 3, (**F**) correlation between uDkk3 and creatinine in group 5. r-Spearman's correlation coefficient; N = 20 for each group.

4. Discussion

Recently published data suggest that urinary Dkk3 is a sensitive indicator of ongoing tubular injury independent of eGFR and albuminuria [1,3]. Sustained increase of this protein via WNT/β-catenin pathway activation alters TEC phenotype and promotes their transformation into pro-fibrotic and pro-inflammatory phenotype. That in turn triggers EMT, leads to irreversible changes in kidney structure and results in the development and progression of CKD [1,3,5,6]. Our results demonstrate that uDkk3/creat. level is the highest in G3, a group with advanced CKD not yet on RRT (mean eGFR 32.8 ± 11.3 mL/min/1.73 m^2). uDkk3/creat. levels in patients with eGFR ≥ 60 mL/min/1.73 m^2 (G1, G2, and G5) were much lower, which is in agreement with the above-mentioned hypothesis and with results published previously by other research groups. In the study conducted by Zewinger et al. median uDkk3/creat. value was also higher in the CKD cohort than in the general population (431 versus 33 pg/mg). Moreover, they observed that urinary Dkk3/creat. level above 4000 pg/mg was independently associated with a mean annual decline in eGFR of 7.6% over 12 months [1]. In our study median uDkk3/creat. exceeded this threshold in G3 and G5 patients (7050 pg/mg and 5710 pg/mg, respectively). In the case of G3, it may point to an advanced damage of TEC and progressive renal fibrosis, as well as reflect poor prognosis. As G5 is a group after KTx but with otherwise preserved GFR, we hypothesize that in this case elevation of uDkk3 may reflect the ongoing albeit not yet clinically apparent renal damage and predict the long-term risk of CKD and future impaired graft function (in KTx patients TEC injury is always multifactorial and may include chronic immunological response ['chronic rejection'], calcineurin inhibitor toxicity, arterial hypertension, and many others [16]). Of note, UACR in KTx patients was well below not only the threshold defined as microalbuminuria, but within the range defined as 'low-grade albuminuria' (i.e., 3 mg/g) which confirms the excellent graft health in studied transplant patients. Moreover, our study revealed a correlation between uDkk3 and serum creatinine level only in G3 and G5, which supports the potential association between uDkk3 and progressive renal function impairment in those two particular groups.

UACR is a marker of endothelial dysfunction, commonly used in everyday practice [17,18]. Schunk et al. reported that uDkk3/creat. correlated with albuminuria in patients with CKD but not in individuals from the general population. UACR > 300 mg/g was associated with higher uDKK3/creat. levels than UACR < 30 mg/g, although 48.6% of subjects with higher albuminuria had low or even undetectable levels of uDkk3 [3]. Some other studies demonstrated no correlation between uDkk3 and proteinuria in CKD cohorts [12,19]. We observed an inverse correlation between uDkk3 and UACR in G2 (the only proteinuric group) and G5 (despite the fact that UACR was extremely low in this group). Taking into account that the molecular mass of the circulating glycosylated form of Dkk3 and albumin are similar (i.e., 60–70 kDa vs. 67 kDa) [3], it seems ambiguous why urinary excretion of these proteins is so different. A likely explanation is that increased leakage through the damaged glomerular filtration barrier is further modified by tubular reabsorption, fine-tuning the final excretion. Since uDkk3 is mostly derived from tubular secretion, it may not necessarily be linked with its serum concentration. Further research is needed to determine factors that affect glomerular filtration and tubular handling of both proteins.

Piek et al. described sDkk3 as the predictor of a new-onset CKD in patients with low urinary albumin excretion (<6.6 mg/24 h) [20]. In our study, sDkk3 correlated with CK-MB and LDL in G1, i.e., a group of patients with low albuminuria (median UACR 2.7 mg/g) and without cardiovascular disease or even the risk for CVD. This correlation was not confirmed in other patient groups. As Dkk3 is also known for its proatherosclerotic properties [13], more detailed studies are needed to confirm and elucidate its significance.

Our study suffers from several limitations. One of them is a cross-sectional nature of observation with a lack of follow-up. Cardiovascular status assessment was based only on cardiac biomarkers and PWV, with only a few patients with echocardiography assessment available. The size of each subgroup (N = 100 in total, 20 subjects each) is low, nevertheless,

to the best of our knowledge, this is the first study that comprehensively addressed the value of serum and urine Dkk3 as novel biomarker in patients within the whole spectrum of renal disease. It is also important that in all patients from the G2 group we established an exact (biopsy-based) diagnosis of CKD (and we did so for most of the G5 group subjects).

5. Conclusions

Urinary Dkk3 level was the highest in participants with advanced CKD and correlated with serum creatinine level and eGFR only in individuals with CKD and those with functioning kidney grafts. Our results confirm the potential role of Dkk3 as a biomarker of an ongoing renal injury. Future studies are needed to assess the utility of this protein in the short and long-term prognosis of CKD progression.

Author Contributions: Conceptualization, T.S. and P.D.-M.; methodology, T.S., P.W. and P.D.-M.; validation, T.S.; formal analysis, T.S.; investigation, P.D.-M., A.W., A.P. and P.W.; resources, T.S. and A.P.; data curation, P.D.-M. and A.W.; writing-original draft preparation, P.D.-M.; writing-review and editing, T.S.; visualization, P.D.-M.; supervision, T.S.; project administration, P.D.-M.; funding acquisition, T.S. All authors have read and agreed to the published version of the manuscript.

