UNIWERSYTET MEDYCZNY
IM. PIASTÓW ŚLĄSKICH WE WROCŁAWIU

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Przydatność wybranych parametrów w ocenie chorób współistniejących oraz terapii antyretrowirusowej u osób zakażonych HIV

Rozprawa doktorska w oparciu o monotematyczny cykl publikacji
w dziedzinie nauk medycznych i nauk o zdrowiu
w dyscyplinie nauki farmaceutyczne

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Spis treści

1. WYKAZ PRAC WCHODZĄCYCH W SKŁAD ROZPRAWY DOKTORSKIEJ

Uzyskane wyniki z przeprowadzonych badań własnych przedstawiono w trzech pracach oryginalnych, które zostały opublikowane w czasopismach z listy A, o zasięgu międzynarodowym. Razem z czwartą pracą o charakterze przeglądowym, stanowią one monotematyczny cykl publikacyjny, będący podstawą ubiegania się o stopień doktora nauk farmaceutycznych. Poniżej przedstawiono wybrane aspekty teoretyczne oraz najważniejsze wyniki badań, które opublikowano w załączonych pracach:

1. **Jurkowska K**, Szymańska B, Knysz B, Kuźniarski A, Piwowar A. Sirtuins as Interesting Players in the Course of HIV Infection and Comorbidities. Cells. 2021; 10(10):2739. https://doi.org/10.3390/cells10102739
   IF 2020: 6,600 Punkty MEiN: 140
2. **Jurkowska K**, Szymańska B, Knysz B, Piwowar A. The Effect of Antiretroviral Therapy on SIRT1, SIRT3 and SIRT6 Expression in HIV-Infected Patients. Molecules. 2022; 27(4):1358. https://doi.org/10.3390/molecules27041358
   IF 2020: 4,412 Punkty MEiN: 140
3. **Jurkowska K**, Szymańska B, Knysz B, Piwowar A. Effect of Combined Antiretroviral Therapy on the Levels of Selected Parameters Reflecting Metabolic and Inflammatory Disturbances in HIV-Infected Patients. Journal of Clinical Medicine. 2022; 11(6):1713. https://doi.org/10.3390/jcm11061713
   IF 2020: 4,242 Punkty MEiN: 140
4. Szymańska B, **Jurkowska K**, Knysz B, Piwowar A. Differences in Expression of Selected Interleukins in HIV-Infected Subjects Undergoing Antiretroviral Therapy. Viruses. 2022; 14(5):997. https://doi.org/10.3390/v14050997
   IF 2020: 5,048 Punkty MEiN:100

Sumaryczny współczynnik IF za cykl publikacji: 20,302
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2. STRESZCZENIE

Około 37,7 miliona osób na świece zakażonych jest ludzkim wirusem upośledzenia odporności (*ang. human immunodeficiency virus*, HIV). W 2020 r. rozpoznano 1,5 mln nowych zakażeń, a około 680 tysięcy osób zmarło z powodu chorób związanych z zespołem nabytego niedoboru odporności (*ang. aquired immunodeificiency syndrome*, AIDS). Dzięki znacznemu postępowi w terapii, infekcja wirusem HIV stała się chorobą przewlekłą, a długość życia osób zakażonych jest obecnie zbliżona do średniej długości życia w populacji ogólnej. Obserwuje się także znacząco zmniejszoną śmiertelność z powodu AIDS. Konieczność stałego stosowania leków w skojarzonej terapii antyretrowirusowej (ang. *combined antiretroviral therapy*, cART), związana jest jednak ze zwiększonym rozwojem towarzyszących zaburzeń metabolicznych i większym ryzykiem występowania m.in. cukrzycy typu 2, insulinooporności, chorób nerek czy chorób sercowo-naczyniowych. Zaburzenia metaboliczne dotyczą około 90% pacjentów zakażonych HIV powyżej 50 roku życia, długotrwale leczonych antyretrowirusowo. Mimo powszechności tego problemu, brak jest kompleksowych badań wieloprofilowych dotyczących rozwoju powyższych zaburzeń w przebiegu zakażenia HIV, co stało się obiektem moich badań.

Celem pracy była ocena wpływu zakażenia HIV oraz skojarzonej terapii antyretrowirusowej cART na zmiany stężeń wybranych parametrów w osoczu osób zakażonych HIV (mężczyźni) w odniesieniu do osób niezakażonych. U osób zakażonych HIV pomiaru stężeń tych parametrów przeprowadzono dwukrotnie: przed zastosowywaniem określonej terapii cART, dla oceny wpływu samego zakażenia HIV na zmiany stężeń powyższych parametrów, oraz po rocznym okresie leczenia antyretrowirusowego. Oceniono zależność zmian stężenia badanych parametrów w aspekcie zastosowanego schematu terapeutycznego oraz miana replikacji wirusa HIV RNA i liczby limfocytów T CD4+ i CD8+ u tych osób.

W toku przeprowadzonych badań oceniono stężenia 15 nieoznaczanych rutynowo parametrów laboratoryjnych, które pogrupowano w 3 panele diagnostyczne, związane z regulacją gospodarki węglowodanowej, lipidowej, rozwojem stanu zapalnego oraz odpowiedzi układu immunologicznego w przebiegu zakażenia HIV. Dostępne dane z piśmiennictwa wskazują na potencjalną rolę tych parametrów w patogenezie i rozwoju chorób współistniejących związanych z HIV. Badanymi parametrami związanymi z zaburzeniami metabolicznymi były: sirtuina 1 (SIRT1), sirtuina 3 (SIRT3), sirtuina 6 (SIRT6), peptyd glukagonopodobny 1 (GLP-1), dipeptydylopeptydaza 4 (DPP4), peptyd YY (PYY), miostatyna (MSTN) i iryzyna (IRS). Parametrami związanymi z rozwojem stanu zapalnego oraz z odpowiedzią układu immunologicznego były: fetuina A (FETU-A), czynnik pochodzenia stromalnego 1 (SDF-1), chemokina RANTES, pentraksyna 3 (PTX3), interleukina 4 (IL-4), interleukina 7 (IL-7) i interleukina 15 (IL-15).

Przeprowadzone badania wykazały zmiany stężeń, różnego charakteru i stopnia ww. parametrów u osób zakażonych HIV w stosunku do osób zdrowych oraz w okresie rocznej obserwacji stosowanej terapii. Zaobserwowano istotnie niższe stężenia SIRT6, IRS, MSTN, GLP-1, PTX3, RANTES oraz istotnie wyższe stężenia IL-4 w osoczu krwi osób zakażonych HIV w momencie rozpoznania choroby i przed rozpoczęciem terapii, w porównaniu do grupy kontrolnej. Wykazano istotnie niższe stężenie SIRT6 oraz istotnie statystycznie wyższe stężenia PYY, IL-4 i IL-7 u osób zakażonych HIV przed zastosowaniem cART w porównaniu do wyników uzyskanych po rocznym okresie leczenia. Zauważono obniżone stężenia wszystkich badanych parametrów (za wyjątkiem IL-15) u osób zakażonych HIV leczonych inhibitorami proteazy (*ang. protease inhibitors*, PIs), w porównaniu do terapii z użyciem inhibitorów integrazy (*ang, integrase strand transfer inhibitors*, INSTIs), ale jedynie dla SIRT1 różnica ta była istotna statystycznie.

Otrzymane wyniki wskazują, iż przebiegowi zakażenia HIV oraz stosowanej terapii antyretrowirusowej towarzyszą zmiany stężeń badanych parametrów laboratoryjnych, odzwierciedlających status zaburzeń metabolicznych, zapalnych i immunologicznych o różnym charakterze i stopniu, mimo relatywnie krótkiego, rocznego okresu obserwacji. Obniżone stężenia badanych parametrów u mężczyzn leczonych schematami terapeutycznymi zawierającymi PIs, wskazują na bardziej wyraźny wpływ tej grupy leków na potencjalny rozwój zaburzeń metabolicznych w przebiegu zakażenia wirusem HIV, w porównaniu do INSTIs.

Kontynuacja badań wydaje się być wysoce uzasadniona, a zgłębienie wiedzy na temat zmian będących obiektem pracy parametrów umożliwi w przyszłości ich wykorzystanie w monitorowaniu progresji choroby, ocenie ryzyka wystąpienia schorzeń towarzyszących oraz optymalizacji terapii antyretrowirusowej, aby minimalizować ryzyko skutków ubocznych terapii oraz zachować jej wysoką skuteczność.

3. SUMMARY

About 37.7 million people worldwide are infected with the human immunodeficiency virus (HIV). In 2020, 1.5 million new infections were diagnosed. About 680,000 people died from diseases associated with the acquired immunodeficiency syndrome (AIDS). Thanks to significant progress in diagnostics and therapy, HIV infection has become a chronic disease, and the life expectancy of infected people is now close to the average life expectancy in the general population. Significantly reduced AIDS mortality is also observed. However, the need for constant use of drugs in combined antiretroviral therapy (cART) is associated with an increased development of accompanying metabolic disorders: type 2 diabetes, insulin resistance, kidney diseases or cardiovascular diseases. Metabolic disorders occur in approximately 90% of HIV-infected patients over 50 years of age receiving long-term antiretroviral therapy. Despite the prevalence of this problem, there is no comprehensive multi-profile research on the development of the above-mentioned disorders in the course of HIV infection, which has become the subject of my research.

The aim of the study was to evaluate the effect of HIV infection and cART therapy on changes in plasma concentrations of selected parameters in HIV-infected individuals (men) in relation to HIV-uninfected men. In HIV-infected individuals, the concentration was measured twice: before the administration of the specified cART therapy and after one year of antiretroviral treatment. The dependence of changes in the concentration of the tested parameters on the HIV RNA, the number of CD4+ and CD8+ T lymphocytes as well as the applied therapeutic regimen was also assessed.

The study assessed the concentrations of 15 selected non-routine parameters, grouped into 3 diagnostic panels, related to the regulation of carbohydrate and lipid metabolism, the development of inflammation and the immune system response in the course of HIV infection. The available literature data suggest a potential role for these parameters in the pathogenesis of HIV-related comorbidities. The parameters studied related to metabolic disorders were: sirtuin 1 (SIRT1), sirtuin 3 (SIRT3), sirtuin 6 (SIRT6), glucagon-like peptide 1 (GLP-1), dipeptidyl peptidase 4 (DPP4), YY peptide (PYY), myostatin (MSTN), irisine (IRS). The parameters related to the development of inflammation and the immune system response were: fetuin A (FETU-A), stromal cell-derived factor 1 (SDF-1), chemokine RANTES, pentraxin 3 (PTX3), interleukin 4 (IL-4), interleukin 7 (IL-7), interleukin 15 (IL-15).

The conducted studies showed significantly lower concentrations of SIRT6, IRS, MSTN, GLP-1, PTX3, RANTES and significantly higher concentrations of IL-4 in the blood plasma of HIV-infected people before cART, compared to the control group. SIRT6 concentrations was found to be significantly lower, as well as statistically significantly higher concentrations of PYY, IL-4 and IL-7 in HIV-infected people before cART compared to the results obtained after one year of treatment. There were lower levels of all parameters tested (except IL-15) in

HIV-infected individuals treated with protease inhibitors (PIs) compared to integrase strand transfer inhibitors (INSTIs), but only for SIRT1 this difference was significant statistically.

The obtained results indicate that the course of HIV infection and antiretroviral therapy is accompanied by changes in the concentrations of the studied metabolic, inflammatory and immune parameters, despite the relatively short one-year follow-up. The decreased concentrations of the studied parameters in men treated with PIs indicate a significant influence of this group of drugs on the development of metabolic disorders in the course of HIV infection. Continuation of the research seems to be highly justified, and the deepening of the knowledge about the presented parameters will enable use them in the future in monitoring disease progression, assessing the risk of comorbidities and optimizing antiretroviral therapy in order to minimize the risk of side effects of the therapy and maintain its high effectiveness.

4. WPROWADZENIE

4.1. Dane epidemiologiczne dotyczące zakażeń wirusem HIV

Zakażenia wirusem ludzkim wirusem niedoboru odporności (*ang. human immunodeficiency virus,* HIV) wciąż stanowią poważny problem medyczny, społeczny oraz ekonomiczny. Na świecie około 37,7 miliona osób żyje z HIV, a w 2020 r. rozpoznano 1,5 mln nowych zakażeń. Około 680 tysięcy osób zmarło z powodu chorób związanych z zespołem nabytego niedoboru odporności (ang. *aquired immunodeificiency syndrome,* AIDS) [1]. Do 2020 roku w Polsce zarejestrowano 26 486 przypadków zakażenia wirusem HIV. Istotnym problemem pozostaje niewielki odsetek osób nieświadomych zakażenia, u których wykrywane jest ono dopiero po 2-4 latach, ze względu na zwiększone ryzyko transmisji zakażenia, które następuje po około 6-24 miesiącach od zakażenia pierwotnego [2]. Zbyt późne rozpoznanie bezpośrednio przyczynia się do rozwoju AIDS. Około 75% osób zakażonych jest świadomych swojego statusu serologicznego, a spośród nich 79% stosuje skojarzoną terapię antyretrowirusową (*ang. combined antiretroviral therapy,* cART)[1]. Do większości nowych zachorowań dochodzi w Afryce Subsaharyjskiej, w mniejszym stopniu Azji Południowo-Wschodniej i Południowej oraz krajach Europy Wschodniej. Najwyższe ryzyko transmisji HIV dotyczy homoseksualnych mężczyzn (MSM), osób stosujących dożylnie narkotyki oraz osób świadczących usługi seksualne [2,3]. Inne drogi transmisji to m.in.: transfuzje krwi lub preparatów krwiopochodnych, kontakt z materiałem zakaźnym, np. w warunkach zawodowych (personel medyczny), droga wertykalna [2]. Według danych z 2020 roku, wciąż jednak tylko około 84% osób było świadomych swojego zakażenia, natomiast liczba osób nieświadomych swojego statusu serologicznego szacowana jest na 6.1 mln osób [1,4].

4.2. Przebieg zakażenia HIV oraz schorzenia towarzyszące

Około 2/3 (40-90%) osób zakażonych wirusem HIV rozwija tzw. ostrą infekcję retrowirusową, w okresie około 2-6 tygodni, będącą pierwszym etapem zakażenia. Objawy ostrej infekcji retrowirusowej są niespecyficzne, a ich nasilenie zróżnicowane, są to m.in.: gorączka, utrata masy ciała, ból gardła, nocne poty, wysypka o charakterze grudkowo plamistym, nudności, wymioty, biegunka. Przedłużenie czasu trwania wyżej wymienionych objawów lub ich znaczne nasilenie jest czynnikiem prognostycznym związanym z szybszym postępem zakażenia. w przebiegu ostrej infekcji retrowirusowej dochodzi do intensywnej replikacji wirusa, a wiremia osiąga ponad 100 milionów kopii/ml. Zakażone zostają komórki posiadające receptor CD4 tj. limfocyty, makrofagi, monocyty oraz komórki bez receptora CD4: komórki śródbłonkowe, nabłonkowe, oligodendroglej, komórki Langerhansa, komórki dendrytyczne, stanowiące rezerwuar wirusa. w wyniku wysokiej wiremii, która pojawia się w pierwszych dniach zakażenia dochodzi także do aktywacji układu immunologicznego, objawiającej się tzw. burzą cytokinową, czyli nadmiernym poziomem m.in.: interferonu α (*ang. Interferon alpha*, IFNα), Interleukiny 15 (*ang. Interleukin 15,* IL-15) [2]. Po ustąpieniu objawów, wiremia oraz liczba