Funding: This research was funded by statutory grant form University of Warmia and Mazury in Olsztyn (25.016.002-25.620.023-300).

Institutional Review Board Statement: The study was conducted in accordance with the Declaration of Helsinki, and approved by the Ethics Committee of the local Chamber of Physician in Olsztyn (WMIL-KB/413/2017, 20 July 2017).

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

Data Availability Statement: Data sharing is not applicable to this article.

Acknowledgments: The authors would like to thank Jan Macioszczyk for help with statistical analysis and graphical content.

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

References

1. Zewinger, S.; Rauen, T.; Rudnicki, M.; Federico, G.; Wagner, M. Dickkopf-3 (DKK3) in Urine Identifies Patients with Short-Term Risk of eGFR Loss. *J. Am. Soc. Nephrol.* **2018**, *29*, 2722–2733. [CrossRef] [PubMed]
2. Boor, P.; Ostendorf, T.; Floege, J. Renal fibrosis: Novel insights into mechanisms and therapeutic targets. *Nat. Rev. Nephrol.* **2010**, *6*, 643–656. [CrossRef] [PubMed]
3. Schunk, S.J.; Speer, T.; Petrakis, I.; Fliser, D. Dickkopf 3—A novel biomarker of the 'kidney injury continuum'. *Nephrol. Dial. Transplant.* **2021**, *36*, 761–767. [CrossRef] [PubMed]
4. Falodia, J.; Singla, M.K. CKD epidemiology and risk factors. *Clin. Queries Nephrol.* **2012**, *1*, 249–252. [CrossRef]
5. Schunk, S.J.; Floege, J.; Fliser, D.; Speer, T. WNT–β-catenin signaling—A versatile player in kidney injury and repair. *Nature* **2021**, *17*, 172–184.
6. Lipphardt, M.; Dihazi, H.; Jeon, N.L.; Dadafarin, S.; Ratliff, B.B.; Rowe, D.W.; Müller, G.A.; Goligorsky, M.S. Dickkopf-3 in aberrant endothelial secretome triggers renal fibroblast activation and endothelial–mesenchymal transition. *Nephrol. Dial. Transplant.* **2019**, *34*, 49–62. [CrossRef] [PubMed]
7. Fang, X.; Hu, J.; Chen, Y.; Shen, W.; Ke, B. Dickkopf-3: Current Knowledge in Kidney Diseases. *Front. Physiol.* **2020**, *11*, 533344. [CrossRef] [PubMed]
8. Wong, D.W.L.; Yiu, W.H.; Wu, H.J.; Li, R.X.; Liu, Y.; Chan, K.W.; Leung, J.C.K.; Chan, L.Y.Y.; Lai, K.N.; Tang, S.C.W. Downregulation of renal tubular Wnt/β-catenin signaling by Dickkopf-3 induces tubular cell death in proteinuric nephropathy. *Cell Death Dis.* **2016**, *7*, e2155. [CrossRef] [PubMed]
9. Liu, M.; Shi, S.; Senthilnathan, S.; Yu, J.; Wu, E.; Bergmann, C.; Zerres, K.; Bogdanova, N.; Coto, E.; Deltas, C.; et al. Genetic variation of DKK3 may modify renal disease severity in ADPKD. *J. Am. Soc. Nephrol.* **2010**, *21*, 1510–1520. [CrossRef] [PubMed]
10. Li, H.; Liu, X.; Li, X.; Zhao, Y.; Wang, Q.; Zhong, H.; Liu, D.; Yuan, C.; Zheng, T. Dickkopf1 (Dkk1) Alleviates Vascular Calcification by Regulating the Degradation of Phospholipase D1 (PLD1). *J. Cardiovasc. Trans. Res.* **2022**, *15*, 1327–1339. [CrossRef] [PubMed]
11. Niehrs, C. Function and biological roles of the Dickkopf family of Wnt modulators. *Oncogene* **2006**, *25*, 7469–7481. [CrossRef] [PubMed]

12. Federico, G.; Meister, M.; Mathow, D.; Heine, G.H.; Moldenhauer, G.; Popovic, Z.H.; Nordström, V.; Kopp-Schneider, A.; Hielscher, T.; Nelson, P.J.; et al. Tubular Dickkopf-3 promotes the development of renal atrophy and fibrosis. *JCI Insight* **2016**, *1*, e84916. [CrossRef] [PubMed]
13. Cheng, W.L.; Yang, Y.; Zhang, X.J.; Guo, J.; Gong, J.; Gong, F.H.; She, Z.K.; Huang, Z.; Xia, H.; Li, H. Dickkopf-3 Ablation Attenuates the Development of Atherosclerosis in ApoE-Deficient Mice. *J. Am. Heart Assoc.* **2017**, *6*, e004690. [CrossRef] [PubMed]
14. Sciascia, S.; Barinotti, A.; Radin, M.; Cecchi, I.; Menegatti, E.; Terzolo, E.; Rossi, D.; Baldovino, S.; Fenoglio, R.; Roccatello, D. Dickkopf Homolog 3 (DKK3) as a Prognostic Marker in Lupus Nephritis: A Prospective Monocentric Experience. *J. Clin. Med.* **2022**, *11*, 2977. [CrossRef] [PubMed]
15. Satrapova, V.; Sparding, N.; Genovese, F.; Karsdal, M.A.; Bartonova, L.; Frausova, D.; Honsova, E.; Kollar, M.; Suchanek, M.; Koprivova, H.; et al. Biomarkers of fibrosis, kidney tissue injury and inflammation may predict severity and outcome of renal ANCA–associated vasculitis. *Front. Immunol.* **2023**, *14*, 1101. [CrossRef] [PubMed]
16. Nankivell, B.J.; Borrows, R.J.; Fung, C.L.S.; O'Connell, P.J.; Allen, R.D.; Chapman, J.R. The natural history of chronic allograft nephropathy. *N. Engl. J. Med.* **2003**, *349*, 2326–2333. [CrossRef] [PubMed]
17. Eknoyan, G.; Lameire, N.; Eckardt, K.; Kasiske, B.; Wheeler, D.; Levin, A.; Coresh, J.J.K.I.; Abboud, O.I.; Adler, S.; Agarwal, R.; et al. KDIGO 2012 clinical practice guideline for the evaluation and management of chronic kidney disease. *Kidney Int.* **2013**, *3*, 5–14.
18. Ix, J.H.; Shlipak, M.G. The promise of tubule biomarkers in kidney disease: A review. *Am. J. Kidney Dis.* **2021**, *78*, 719–727. [CrossRef] [PubMed]
19. Gröne, E.F.; Federico, G.; Nelson, P.J.; Arnold, B.; Gröne, H.J. The hormetic functions of Wnt pathways in tubular injury. *Arch.-Eur. J. Physiol.* **2017**, *469*, 899–906. [CrossRef] [PubMed]
20. Piek, A.; Smit, L.; Suthahar, N.; Bakker, S.J.L.; de Boer, R.A.; Silljé, H.H.W. The emerging plasma biomarker Dickkopf-3 (DKK3) and its association with renal and cardiovascular disease in the general population. *Sci. Rep.* **2021**, *11*, 8642. [CrossRef] [PubMed]

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

ACTA ABP BIOCHIMICA POLONICA

Vol. 66, No 4/2019
389–392
https://doi.org/10.18388/abp.2019_2844

Review

Versatility of USP18 in physiology and pathophysiology

Paulina Dziamałek-Macioszczyk[1]✉, Joanna M. Harazny[2] and Tomasz Stompór[1]

[1]Department of Nephrology, Hypertension and Internal Medicine, University of Warmia and Mazury, Olsztyn, Poland; [2]Department of Pathophysiology, University of Warmia and Mazury, Olsztyn, Poland

Ubiquitin-specific peptidase 18 (USP18) is a multifunctional protein and its roles are still being investigated. This enzyme removes ubiquitin-like molecules from their substrates and the only known interferon-stimulated gene 15 (ISG15) specific protease. Apart from its enzymatic function, it also inhibits interferon type I and III signalling pathways. USP18 is known to regulate multiple processes, such as: cell cycle, cell signalling and response to viral and bacterial infections. Moreover, it contributes to the development of several autoimmune diseases and carcinogenesis, and recently was described as a cardiac remodelling inhibitor. This review summarizes the current knowledge on USP18 functions, highlighting its contribution to the development of heart failure, given the fact that this disease's etiology is now considered to be inflammatory in nature.

Key words: peptidase, deISGylation, interferon, cardiac remodelling, heart failure, biomarker

Received: 2 July, 2019; revised: 03 October, 2019; accepted: 23 October, 2019; available on-line: 20 November, 2019

✉e-mail: p.dziamalek@gmail.com

Abbreviations: DCs, dendritic cells; IFN, interferon; IFNAR, interferon type I receptor; ISG15, interferon-stimulated gene 15; ISRE, interferon-stimulated response element; JAK1, Janus kinase 1; JAK-STAT, Janus-activated kinase/signal transducer and activator of transcription; MAPKs, mitogen-activated protein kinases; ROS, reactive oxygen species; STING, stimulator of interferon genes; TNF-α, tumour necrosis factor alpha; T1D, type 1 diabetes; TYK2, tyrosine kinase 2; Ubls, ubiquitin-like molecules; USP18, Ubiquitin-specific peptidase 18; UBPs, ubiquitin-specific proteases

INTRODUCTION

Ubiquitin-specific peptidase 18 (USP18) is a cysteine protease of a dual nature. As an enzyme, it cleaves ubiquitin-like molecules (Ubls) from their target proteins, while as a substrate for an interferon (IFN) receptor it acts as an IFN type I and III signalling suppressor (Hoeller *et al.*, 2006; Manini at al., 2013; Hong *et al.*, 2014; Ketscher & Knobeloch, 2015; Honke *et al.*, 2016; MacParland *et al.*, 2016; An *et al.*, 2017; Basters *et al.*, 2017; Mustachio *et al.*, 2017; Basters *et al.*, 2018; Shaabani *et al.*, 2018; Gu *et al.*, 2019). These diverse functions make USP18 an interesting object for research and many studies describing this unique molecule have been published recently. Among others, its contribution to cell signalling, response to viral and bacterial infections, development of several malignancies and development of autoimmune diseases have been described (Liu *et al.*, 1999; Ritchie *et al.*, 2004; Hoeller *et al.*, 2006; Catic *et al.*, 2007; Duex & Sorkin, 2009; François-Newton *et al.*, 2011; Murray *et al.*, 2011; Burkart *et al.*, 2012; Guo *et al.*, 2012; Yim *et al.*, 2012; Coit *et al.*, 2013; Honke *et al.*, 2013; Hong *et al.*, 2014; Zhang *et al.*, 2015; Honke *et al.*, 2016; Shaabani *et al.*, 2016; Ying *et al.*, 2016; Zhang at al., 2016; An *et al.*, 2017; Arimoto *et al.*, 2017; Basters *et al.*, 2017; Mustachio *et al.*, 2017; Basters *et al.*, 2018; Shaabani *et al.*, 2018; Gu *et al.*, 2019). USP18 was also found to serve as an inhibitor of cardiac remodelling (Ying *et al.*, 2016). This review summarizes the abovementioned functions, emphasizing the role of USP18 as a biomarker with potential in cardiovascular disease monitoring.