limfocytów T CD4+ obniża się, jednak nie do stanu sprzed zakażenia, a ustalony na tym etapie zakażenia ich poziom może utrzymywać się przez wiele lat. Ustalony zostaje tzw. *set point* i dokonuje się stabilizacja zakażenia. Jest to etap tzw. „pierwotnej infekcji retrowirusowej". Pacjenci ze względnie niską wiremią mają lepsze rokowania i niskie ryzyko zapadalności na AIDS w ciągu kolejnych 10 lat, natomiast jeżeli wiremia na tym etapie wynosi ponad 100 000 kopii/ml, istnieje znacznie wyższe ryzyko wystąpienia AIDS w ciągu najbliższych dwóch lat. Następujące stadium bezobjawowej infekcji może trwać ponad 10-12 lat [2,3]. Liczba komórek CD4+ systematycznie obniża się, a tempo tego zjawiska jest czynnikiem warunkującym dalszy przebieg choroby. w przypadku braku rozpoznania i wdrożenia terapii, po tym czasie rozwija się stadium objawowe infekcji HIV, w konsekwencji prowadzące do rozwoju AIDS [5].

Jak pokazują dane z piśmiennictwa, liczba osób zakażonych wirusem HIV na świecie w wieku ponad 50 lat wynosi ponad 10%, a w regionach bardziej rozwiniętych sięga nawet 50% i szacuje się, że będzie ona rosła [6]. Według badań kohortowych, przeprowadzonych w latach 1999-2004, wśród pacjentów HIV-pozytywnych zanotowano znaczny wzrost, o około 33% śmiertelności z przyczyn niezwiązanych bezpośrednio z zakażeniem HIV, przy jednoczesnym istotnym spadku śmiertelności w tej kohorcie [7]. Oczekiwana długość życia była dłuższa u pacjentów z liczbą limfocytów T CD4+ w zakresie 200-350 komórek/µl i krótsza u pacjentów rozpoczynających terapię z liczbą limfocytów T CD4+ <100 komórek/µl. Szybkie wdrożenie cART, dzięki zahamowaniu wiremii i transmisji wirusa, znacząco zmniejsza nie tylko ryzyko wystąpienia pełnoobjawowego AIDS, ale także minimalizuje ryzyko wystąpienia chorób współistniejących takich jak: nowotwory, choroby sercowo-naczyniowe (*ang. cardiovascular diseases*, CVD) i choroby nerek. Mimo to, u osób zakażonych istnieje także wyższe ryzyko wystąpienia zaburzeń metabolicznych, związanych z przewlekłym stosowaniem cART [8]. Wieloośrodkowe badanie przeprowadzone przez Serrão et al. [9] wśród pacjentów zakażonych wirusem HIV w wieku powyżej 50 lat wykazało pozytywną korelację pomiędzy wiekiem pacjentów, a występowaniem schorzeń współistniejących niezwiązanych bezpośrednio z HIV lub AIDS. Warto również zauważyć, że prawie 90% pacjentów miało co najmniej jedno schorzenie współistniejące, a 35% pacjentów cierpiało na trzy lub więcej schorzeń [9].

Do najczęstszych zaburzeń u chorych z HIV należą: hipercholesterolemia, nadciśnienie tętnicze, depresja/przewlekły lęk, przewlekłe wirusowe zapalenie wątroby typu C, cukrzyca typu 2 (*ang. type 2 diabetes mellitus*, T2DM) oraz insulinooporność. Wykazano także, że osoby zakażone HIV zapadają na T2DM częściej oraz w młodszym wieku, w porównaniu do populacji ogólnej. Zaburzenia gospodarki węglowodanowej, w przebiegu infekcji HIV są związane z przewlekłym stosowaniem cART, szczególnie w schematach opartych na PIs oraz NRTIs. Jako potencjalne mechanizmy rozwoju tych zaburzeń uważa się interakcję z transporterem glukozy typu 4 (*ang. glucose transporter type 4*, GLUT-4), ograniczenie translokacji GLUT-4,

zaburzenia funkcji i różnicowania adipocytów, hamowanie syntezy oraz produkcji adiponektyny oraz zmniejszenie fosforylacji kinazy aktywowanej mitogenami (*ang. mitogen-activated protein kinases*, MAPK), co skutkuje zaburzeniem postreceptorowego procesu przekazywania sygnału [10,11]. Ponadto wykazano, że leki z grupy NRTIs powodują zaburzenia funkcji DNA mitochondrialnego w mięśniach, co obniża ekspresję genów uczestniczących w metabolizmie [12].

Wśród osób zakażonych wirusem HIV obserwuje się także zwiększone ryzyko chorób sercowo-naczyniowych. Wiąże się to z występowaniem zaburzeń metabolicznych i chorób towarzyszących m.in.: insulinooporności, dyslipidemii czy wirusowego zapalenia wątroby typu C oraz zmian w układzie odpornościowym związanych z zakażeniem HIV [13,14]. Wykazano także działanie proaterogenne cART. Pokazano też wpływ PIs lub abakawiru na ryzyko rozwoju choroby niedokrwiennej, które zwiększają się wraz z czasem stosowania terapii [13]. Jak pokazują dane epidemiologiczne, obserwuje się zwiększoną śmiertelność z powodu CVD osób zakażonych HIV i jest to jedna z najczęstszych przyczyn zgonów niezwiązanych z AIDS u osób leczonych skutecznie terapią antyretrowirusową [15].

4.3. Leczenie antyretrowirusowe

Według rekomendacji Europejskiego Stowarzyszenia Badań nad AIDS (*ang. European AIDS Clinical Society*, EACS), leczenie antyretrowirusowe powinno być wdrażane u każdej osoby niezwłocznie po rozpoznanym zakażeniu i kontynuowane przez całe życie. Obecnie stosowane schematy terapeutyczne pozwalają na niemal całkowite obniżenie wiremii do poziomu niewykrywalnego rutynowymi metodami. Osoby skutecznie leczone nie stanowią też znacznego ryzyka transmisji zakażenia [5,16].

Obecnie stosowane leki antyretrowirusowe należą do jednej z 7 klas:

• nukleozydowe inhibitory odwrotnej transkryptazy (*ang. nucleoside reverse transcriptase inhibitors*, NRTIs): abakawir (ABC), emtrycytabina (FTC), lamiwudyna (3TC), dizaproksyl tenefowiru (TDF), alafenamid tenofowiru (TAF), zydowudyna (AZT)

• nienukleozydowe inhibitory odwrotnej transkryptazy (*ang. non-nucleoside reverse transcriptase inhibitors*, NNRTIs): efawirenz (EFV), etrawiryna (ETV), newirapina (NVP), rylpiwiryna (RPV), dorawiryna (DOR)

• inhibitory integrazy (*ang. integrase strand transfer inhibitors*, INSTIs): raltegrawir (RAL), elwitegrawir (EVG), dolutegrawir (DTG), bikategrawir (BIC), kabotegrawir (CAB)

• inhibitory proteazy (*ang. protease inhibitors*, PIs: atazanawir (ATV), darunawir (DRV), lopinawir (LPV/r), ritonawir (r), sakwinawir (SQV)

• inhibitory koreceptora C-C chemokin typu 5 (*ang. C-C Motif Chemokine Receptor 5*, CCR5): marawirok (MVC)

• inhibitory fuzji (*ang. fusion inhibitors*): enfuwirtyd (ENF)

• inhibitor przyłączania (ang. *attachment inhibitors*, AI)- fostemsawir (FOS)

Rekomendowane schematy terapeutyczne zawierają zazwyczaj trzy leki: dwa analogi nukleozydowe w połączeniu z nienukleozydowym inhibitorem odwrotnej transkryptazy lub dwa analogi nukleozydowe w połączeniu z inhibitorem proteazy wzmacnianym rytonawirem lub kobicystatem lub dwa analogi nukleozydowe w połączeniu z inhibitorem integrazy [2,17].