CHARACTERISTICS

The USP18 protein belongs to a large family of ubiquitin-specific proteases (UBPs). Initially, the Usp18 gene was called Ubp43 since it encodes a 43-kDa protein with UBP properties, i.e. it cleaves ubiquitin or Ubls from their target molecules (Honke *et al.*, 2016). Initially, USP18 was cloned from mice expressing leukemia-fusion protein AML1-ETO and, afterwards, from virus-infected porcine alveolar macrophages and human melanoma cell lines (Honke *et al.*, 2016; Gu *et al.*, 2019).

Human USP18 has two isoforms which differ in the N-terminal region of the polypeptide chain. Both isoforms are localized in the cell cytoplasm and one of them, USP18-sf, is also detected in the nucleus. This is the main nuclear ISG15-specific protease, which suggests that USP18-sf may exert additional, more specific functions (Burkart *et al.*, 2012; Honke *et al.*, 2016). High expression of USP18 was detected in the liver, thymus and spleen. Lower amounts were found in the bone marrow, lungs and adipose tissue. Synthesis of USP18 is strongly induced by IFNs I and III, viral infections, lipopolysaccharide, tumour necrosis factor alpha (TNF-α) and genotoxic stress (Honke *et al.*, 2016).

USP18 presents the same three-dimensional structure as other USPs. It consists of three catalytic domains, described as: the finger, the palm and the thumb. The area between the thumb and palm domains contains a catalytic triad composed of a cysteine, an aspartate and a histidine residue. Unlike other USPs, this catalytic core is the only protein interaction domain in the USP18 structure (Basters *et al.*, 2018).

USP18 is the only known protease which specifically deconjugates interferon-stimulated gene 15 (ISG15), one of the Ubls, from modified proteins *in vivo*. According to some reports, under specific circumstances USP18 also acts as an ubiquitin protease. DeISGylation, which is analogous to deubiquitination, was shown to influence antiviral response mechanisms, genome stability and carcinogenesis. Similarly to USP18, ISG15 synthesis is induced by IFN-I, especially during viral and bacterial infections (Zhang & Zhang, 2011). The role of this protein is not fully understood, but it is known that ISGylation of viral proteins inhibits replication of many virus-

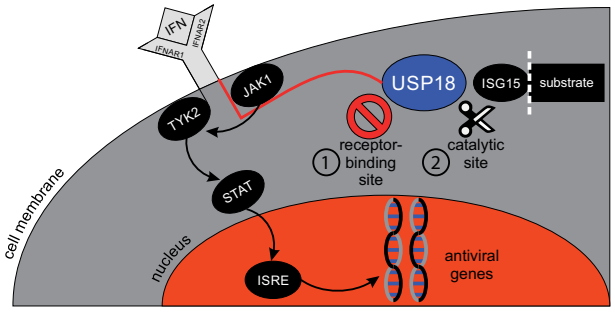


Figure 1. Schematic presentation of two types of USP18 activity: 1) IFN signalling pathway inhibition through binding with IFNAR2 subunit of type I IFN receptor and blocking activation of JAK-STAT cascade; 2) enzymatic deISGylating function.
Abbreviations: IFN, interferon; IFNAR1, first subunit of type I interferon receptor; IFNAR2, second subunit of type I interferon receptor; ISG15, interferon-stimulated gene 15; ISRE, interferon-stimulated response element; JAK1, Janus kinase 1; STAT, signal transducer and activator of transcription; TYK2, tyrosine kinase 2; USP18, ubiquitin-specific peptidase 18 (for details see text).

es. Free ISG15 was also described as an antiviral factor (Zhao *et al*., 2013).

Another USP18 role seems to be structurally separated from its enzymatic function. It attenuates type I IFN signalling by interaction with the IFNAR2 subunit of the IFN-I receptor (IFNAR) (Basters *et al*., 2017; Basters *et al*., 2018). As a result, it inhibits the Janus-activated kinase/signal transducer and activator of transcription (JAK-STAT) pathway, acting in a negative feedback loop (see Fig. 1). Type I IFNs are well-known for their antiviral, antineoplastic and immunomodulatory effects, which makes USP18 a factor that facilitates viral infections and that may promote carcinogenesis, but also prevents INF hypersensitivity and autoimmune disease development under physiological conditions (Arimoto *et al*., 2017). Recent studies described this activity as protease-independent. USP-18 knock-out mice, which presented an increased IFN response and died after administration of the IFN-inducing agent poly I:C, were saved by an enzymatically inactive USP18. Moreover, IFN hypersensitivity and interferonopathy were not observed in mice with USP18 inactivation that was limited to its catalytic properties (Basters *et al*., 2018).

INFECTIONS

USP18 is known for its proviral effect, which is exerted by a negative regulation of the IFN-I signalling pathway. IFN-I binds to IFNAR on the cell surface, which due to its kinase activity triggers Janus kinase 1 (JAK1) and tyrosine kinase 2 (TYK2) activation, which then initiates STATs phosphorylation. STATs activate the interferon-stimulated response element (ISRE) in the nucleus which, in turn, starts antiviral gene transcription. USP18 binds with IFNAR2, one of the two IFNAR subunits, and prevents JAK1 phosphorylation (Honke *et al*., 2016; Basters *et al*., 2017; Gu *et al*., 2019). Apart from IFNs, other factors, such as TNF-α or LPS, can also boost USP18 expression, reduce antiviral defence and facilitate opportunistic viral infections. Upon absence of USP18, phosphorylation of STATs is enhanced and there is an increase in expression of genes which encode antiviral proteins, cytokines, chemokines and genes for proteins that contribute to antigen presentation. Such an effect was described for viruses, such as: LCMV, VSV, HBV, HCV, HIV, Coronavirus, Sindbis, influenza B virus and the Herpes virus. Isg15-deficient mice do not present enhanced susceptibility to viruses, so this activity is not associated with the ISGylation process. Nevertheless, USP18's influence on antiviral mechanisms is ambiguous. Although IFN-I limits viral spread in the organism, it diminishes presentation of viral antigens to immune cells, which impairs the adaptive immune system response. Without efficient antigen presentation, the immune reaction is impaired and this can lead to the autoimmune process (Honke *et al*., 2016).

Another issue is USP18's complicated impact on antibacterial mechanisms. It has been demonstrated that Usp18 knock-out mice are characterized by less-pronounced growth of *Salmonella typhimurium* than the control animals. In contrast, mice with a missense mutation in USP18 ($Usp18_{lty9}$) present lethal susceptibility to *S. typhimurium* (Honke *et al*., 2016). According to Shaabani *et al*., IFN-I signalling attenuates the immune response in the course of several primary bacterial infections and promotes a secondary bacterial invasion following viral infections. Lack of IFN-I signalling leads to a reduction in bacterial replication and survival. Interestingly, it has been demonstrated that USP18 is responsible for the harmful effect of IFN-I during *Listeria monocytogenes* or *Staphylococcus aureus* infection of mice. The mechanism of this phenomenon is different than those described above. In this case, the authors suggest that USP18 inhibits the antibacterial TNF-α signalling and, as a result, reduces reactive oxygen species (ROS) production and facilitates proliferation of bacteria. IFNAR1 or Usp18 knock-out CD11c-Cre+ cells were observed to mitigate bacterial titres in several organs and to prolong survival. This phenomenon was proven to be independent of the IFNAR2 binding and isopeptidase domains (Shaabani *et al*., 2018). It is possible that USP18 may also involve other USPs and suppress TNF-α in different pathways. Such a relationship was described for USP20 which deubiquitinates mitochondrial adaptor protein STING (stimulator of interferon genes) (Zhang *et al*., 2016; Shaabani *et al*., 2018). However, other authors have shown that this recruitment, leading to STING stabilization, sustains cellular antiviral responses and decreases susceptibility to HSV-1 (Zhang *et al*., 2016). Without a doubt, further studies are needed to define this effect in more detail.

CARCINOGENESIS

Several studies have been published on the role of USP18 in carcinogenesis. Their diverse results suggest that USP18 can act both, as an oncogene and tumour suppressor, and that the ultimate effect depends on the tissue type and cellular background (Mustachio *et al*., 2017).

USP18's oncogenic role was defined for several types of tumours. Mustachio and others (Mustachio *et al*., 2017) had shown that expression of USP18 in the lung cancer tissue is higher than in a normal lung. Likewise, loss of USP18 expression led to reduced lung cancer cell growth, induced apoptosis and increased cell response to treatment. That and other studies revealed that USP18 directly stabilizes cyclin D1 and KRAS proto-oncogene GTPase, which are over-expressed or continuously activated in the lung cancer cells (Hoeller *et al*., 2006; Guo *et al*., 2012; Mustachio *et al*., 2017). Similar effects were described for the breast cancer cell line MCF-7 and glioblastoma cells (Honke *et al*., 2016). According to Guo and others (Guo *et al*., 2012) acute promyelocytic leukemia cells also present increased levels of USP18 and a

gain of USP18 expression stabilized the oncogenic protein PML/RARα in these cells (Ritchie *et al*., 2004). In turn, Duex and Sorkin had stated that due to Usp18 knock-out, EGFR expression was reduced up to 50–80% in several cell lines. Since this effect was assigned to EGFR mRNA translation inhibition, USP18 contribution to squamous cell carcinoma development is also possible (Duex & Sorkin, 2009).

On the contrary, Hong and others (Hong *et al*., 2014) had found an opposite role of USP18 in the context of cancer development. They stated that after induction by INF-γ in tumour cells, USP18 inhibits carcinogenesis and enhances anti-neoplastic immunity. Moreover, it not only modifies malignant cells, but also the tumour microenvironment, by regulating the synthesis of IFN-γ and activation of antigen-specific cytotoxic T lymphocytes (Hong *et al*., 2014; Honke *et al*., 2016). Subsequent studies may elucidate and highlight this controversial nature of USP18.