Obecnie istnieje wiele preparatów złożonych, które znacząco ułatwiają dawkowanie, co skutkuje zwiększoną skutecznością i lepszą adherencją, m.in.: TAF/FTC, ABC/3TC/DTG, TDF/FTC/EFV lub TAF/FTC/RPV [3].

Dobór odpowiedniego schematu terapeutycznego zależy od indywidualnych potrzeb HIV zakażonych osób, obecności chorób współistniejących oraz potencjalnych interakcji z pozostałymi lekami stosowanymi przez pacjenta, szczególnie leków metabolizowanych za pośrednictwem enzymów cytochromu P450: niektóre statyny, klarytromycyna, ryfampicyna, blokery kanału wapniowego, itrakonazol, warfaryna, flukonazol, paroksetyna, sertralina, leki stosowane w terapii zapalenia wątroby typu C [18].

Od niedawna wprowadzono także schematy dwulekowe, które wykazują podobną skuteczność w porównaniu do schematów standardowych, jednocześnie z mniejszym ryzykiem działań niepożądanych [19]. Pierwszym zarejestrowanym preparatem dwuskładniowym było połączenie dolutegrawiru i rylpawiryny w 2017 roku [20,21]. w randomizowanych wieloośrodkowych badaniach III fazy, z podwójnie zaślepioną próbą wykazano, że także połączenie dolutegrawiru z lamiudyną, u pacjentów zakażonych HIV, nieleczonych wcześniej antyretrowirusowo wykazuje jednakową skuteczność porównując schemat tradycyjny: dolutegrawir, dizaproksyl tenofowiru i emtrycytabinę [22].

W ostatnich latach zatwierdzone zostało przez amerykańską Agencję Żywności i Leków (*ang. Food and Drug Administration*, FDA) i Europejską Agencję Leków (*ang. European Medicine Agency, EMA*) pierwsze humanizowane przeciwciało monoklonalne- Ibalizumab, skierowane przeciw drugiej domenie CD4, uniemożliwiając zakażanie limfocytów T CD4+, do leczenia zakażeń HIV u osób z opornością wielolekową, w połączeniu ze standardową terapią, co otwiera nowe możliwości w zwiększeniu skuteczności terapii [23].

5. CELE PRACY

Szacuje się, że wprowadzenie skutecznego leczenia antyretrowirusowego oraz znaczący wzrost dostępu do terapii spowodowało zmniejszenie liczby zgonów z powodu HIV/AIDS o około 43%, w porównaniu do roku 2001 [1]. Mimo znacznego postępu w leczeniu zakażania wirusem HIV, nadal niemożliwa jest całkowita eradykacja wirusa, co wiąże się z koniecznością dożywotniego przyjmowania leków antyretrowirusowych. w dalszym ciągu, mimo rejestracji nowych, bezpieczniejszych leków, terapia ta obarczona jest licznymi działaniami niepożądanymi, w tym także efektami długofalowymi, co pogarsza ogólny stan pacjenta i może skrócić okres przewidywanego przeżycia takich osób [3,18]. u osób zakażonych HIV, mimo dobrego stanu klinicznego, często obserwowana jest tendencja do zwiększonego rozwoju insulinooporności, zespołu metabolicznego, zaburzeń lipidowych czy stanu zapalnego [10,16,24].

W pracy oznaczono 15 wybranych nierutynowych parametrów związanych z regulacją gospodarki węglowodanowej, lipidowej, rozwojem stanu zapalnego oraz odpowiedzi układu immunologicznego w osoczu osób zakażonych HIV (mężczyźni) przed terapią cART, po okresie rocznego leczenia oraz u osób niezakażonych HIV (mężczyźni), stanowiących grupę kontrolną. Parametry te pogrupowane zostały w 3 panele:

1 panel stanowiły – sirtuina 1 (*ang. sirtuin 1*, SIRT1), sirtuina 3 (*ang. sirtuin 3*, SIRT3), sirtuina 6 (*ang. sirtuin 6*, SIRT6);

2 panel obejmował parametry związane z zaburzeniami metabolicznymi oraz rozwojem stanu zapalnego - iryzyna (*ang. irisin*, IRS), miostatyna (*ang. myostatin*, MSTN), ludzki peptyd YY (*ang. Peptide YY*, PYY), peptyd glukagonopodobny 1 (*ang. glucagon-like peptide-1*, GLP- 1), dipeptydylopeptydaza 4 (DPP4), fetuina A (*ang. fetuin A*, FETU-A), pentraksyna 3 (*ang. pentraxin 3*, PTX3), czynnik pochodzenia stromalnego 1 (ang. *stromal cell-derived factor 1*, SDF-1), chemokina RANTES (*ang. regulated upon activation, normal t cell expressed and presumably secreted*);

3 panel dotyczył parametrów związanych z odpowiedzią układu immunologicznego - interleukin - interleukina 4 (*ang. interleukin 4*, IL-4), interleukina 7 (*ang. interleukin 7*, IL-7), interleukina 15 (*ang. interleukin 5*, IL-15).

Celem pracy była:

1. Ocena wpływu zakażenia HIV na stężenia wybranych parametrów w osoczu osób zakażonych w ww. 3 panelach, w porównaniu do osób niezakażonych HIV, stanowiących grupę kontrolną.
2. Porównanie zmian stężeń wybranych parametrów w obserwacji jednorocznej u pacjentów zakażonych HIV, po zastosowaniu cART.
3. Ocena zmian stężenia badanych parametrów zależnych od miana replikacji wirusa HIV RNA, liczby limfocytów T CD4+ i CD8+ oraz od zastosowanego schematu terapeutycznego.

4. Uzyskanie wstępnej informacji dotyczącej zasadności prowadzenia badań nad wykorzystaniem badanych parametrów jako potencjalnych wskaźników diagnostycznych i/lub prognostycznych u osób zakażonych HIV.
5. Poszerzenie wiedzy dotyczącej udziału i roli terapii cART w rozwoju zaburzeń i schorzeń współistniejących.

W dostępnym piśmiennictwie naukowym nie znaleziono informacji na temat podobnych badań, obejmujących tak szeroką analizę nierutynowo oznaczanych parametrów w takim układzie badań panelowych charakteryzujących zaburzenia metaboliczne, odpowiedź immunologiczną oraz zmiany zapalne u osób zakażonych HIV w obserwacji 12-sto miesięcznej oraz ocenę wpływu zakażenia na zmiany ich stężeń, co czyni przedłożoną pracę nowatorską oraz stanowiącą solidne podstawy do badań długofalowych i stworzenia optymalnego algorytmu prognostycznego dla utrzymania dobrego stanu zdrowia takich osób.

6. MATERIAŁ i METODY

6.1. Charakterystyka grupy badanej oraz grupy kontrolnej

Badaniami objęto 53 mężczyzn (średnia wieku 33 lata), zakażonych HIV, będących pod opieką Ośrodka Profilaktyczno-Leczniczego Chorób Zakaźnych i Terapii Uzależnień we Wrocławiu i Kliniki Chorób Zakaźnych, Chorób Wątroby i Nabytych Niedoborów Odpornościowych Uniwersytetu Medycznego we Wrocławiu oraz 35 mężczyzn dopasowanych wiekiem, niezakażonych HIV, stanowiących grupę kontrolną.

Kryteriami włączenia w grupie badanej były: potwierdzone zakażenie wirusem HIV oraz przyjmowanie leków antyretrowirusowych po pierwszej fazie badania.