AUTOIMMUNE DISEASES

Another group of pathologies influenced by the USP18 and IFN-I pathway are autoimmune diseases. Type 1 diabetes (T1D) is a well-described example. A crucial role in its pathogenesis is played by dendritic cells (DCs), which are known for their considerable USP18 expression. In terms of a viral infection, when viral antigens are homologous to epitopes of the β islet cells, DCs present autoantigen to autoreactive CD8+ cells, which induce islet damage. It was shown in mice that the depletion of DCs diminishes viral replication and autoantigen presentation, which inhibits T1D development. This effect is thought to be explained by augmented IFN-I signalling and effective antiviral defence, resulting from suppression of USP18. Therefore, USP18 increases viral replication in DCs and can trigger an autoimmune reaction (Honke *et al*., 2013; Honke *et al*., 2016). On the contrary, other studies revealed that sufficient USP18 synthesis in β islet cells is mandatory to prevent them from autoimmune damage and the development of T1D. Increased expression of USP18 inhibits insulitis by limiting synthesis of proinflammatory chemokines. Moreover, USP18 influences the mitochondrial pathway of cell apoptosis and prevents IFN-induced β cell apoptosis (Santin *et al*., 2012; Honke *et al*., 2016).

Coit and others (Coit *et al*., 2013) had suggested that USP18 participates in pathogenesis of lupus. They noted a substantial hypomethylation of genes regulated by IFN in naïve T cells obtained from lupus patients; this included, among others, the Usp18 gene. These genes appeared to be over-expressed in all CD4+ T cells. Based on these findings, the authors hypothesised IFN-I hyperresponsiveness in lupus T cells.

Furthermore, some studies suggest that USP18 is crucial for the balanced function of microglia in the central nervous system. Without USP18, STATs activation is prolonged, which may lead to microgliopathy. USP18 may also be involved in pathogenesis of multiple sclerosis (Honke *et al*., 2016).

CARDIAC REMODELLING

Ying and others (Ying *et al*., 2016) had shed a new light on activity of USP18 and extended its action to the cardiovascular system. Their study demonstrated that USP18 inhibits cardiac remodelling and protects from the development of heart failure. In hearts of patients with dilated cardiomyopathy, USP18 expression was significantly higher than in hearts from healthy donors. Moreover, in murine hearts exposed to increased afterload, augmented expression of USP18 in cardiomyocytes was related to less pronounced myocardial fibrosis, reduced left ventricular wall hypertrophy and delayed onset of heart failure (Ying *et al*., 2016). In contrast, USP18-deficient mice presented advanced cardiac remodelling (Ying *et al*., 2016). According to Ying and others (Ying *et al*., 2016) cardioprotective effect of USP18 is a consequence of the TAK1-p38-/JNK1/2 signalling pathway inhibition, which is an example of MAPKs (mitogen-activated protein kinases) – dependent pathway (Ying *et al*., 2016). MAPKs are involved in myocardial hypertrophy and heart failure pathogenesis, since upon prolonged pressure overload they contribute to structural modification of the contractile proteins (Dick & Epelman, 2016). Our unpublished data indicate that the serum USP18 level is up to 10-fold higher in drug-naïve patients with early stage systolic-diastolic arterial hypertension than in normotensive subjects, and also greater than in patients with isolated diastolic hypertension. It is known that inflammatory processes take part in the development of heart failure and contribute to cardiac injury and repair (Dick & Epelman, 2016). Considering the above-mentioned facts, USP18 may indicate a counter-regulatory regenerative process to increased afterload, aiming at maintaining the proper cardiomyocyte structure and contractile function. In these terms, elevated serum USP18 level may result from intensified synthesis and/or cardiomyocyte cellular membrane damage and release into serum. If this thesis is true, it might be used as a cardiac remodelling biomarker in the future.

CONCLUSIONS

USP18 is a multifunctional protein which exerts both, enzymatic and IFN-I suppressing functions. Due to this multi-faceted nature, USP18 participates in several intra- and extracellular pathways, response to viral and bacterial infections, oncogenesis and autoimmune disease development. Although its role is not fully understood, recent studies suggest that USP18 may also serve as a cardiac remodelling biomarker. Expression of USP18 is significantly higher in damaged cardiomyocytes than in healthy cells. Moreover, it suppresses hypertrophy and fibrosis of the cells and delays the onset of heart failure in a murine model. Since cardiac diseases are now considered inflammatory in nature, the role of USP18 in their pathogenesis is very likely. Further studies are needed to resolve this issue.

Acknowledgements

The authors would like to thank Jan Macioszczyk for help with graphical content.