Kryteriami wyłączenia dla grupy badanej były: występowanie chorób przewlekłych: cukrzycy, chorób nowotworowych, chorób sercowo-naczyniowych, chorób układu moczowego, nowotworowych, stosowanie innych leków niż antyretrowirusowe.

Kryteriami włączenia dla grupy kontrolnej były: potwierdzony brak zakażenia wirusem HIV, brak czynnych infekcji wirusowych, bakteryjnych oraz brak chorób przewlekłych: cukrzycy, chorób nowotworowych, chorób sercowo-naczyniowych, chorób układu moczowego, a także stałe stosowanie leków oraz wiek zbliżony do grupy badanej.

Wyniki analizowano całościowo, w grupie badanej osób chorych oraz w grupie kontrolnej osób zdrowych, oraz w grupie badanej w zależności od miana wirusa HIV-RNA (poniżej i powyżej 100 000 kopii RNA/ml), liczby limfocytów T CD4+ (poniżej i powyżej 300 komórek/µl), liczby limfocytów T CD8+ (poniżej i powyżej 1000 komórek/µl) oraz zastosowanego schematu terapeutycznego (INSTIs lub PIs). Badania przeprowadzono zarówno przed (wyniki oznaczone jako A), jak i po rocznej terapii cART (wyniki oznaczone jako B). Wyniki dla grupy kontrolnej oznaczono jako C.

U mężczyzn zakażonych HIV zastosowano dwa schematy leczenia. w pierwszym schemacie zastosowano lek z grupy inhibitorów proteazy (PIs): ritonavir wzmocniony cobicystatem lub darunavir z cobicystatem w połaczeniu z nukleozydowymi inhibitorami odwrotnej transkryptazy (NRTIs): emtrycytabiną + alafenamidem tenofowiru. Drugi schemat leczenia zawierał inhibitor integrazy (INSTIs): dolutegravir w połączaniu z powyższymi NRTIs.

Dane dotyczące poziomów wiremii HIV RNA oraz liczby limfocytów T CD4+ i CD8+ uzyskano z dokumentacji medycznej. Miano wirusa HIV mierzono za pomocą testu PCR w czasie rzeczywistym (test COBAS TaqMan HIV-1 v. 2.0, Roche Diagnostics, Basel, Szwajcaria). Granica wykrywalności wynosiła 40 kopii/ml. Zliczenia limfocytów T CD4+ i CD8+ dokonano metodą cytometrii przepływowej przy użyciu systemu FACSCount Becton Dickinson (BD Biosciences, San Jose, CA, USA).

Średnia wieku, indeks masy ciała (*ang. Body mass index*, BMI), stężenie glukozy na czczo (*ang. Fasting blood glucose*, FBG), triglicerydów (*ang. Triglycerides*, TG), cholesterolu LDL (LDL-C), cholesterolu (HDL-C) u mężczyzn zakażonych wirusem HIV przed (A) i po

leczeniu (B) były zbliżone w badanej grupie pacjentów i grupie kontrolnej i nie różniły się istotne statystycznie. Zauważono statystycznie istotny wzrost liczby limfocytów T CD4+ ($p<0,001$) i spadek liczby limfocytów TCD8+ ($p=0,004$) oraz kopii HIV RNA ($p<0,001$) u mężczyzn zakażonych HIV, po rocznej terapii antyretrowirusowej, co świadczy o skuteczności leczenia i odbudowie funkcji układu immunologicznego.

6.2. Materiał do badań

Materiałem badawczym było osocze pozyskane z krwi pełnej osób biorących udział w badaniach, pobranej na czczo do probówek zawierających antykoagulant (Sarstedt, Poland), za pomocą sterylnego sprzętu medycznego jednorazowego użytku. Uzyskane osocze przechowywano w temperaturze -80°C, do czasu wykonania oznaczeń. u mężczyzn zakażonych HIV krew pobrano dwukrotnie przed i po rocznej terapii cART. Wszystkie badania przeprowadzono w Katedrze i Zakładzie Toksykologii Wydziału Farmaceutycznego Uniwersytetu Medycznego we Wrocławiu.

Badanie zostało przeprowadzone zgodnie z Deklaracją Helsińską, a protokół został zatwierdzony przez Komisję Bioetyczną Uniwersytetu Medycznego we Wrocławiu (KB-597/2019). Od wszystkich uczestników uzyskano pisemną świadomą zgodę na udział w badaniu.

Badania zostały sfinansowane ze środków uzyskanych w ramach Grantu dla Młodych Naukowców nr STM.D150.20.049 Uniwersytetu Medycznego we Wrocławiu w latach 2019-2020, działalności statutowej ST.D150.18.004 na rok 2019 oraz subwencji na utrzymanie i rozwój potencjału badawczego w 2022 roku SUBZ.C170.22.071.

6.3. Wykonanie oznaczeń

Pomiar stężenia badanych parametrów przeprowadzono metodą immunoenzymatyczną, za pomocą dostępnych komercyjnie testów ELISA. Wszystkie oznaczenia wykonano zgodnie z instrukcjami producentów. Do badań użyto następujacych testów: Human Irisin ELISA Kit (Cat.No E3253Hu), Human Growth Differentiation Factor8 ELISA Kit (Cat.No E3058Hu), Human Peptide YY ELISA Kit (Cat.No E1369Hu), Human Glucagon-like Peptide 1 ELISA Kit (Cat.No E0022Hu), Human Dipeptidyl peptidase 4 ELISA Kit (Cat.No E6631Hu), Human Fetuin a ELISA Kit (Cat.No E1386Hu), Human Pentraxin 3 ELISA Kit (Cat.No E1938Hu), Human Stromal Cell Derived Factor 1 ELISA Kit (Cat.No E3353Hu), Human Regulated on Activation in Normal T-cell Expressed and Secreted/C-C Motif Chemokine 5 ELISA Kit (Cat.No E3663Hu) Bioassay Technology Labora-tory (BT Lab; Shanghai Korain Biotech Co Ltd), Human IL-4 Immunoassay (R&D Systems, Inc., Minneapolis, MN, USA); Human IL-7 ELISA Kit (Thermo Fisher Scientific Inc., Waltham, MA, USA); Human IL-15 Immunoassay (R&D Systems, Inc., Minneapolis, MN, USA).

6.4. Analiza statystyczna

Analizę statystyczną uzyskanych wyników wykonano za pomocą programu Statistica 13.3 (StatSoft, Polska). Wszystkie badane zmienne typu ilościowego sprawdzono testem Shapiro-Wilka dla ustalenia typu rozkładu. Porównanie zmiennych jakościowych pomiędzy grupami dokonano przy wykorzystaniu testu chi-kwadrat ($\chi 2$). Porównanie zmiennych typu ilościowego pomiędzy grupami wykonano wykorzystując test U-Mann-Whitney oraz test Kruskala-Wallisa. Test Kruskala-Wallisa posłużył do porównania trzech grup (mężczyzn zakażonych HIV przed cART, po cART i grupy kontrolnej) pod względem badanych zmiennych ilościowych. Istotny statystycznie wynik testu Kruskala-Wallisa wskazywał, że przynajmniej jedna grupa różni się od drugiej. w związku z tym przeprowadzono następnie test post-hoc (test Dunna z poprawką Bonferroniego), aby dokładnie określić które grupy różnią się od siebie. Porównanie wewnątrzgrupowe pomiędzy wynikami przed i po przeprowadzono za pomocą testu Wilcoxona. Korelacje między parametrami oceniano za pomocą testu Spearmana. We wszystkich analizach za wartość istotną przyjęto $p < 0,05$.