REFERENCES

An S, Zhao LP, Shen LJ, Wang S, Zhang K, Qi Y1, Zheng J, Zhang XJ, Zhu XY, Bao R, Yang L, Lu YX, She ZG, Tang YD (2017) USP18 protects against hepati steatosis and insulin resistance through its deubiquitinating activity. Hepatology **66**: 1866–1884. https://doi.org/10.1002/hep.29375

Arimoto K, Löchte S, Stoner SA, Burkart C, Zhang Y, Miyauchi S, Wilmes S, Fan JB, Heinisch JJ, Li Z, Yan M, Pellegrini S, Colland F, Piehler J, Zhang DE (2017) STAT2 is an essential adaptor in USP18-mediated suppression of type I interferon signalling. *Nat Struct Mol Biol* **24**: 279–289. https://doi.org/10.1038/nsmb.3378

Basters A, Geurink PP, Röcker A, Witting KF, Tadayon R, Hess S, Semrau MS, Storici P, Ovaa H, Knobeloch KP, Fritz G (2017)

Structural basis of the specificity of USP18 toward ISG15. *Nat Struct Mol Biol* **24**: 270–278. https://doi.org/10.1038/nsmb.3371

Basters A, Knobeloch KP, Fritz G (2018) How USP 18 deals with ISG 15-modified proteins: structural basis for the specificity of the protease. *FEBS J* **285**: 1024–1029. https://doi.org/10.1111/febs.14260

Burkart C, Fan JB, Zhang DE (2012) Two independent mechanisms promote expression of an N-terminal truncated USP18 isoform with higher DeISGylation activity in the nucleus. *J Biol Chem* **287**: 4883–4893. https://doi.org/10.1074/jbc.M111.255570

Catic A, Fiebiger E, Korbel GA, Blom D, Galardy PJ, Ploegh HL (2007) Screen for ISG15-crossreactive deubiquitinases. *PloS One* **2**: e679. https://doi.org/10.1371/journal.pone.0000679

Coit P, Jeffries M, Altorok N, Dozmorov MG, Koelsch KA, Wren JD, Merrill JT, McCune WJ, Sawalha AH (2013) Genome-wide DNA methylation study suggests epigenetic accessibility and transcriptional poising of interferon-regulated genes in naive CD4+ T cells from lupus patients. *J Autoimmunity* **43**: 78–84. https://doi.org/10.1016/j.jaut.2013.04.003

Dick SA, Epelman S (2016) Chronic heart failure and inflammation: what do we really know? *Circ Res* **119**: 159–176. https://doi.org/10.1161/CIRCRESAHA.116.308030

Duex JE, Sorkin A (2009) RNA interference screen identifies Usp18 as a regulator of epidermal growth factor receptor synthesis. *Mol Biol Cell* **20**: 1833–1844. https://doi.org/10.1091/mbc.e08-08-0880

François-Newton V, de Freitas Almeida GM, Payelle-Brogard B, Monneron D, Pichard-Garcia L, Piehler J, Pellegrini S, Uzé G (2011) USP18-based negative feedback control is induced by type I and type III interferons and specifically inactivates interferon α response. *PloS One* **6**: e22200. https://doi.org/10.1371/journal.pone.0022200

Gu T, Lu L, An C, Zhang Y, Wu X, Xu Q, Chen G (2019) Negative regulation of the RLR-mediated IFN signaling pathway by duck ubiquitin-specific protease 18 (USP18). *J Cell Physiol* **234**: 3995–4004. https://doi.org/10.1002/jcp.27208

Guo Y, Chinyengetere F, Dolinko AV, Lopez-Aguiar A, Lu Y, Galimberti F, Ma T, Feng Q, Sekula D, Freemantle SJ, Andrew AS, Memoli V, Dmitrovsky E (2012) Evidence for the ubiquitin protease UBP43 as an antineoplastic target. *Mol Cancer Ther* **11**: 1968–1977. https://doi.org/10.1158/1535-7163.MCT-12-0248

Hoeller D, Hecker CM, Dikic I (2006) Ubiquitin and ubiquitin-like proteins in cancer pathogenesis. *Nat Rev Cancer* **6**: 776–788. https://doi.org/10.1038/nrc1994

Hong B, Li H, Lu Y, Zhang M, Zheng Y, Qian J, Yi Q (2014) USP18 is crucial for IFN-γ-mediated inhibition of B16 melanoma tumorigenesis and antitumor immunity. *Mol Cancer* **13**: 132. https://doi.org/10.1186/1476-4598-13-132

Honke N, Shaabani N, Zhang DE, Iliakis G, Xu HC, Häussinger D, Recher M, Löhning M, Lang PA, Lang KS (2013) Usp18 driven enforced viral replication in dendritic cells contributes to break of immunological tolerance in autoimmune diabetes. *PLoS Pathogens* **9**: e1003650. https://doi.org/10.1371/journal.ppat.1003650

Honke N, Shaabani N, Zhang DE, Hardt C, Lang KS (2016) Multiple functions of USP18. *Cell Death Dis* **7**: e2444. https://doi.org/10.1038/cddis.2016.326

Ketscher L, Knobeloch KP (2015) ISG15 uncut: dissecting enzymatic and non-enzymatic functions of USP18 *in vivo*. *Cytokine* **76**: 569–571. https://doi.org/10.1016/j.cyto.2015.03.006

Liu LQ, Ilaria R, Kingsley PD, Iwama A, van Etten RA, Palis J, Zhang DE (1999) A novel ubiquitin-specific protease, UBP43, cloned from leukemia fusion protein AML1-ETO-expressing mice, functions in hematopoietic cell differentiation. *Mol Cell Biol* **19**: 3029–3038. https://doi.org/10.1128/mcb.19.4.3029