7. OMÓWIENIE PUBLIKACJI WCHODZĄCYCH w SKŁAD ROZPRAWY

W skład przedłożonej rozprawy doktorskiej wchodzą cztery publikacje, w tym jedna przeglądowa i trzy oryginalne, opublikowane w recenzowanych czasopismach naukowych o zasięgu międzynarodowym, takich jak: *Cells* (IF 2020: 6,600, Punkty MEiN:140), *Journal of Clinical Medicine* (IF 2020: 4,242, Punkty MEiN:140) , *Molecules* (IF 2020: 4,412, Punkty MEiN: 140), *Viruses* (IF 2020: 5,048, Punkty MEiN:100).

Pierwsza praca, o charakterze przeglądowym, przedstawia stan wiedzy o enzymach z rodziny Sirtuin (SIRTs), z grupy deacet ylaz zależnych od NAD+ i ich potencjalnego udziału w rozwoju schorzeń towarzyszących zakażeniu wirusem HIV. Działanie SIRT wiąże się z kontrolą metaboliczną, stresem oksydacyjnym, stanem zapalnym, apoptozą, a także wpływa na przebieg infekcji wirusowych. z tego powodu mogą one uczestniczyć w patogenezie i rozwoju wielu chorób, jednak niewiele wiadomo o ich roli w przebiegu zakażenia HIV, co stało się przedmiotem niniejszego przeglądu.

Druga publikacja jest pracą oryginalną gdzie badano wpływ zakażenia HIV oraz terapii antyretrowirusowej cART na ekspresję SIRT1, SIRT3 i SIRT6 w osoczu krwi mężczyzn zakażonych HIV w obserwacji jednorocznej.

Trzecia publikacja jest także pracą oryginalną, w której przedstawiono wpływ zakażenia HIV oraz zastosowanej rocznej terapii cART na stężenia wybranych parametrów związanych z metabolizmem węglowodanów i lipidów, CVD i stanem zapalnym w osoczu krwi mężczyzn zakażonych HIV.

Czwarta publikacja omawia zależność między zakażeniem HIV oraz wpływem terapii cART na stężenia trzech wybranych interleukin: IL-4, IL-7 i IL-15 w osoczu krwi mężczyzn zakażonych HIV jako wskaźników procesu zapalnego w przebiegu zakażenia HIV.

W pracach oryginalnych uwzględniono także związek miana replikacji wirusa HIV RNA, liczby limfocytów T CD4+ i CD8+ oraz rodzaju zastosowanego schematu terapeutycznego na zmiany stężeń badanych parametrów.

Kolejność przedstawionych artykułów w logiczny sposób wprowadza w temat rozprawy doktorskiej i stanowi logiczny ciąg powiązanych ze sobą, spójnych tematycznie prac.

7.1. Publikacja 1

Publikacja pierwsza pt.: ***Sirtuins as Interesting Players in the Course of HIV Infectionand Comorbidities*** jest pracą przeglądową opublikowaną w czasopiśmie *Cells* (IF 2020: 6,600 Pkt. MEiN:140) *i* stanowi wprowadzenie do drugiego artykułu z cyklu, będącego pracą oryginalną. Manuskrypt opisuje grupę enzymów z rodziny sirtuin (*ang. sirtuins*), będących NAD+ zależnymi deacetylazami, których głównym zadaniem jest odszczepianie grupy acetylowej z histonów, czynników transkrypcyjnych i innych białek, wyciszając ekspresję wybranych genów. u ssaków znanych jest siedem sirtuin (SIRT1-7), które oprócz aktywności deacetylazy, katalizaują także inne reakcje, takie jak: mono-ADP-rybozylacja, desukcynylacja, demalonylacja, demirystylacja lub depalmitylacja [25,26]. Sirtuiny są enzymami o wielokierunkowym działaniu. Regulują proces starzenia organizmu, proces apoptozy, odporność na stres oksydacyjny, stan zapalny, procesy transkrypcji oraz naprawy uszkodzeń DNA. Zaangażowane są też w regulację metaboliczną, zwłaszcza w stanach zmniejszonej podaży kalorii, zwiększając wydajność procesu pozyskiwania energii [27,28]. *u* ssaków, siedem sirtuin rozlokowanych jest w różnych kompartmentach komórkowych: w jądrze komórkowym (SIRT1, SIRT6, SIRT7), w cytoplazmie (SIRT2), w mitochondriach (SIRT3, SIRT4, SIRT5) [29].

Dane omówione w przeglądzie wskazują na potencjalny udział SIRTs w komórkowych szlakach sygnałowych wspólnych dla niektórych białek wirusa HIV, prowadzących do: osteoporozy – tu szczególną rolę odgrywa SIRT1i SIRT6, cukrzycy typu 2 i insulinooporności, gdzie największe znaczenie przypisano SIRT1, SIRT2, SIRT3 i SIRT6, niealkoholowej stłuszczeniowej chorobie wątroby i zaburzeniach metabolizmu lipidów, w których największe znaczenie przypisuje się SIRT1, SIRT3 i SIRT6, chorób układu krążenia – ze wskazywaną rolą największej liczby sirtuin - SIRT1, SIRT2, SIRT4, SIRT5 i SIRT6, chorób nerek, gdzie szczególną rolę przypisuje się SIRT1 i SIRT3, oraz chorób jamy ustnej, gdzie podkreślana jest rola SIRT1 i SIRT4 [31].

Podsumowując, publikacja ta przedstawia wiele istotnych informacji dotyczących potencjalnej roli sirtuin w przebiegu zakażenia HIV, mechanizmów ich działania oraz potencjalnych punktów uchwytu regulacji ich aktywności.

7.2. Publikacja 2

Wprowadzenie

Publikacja 2 pt.: ***The Effect of Antiretroviral Therapy on SIRT1, SIRT3 and SIRT6 Expression in HIV-Infected Patients*** jest pracą oryginalną opublikowaną w czasopiśmie *Molecules* (IF 2020: 4,412 Pkt. MEiN:140). w artykule oceniono wpływ zakażenia HIV oraz rocznej terapii cART na stężenie trzech wybranych sirtuin: SIRT1, SIRT-3 i SIRT-6 w osoczu krwi mężczyzn zakażonych HIV w odniesieniu do grupy kontrolnej, złożonej ze zdrowych mężczyzn, niezakażonych HIV ani nieobarczonych innymi chorobami przewlekłymi czy ostrym stanem zapalnym.

Charakterystyka badanych sirtuin

Sirtuina 1

Ekspresję SIRT1 wykazano w większości tkanek, w tym w mięśniach szkieletowych, wątrobie, adipocytach, nerkach itp. Wykazano, że SIRT1 uczestniczy w sygnalizacji komórkowej szlaków związanych z glukoneogenezą, glikolizą, wydzielaniem insuliny, a także naprawą DNA czy starzeniem się [32]. Reguluje aktywność wielu czynników transkrypcyjnych, np. białka należące do rodziny forkhead box o (*ang. forkhead box O*, FOXO), czynnika indukowanego hipoksją 1-alfa (*ang. hypoxia-inducible factor-1 alpha,* HIF-1α), receptora wątrobowego X (*ang. liver X receptor,* LXR), białka wiążącego element regulatorowy steroli 1 (*ang. sterol regulatory element-binding protein 1,* SREBP-1), koaktywatora 1α receptora γ aktywowanego przez proliferatory peroksysomów (*ang. peroxisome proliferator-activated receptor gamma coactivator 1-alpha,* PGC-1α), białka p53 i jądrowego czynnika transkrypcyjnego NF kappa B (*ang. nuclear factor kappa-light-chain-enhancer of activated B cells,* NF-kB) [31,32]. Opisano również interakcję SIRT1 z białkiem Tat wirusa HIV (*ang. Trans-Activator of Transscription)*, który jest transaktywatorem transkrypcji zintegrowanego prowirusowego mRNA [33]. Deacetylacja Tat zwiększa wydajność transkrypcji HIV poprzez kontynuowanie wydłużania wirusowego mRNA. Tat blokuje również SIRT1, zmniejszając w ten sposób deacetylację NF-kB, prowadząc do aktywacji procesów zapalnych. SIRT1 może mieć istotny i bezpośredni wpływ na proces zakażenia wirusem HIV i przewlekłą aktywację układu odpornościowego, bedącą jedną z przyczyn rozwoju chorób współistniejących w przebiegu HIV [34].