MacParland SA, Ma XZ, Chen L, Khattar R, Cherepanov V, Selzner M, Feld JJ, Selzner N, McGilvray ID (2016) Lipopolysaccharide and tumor necrosis factor alpha inhibit interferon signaling in hepatocytes by increasing ubiquitin-like protease 18 (USP18) expression. *J Virol* **90**: 5549–5560. https://doi.org/10.1128/JVI.02557-15

Manini I, Sgorbissa A, Potu H, Tomasella A, Brancolini C (2013) The DeISGylase USP18 limits TRAIL-induced apoptosis through the regulation of TRAIL levels: Cellular levels of TRAIL influences responsiveness to TRAIL-induced apoptosis. *Cancer Biol Therapy* **14**: 1158–1166. https://doi.org/10.4161/cbt.26525

Murray EJ, Burden F, Horscroft N, Smith-Burchnell C, Westby M (2011) Knockdown of USP18 increases alpha 2a interferon signaling and induction of interferon-stimulating genes but does not increase antiviral activity in Huh7 cells. *Antimicrob Agents Chem* **55**: 4311–4319. https://dx.doi.org/10.1128/AAC.00644-11

Mustachio LM, Kawakami M, Lu Y, Rodriguez-Canales J, Mino B, Behrens C, Wistuba I, Bota-Rabassedas N, Yu J, Lee JJ, Roszik J, Zheng L, Liu X, Freemantle SJ, Dmitrovsky E (2017) The ISG15-specific protease USP18 regulates stability of PTEN. *Oncotarget* **8**: 3–14. https://doi.org/10.18632/oncotarget.13914

Ritchie KJ, Hahn CS, Kim KI, Yan M, Rosario D, Li L, de la Torre JC, Zhang DE (2004) Role of ISG15 protease UBP43 (USP18) in innate immunity to viral infection. *Nat Med* **10**: 1374–1378. https://doi.org/10.1038/nm1133

Santin I, Moore F, Grieco FA *et al.* (2012) USP18 is a key regulator of the interferon-driven gene network modulating pancreatic beta cell inflammation and apoptosis. *Cell Death Dis* **3**: e419. https://doi.org/10.1038/cddis.2012.158

Shaabani N, Honke N, Nguyen N, Huang Z, Arimoto KI, Lazar D, Loe TK, Lang KS, Prinz M, Knobeloch KP, Zhang DE, Teijaro JR (2018) The probacterial effect of type I interferon signaling requires its own negative regulator USP18. *Sci Immunol* **3**: eaau2125. https://doi.org/10.1126/sciimmunol.aau2125

Shaabani N, Khairnar V, Duhan V, Zhou F, Tur RF, Häussinger D, Recher M, Tumanov AV, Hardt C, Pinschewer D, Christen U, Lang PA, Honke N, Lang KS (2016) Two separate mechanisms of enforced viral replication balance innate and adaptive immune activation. *J Autoimmun* **67**: 82–89. https://doi.org/10.1016/j.jaut.2015.10.004

Yim HY, Yang Y, Lim YS, Lee MS, Zhang DE, Kim KI (2012) The mitochondrial pathway and reactive oxygen species are critical contributors to interferon-α/β-mediated apoptosis in Ubp43-deficient hematopoietic cells. *Biochem Biophys Res Commun* **423**: 436–440. https://doi.org/10.1016/j.bbrc.2012.05.154

Ying X, Zhao Y, Yao T, Yuan A, Xu L, Gao L, Ding S, Ding H, Pu J, He B (2016) Novel protective role for ubiquitin-specific protease 18 in pathological cardiac remodelling. *Hypertension* **68**: 1160–1170. https://doi.org/10.1161/HYPERTENSIONAHA.116.07562

Zhang D, Zhang DE (2011) Interferon-stimulated gene 15 and the protein ISGylation system. *J Interferon Cytokine Res* **31**: 119–130. https://doi.org/10.1089/jir.2010.0110

Zhang M, Zhang MX, Zhang Q, Zhu GF, Yuan L, Zhang DE, Zhu Q, Yao J, Shu HB, Zhong B (2016) USP18 recruits USP20 to promote innate antiviral response through deubiquitinating STING/MITA. *Cell Res* **26**: 1302. https://doi.org/10.1038/cr.2016.125

Zhang X, Bogunovic D, Payelle-Brogard B, Francois-Newton V, Speer SD, Yuan C, Volpi S, Li Z, Sanal O, Mansouri D, Tezcan I, Rice GI, Chen C, Mansouri N, Mahdaviani SA, Itan Y, Boisson B, Okada S, Zeng L, Wang X, Jiang H, Liu W, Han T, Liu D, Ma T, Wang B, Liu M, Liu JY, Wang QK, Yalnizoglu D, Radoshevich L, Uzé G, Gros P, Rozenberg F, Zhang SY, Jouanguy E, Bustamante J, García-Sastre A, Abel L, Lebon P, Notarangelo LD, Crow YJ, Boisson-Dupuis S, Casanova JL, Pellegrini S (2015) Human intracellular ISG15 prevents interferon-α/β over-amplification and auto-inflammation. *Nature* **517**: 89. https://doi.org/10.1038/nature13801

Zhao C, Collins M, Hsiang TY, Krug RM (2013) Interferon-induced ISG15 pathway: an ongoing virus-host battle. *Trends Microbiol* **21**: 181–186. https://doi.org/10.1016/j.tim.2013.01.005