Sirtuina 3

SIRT3 jest najlepiej zbadaną mitochondrialną sirtuiną, występuje głownie w matrix mitochondrialnym. Uważana jest za jeden z kluczowych regulatorów metabolizmu mitochondriów, a główną jej funkcja jest utrzymanie odpowiedniego poziomu ATP w komórkach, ochrona przed uszkodzeniami, regulacja niemal wszystkich procesów metabolicznych zachodzących w mitochondriach. SIRT3 wykazuje działanie antyoksydacyjne, reguluje cykl kwasu cytrynowego, fosforylacje oksydacyjną, cykl mocznikowy, produkcję reaktywnych form tlenu (*ang. reactive oxygen species,* ROS), oksydację kwasów tłuszczowych, apoptozę i inne. Jest

szeroko rozpowszechniona, zwłaszcza w tkankach bogatych w mitochondria, tj. mięśniu sercowym, nerkach, mięśniach szkieletowych, wątrobie. Dlatego też SIRT3 odgrywa kluczową rolę w rozwoju zaburzeń sercowo-naczyniowych, nowotworowych, neurodegeneracyjnych a także metabolicznych [30,31]. Dotychczas nie istnieje zbyt wiele danych dotyczących wpływu infekcji wirusem HIV na aktywność lub stężenie SIRT3 i związku tego białka z przebiegiem choroby. Wiadomo jednak, że SIRT3 wykazuje korzystne działanie przeciwzapalne i przeciwfibrotyczne, co jest korzystne w przebiegu współistniejących chorób wątroby i zakażenia wirusem HIV [37]. Działanie antyoksydacyjne SIRT3 jest także kluczowe w aspekcie zapobiegania chorobom sercowo-naczyniowym [32]. Yu et al. [25] wykazali zwiększone wytwarzanie ROS w limfocytach T CD8+ i CD4+ w przebiegu zakażenia HIV, a także zmiany potencjału błony mitochondrialnej i masy mitochondrialnej w porównaniu z komórkami izolowanymi od osób niezakażonych HIV [33].

Sirtuina 6

SIRT6 jest zlokalizowana głównie w jądrze komórkowym. Bierze udział w procesach naprawy DNA, działa jako supresor procesów nowotworowych oraz zapewnia stabilność genomu. Wpływa także na metabolizm i funkcję telomerów [34]. Poza tym, podobnie do SIRT1, jest regulatorem wielu procesów metabolicznych. Reguluje metabolizm glukozy, między innymi przez wpływ na proces glukoneogenezy poprzez PGC-1α. SIRT6 wykazuje także interakcję z insulinozależnym transporterem glukozy typu 4 (*ang. Glucose transporter type 4*, GLUT-4), zapobiegając hipoglikemii. Leki z grupy PIs hamują działanie GLUT-4 w tkankach obwodowych, powodując zaburzenia wydzielania insuliny i przyczyniając się do rozwoju insulinooporności. SIRT6 reguluje także proces glikolizy [35,36]. Poza tym, podobnie jak SIRT1,wykazuje działanie przeciwfibrotyczne poprzez hamowanie sygnalizacji TGF-β i zmniejszenie ekspresji profibrotycznych genów [37].

Wyniki i ich omówienie

W badaniach własnych zaobserwowano niższe stężenia SIRT1, SIRT3 i SIRT6 w grupie mężczyzn zakażonych HIV zarówno przed, jak i po terapii cART w porównaniu do wartości otrzymanych w grupie kontrolnej. Dla SIRT1 mediana stężenia była 1,5- krotnie wyższa przed leczeniem w porównaniu do wyników uzyskanych po terapii cART, choć różnica ta nie była istotna statystycznie. Mediany stężeń SIRT3 w grupie mężczyzn zakażonych HIV były zbliżone zarówno przed jak i po terapii cART. z kolei mediana stężenia SIRT6 u mężczyzn zakażonych HIV przed terapią cART była 1,6- krotnie niższa w porównaniu do wartości otrzymanej po leczeniu i różniła się istotnie statystycznie ($p=0.022$), a mediana SIRT6 dla mężczyzn zakażonych HIV przed terapią cART była 2,6- krotnie niższa w porównaniu do wartości otrzymanej w grupie kontrolnej i różniła się istotnie statystycznie ($p=0.007$),

Wyniki sugerują, że największy udział w patogenezie zakażenia HIV odgrywa SIRT6. Dotychczas jednak nie ma wystarczających danych literaturowych, dotyczących potencjalnych

mechanizmów działania lub ścieżek sygnałowych, które są modyfikowane w wyniku zakażenia wirusem HIV.

W wyniku terapii cART, zauważono korzystną zmianę parametrów klinicznych badanych mężczyzn, takich jak: wzrost liczby limfocytów CD4+, spadek limfocytów CD8+, redukcja miana replikacji HIV RNA. Mediany stężeń SIRT6 u mężczyzn zakażonych HIV z liczbą limfocytów CD8+≤1000 komórek/µl wzrosła prawie 2-krotnie na skutek terapii cART, a różnica była istotna statystycznie (p=0,04). Podobną zależność dla SIRT6 wykazano u mężczyzn zakażonych HIV z liczbą limfocytów CD8+>1000 komórek/µl (p=0.01). Przedstawione wyniki wskazują na korzystny wpływ terapii cART na ekspresję SIRT6 oraz odpowiedź układu immunologicznego i jego zdolność do kontrolowania infekcji HIV, poprzez aktywność limfocytów T CD8+.

Wykazano wyższe mediany stężeń badanych sirtuin po wdrożeniu rocznego schematu terapeutycznego opartego na INSTIs. Stężenie SIRT1 było prawie 4-krotnie wyższe u mężczyzn zakażonych HIV leczonych INSTIs w porównaniu do wartości uzyskanej w grupie leczonej PIs (p=0.025), co może wskazywać na znaczący wpływ leków z grupy PIs na modyfikacje aktywności SIRT1, co z kolei może być potencjalnym mechanizmem rozwoju schorzeń towarzyszących zakażeniu wirusem HIV, będących także konsekwencją stosowania cART.

Podsumowanie

Przedstawione wyniki sugerują, że spośród badanych sirtuin największy udział w przebiegu zakażenia HIV zaobserwowano dla SIRT6. Również znaczące zmiany w jej ekspresji zauważono po wdrożeniu rocznej terapii antyretrowirusowej, co wskazuje na konieczność dalszych badań w celu poznania szczegółowych mechanizmów działania SIRT6 w przebiegu zakażenia wirusem HIV. Wykazano także znaczące różnice w ekspresji SIRT1, w zależności od rodzaju zastosowanego leczenia, gdzie PIs znacząco redukowały poziom SIRT1, co stanowi interesujący temat dalszych badań. Otrzymane dane wskazują na potencjalną możliwość wykorzystania SIRT1 w monitorowaniu bezpieczeństwa terapii antyretrowirusowej, czy rozwoju schorzeń towarzyszących.

7.3. Publikacja 3

Publikacja 3 pt.: ***Effect of Combined Antiretroviral Therapy on the Levels of Selected Parameters Reflecting Metabolic and Inflammatory Disturbances in HIV-Infected Patients*** jest pracą oryginalną opublikowaną w czasopiśmie *Journal of Clinical Medicine* (IF 2020: 4,242, Pkt. MEiN:140). w artykule oceniono wpływ zakażenia HIV oraz rocznej terapii cART na stężenia wybranych parametrów związanych z metabolizmem węglowodanów i lipidów, chorobami układu krążenia i stanem zapalnym w osoczu krwi mężczyzn zakażonych HIV w odniesieniu do grupy kontrolnej, złożonej z mężczyzn niezakażonych HIV. Badanymi parametrami były: iryzyna (IRS), miostatyna (MSTN), ludzki peptyd YY (PYY), peptyd glukagonopodobny 1 (GLP-1), dipeptydylopeptydaza 4 (DPP4), fetuina A (FETU-A), pentraksyna 3 (PTX3)**,** czynnik pochodzenia stromalnego 1 (SDF-1) i chemokina RANTES.

Charakterystyka badanych parametrów

Iryzyna

IRS jest adipomiokiną obecną głównie w mięśniach szkieletowych oraz w tkance tłuszczowej podskórnej i trzewnej. Jest kluczowym czynnikiem regulującym metabolizm oraz wydatkowanie energii [38]. Jest obecnie przedmiotem badań dotyczących jej roli w rozwoju insulinooporności czy T2DM. Dane literaturowe wskazują na ochronną rolę IRS w rozwoju tych zaburzeń, a także otyłości oraz CVD i chorób nowotworowych. IRS wykazuje działanie przeciwzapalne, zwiększa wrażliwość komórek na insulinę, hamuje proces glukoneogenezy [39]. IRS redukuje także formowanie nowych adipocytów, nasila metabolizm tkanki tłuszczowej oraz termogenezę [45]. Srinivasa i wsp. [32] u zakażonych HIV, ze stwierdzonym zespołem metabolicznym wykazali znacząco obniżone stężenie IRS w porównaniu do grupy kontrolnej [40]. Wciąż jednak nie jest znany receptor dla IRS oraz dokładny mechanizm jej działania. Badania nad aktywnością iryzyny mogą być także przydatne w aspekcie schorzeń współistniejących infekcji HIV [38]. Rozważany jest także pozytywny wpływ IRS na prewencję chorób sercowo-naczyniowych poprzez zapobieganiu dysfunkcji śródbłonka, działanie przeciwzapalne oraz hamowanie apoptozy [45, 47].

Miostatyna

MSTN jest znana jako czynnik hamujacy proliferację mioblastów, determinujący ostateczną liczbę oraz wielkość włókien mięśniowych. Jej ekspresja obserwowana jest w mięśniach szkieletowych, tkance tłuszczowej oraz mięśniu sercowym [41]. MSTN wytwarzana jest przez włókna mięśniowe. Pełni ona funkcje autokrynne, ale może być także wydzielana do osocza krwi i wykazywać działanie endokrynne oraz do przylegających komórek satelitowych (działanie parakrynne), hamując ich proliferację i różnicowanie. MSTN występuje we krwi w postaci nieaktywnego propeptydu. Podwyższone stężenie MST predysponuje do rozwoju zaburzeń metabolicznych, w tym insulinooporności [42]. Wykazano także, że stężenie MSTN

jest pozytywnie skorelowane z poziomem glukozy na czczo (*ang. fasting bloog glucose,* FBG) oraz wskaźnikiem insulinooporności HOMA-IR (*ang. homeostatic model assessment for insulin resistance*) [43]. w badaniu obejmującym pacjentów zakażonych HIV, u których występował zespół wyniszczenia związany z AIDS oraz znacząca utrata masy ciała, obserwowano podwyższony osoczowy poziom MSTN w porównaniu do grupy kontrolnej [44].

Peptyd YY

Peptyd YY jest polipeptydem trzustkowym, składającym się z 38 reszt aminokwasowych, wydzielanym i syntezowanym przez komórki dokrewne L jelita grubego i okrężnicy [57]. PYY jest prawdopodobnie magazynowany oraz wydzielany wspólnie z GLP-1, reguluje oś jelitowo-mózgową, hamuje motorykę jelit i opróżnianie żołądka, wydzielanie soku żołądkowego oraz apetyt [58,59]. PYY hamuje także wydzielanie trzustkowe. Uważany jest także za cel nowych terapii przeciwcukrzycowych [45]. Ponadto wykazano odwrotną korelację między poziomem PYY, a czynnikami ryzyka sercowo-naczyniowego (cukrzyca, nadciśnienie i hipercholesterolemia) oraz ryzykiem zdarzeń sercowo-naczyniowych [46]. Działanie przeciwzapalne PYY wykorzystywane jest w badaniach nad leczeniem zapalenia trzustki [47]. Wciąż jednak nie ma danych literaturowych, dotyczących potencjalnych zmian w aktywności lub stężeniu PYY u pacjentów zakażonych HIV.

Glukagonopodobny peptyd 1

GLP-1 jest peptydem inkretynowym o efekcie hipoglikemicznym. Działa poprzez zwiększenie uwalniania insuliny z komórek β trzustki oraz hamowanie uwalniania glukagonu. Podobnie jak PYY, działa hamująco na motorykę przewodu pokarmowego i redukuje uczucie głodu [48]. Dane literaturowe dotyczące wydzielania i stężenia GLP-1 u pacjentów zakażonych HIV nie są jednoznaczne. Wykazano podwyższone, stymulowane posiłkiem, poziomy GLP-1 u osób zakażonych HIV, z insulinoopornością, w porównaniu do osób z normoglikemią. Wykazano upośledzone wydzielanie inkretyn u zakażonych HIV, u których występowało uszkodzenia nabłonka jelitowego i enteropatia w wyniku zakażenia [49,50].

Dipeptydylopeptydaza 4

DPP-4, inaczej zwana białkiem różnicowania dopełniacza 26 (CD26) jest ektoproteazą serynową należącą do prolilooligopeptydaz. Jest enzymem szeroko rozpowszechnionym w organizmie, jego ekspresja występuje m.in.: w wątrobie, nerkach, płucach, jelitach, itd. [48]. DPP-4 pełni różnorodne funkcje w oragizmie, a substratami dla tego enzymu są liczne neuromodulatory, hormony i chemokiny, m.in. SDF-1, CXCR4, RANTES, dlatego też odgrywa kluczową rolę w regulacji funkcji układu immunologicznego [42,43]. DPP-4 reguluje także funkcję limfocytów CD4+, komórek NK czy makrofagów, poprzez wiązanie deaminazy adenozyny, hamując tym samym przekształcenie adenozyny w inozynę i nasilenie proliferacji limfocytów T i zwiększenie ich migracji. Poza tym reguluje funkcje odpornościowe poprzez hydrolizę bioaktywnych peptydów i białek m.in.: neuropeptyd Y, SDF-1, eotaksyny [51]. DPP-4

uważany jest za kostymulatora aktywacji komórek T, jednak dokładny mechanizm tej interakcji nie jest poznany. Białko Tat wirusa HIV, wykazuje znaczne powinowactwo do DDP-4, osłabiając jego aktywność enzymatyczną, co także tłumaczy jego właściwości immunosupresyjne.

Fetuina A

Fetuina A inaczej zwana alfa-2-HS-glikoproteiną jest wielofunkcyjna glikoproteiną, a jej synteza przebiega głównie w wątrobie, a także w mniejszym stopniu w tkance tłuszczowej. Jest negatywnym białkiem ostrej fazy, a jej zmniejszoną syntezę obserwuje się w przebiegu stanów zapalnych. FETU-A jest inhibitorem kinazy tyrozynowej receptora insulinowego w mięśniach, wątrobie i tkance tłuszczowej, sprzyjając tym samym rozwojowi insulinooporności i T2DM [52,53]. FETU-A hamuje także ekspresję adiponektyny, receptora aktywowanego przez proliferatory peroksysomów γ (ang. *peroxisome proliferator-activated receptor* γ, PPARγ) oraz aktywuje receptor toll-podobny 4 (*ang. Toll-like receptor 4*, TLR 4) na powierzchni adipocytów oraz komórek układu odpornościowego, zmniejszając ekspresję prozapalnego czynnika jądrowego NF-kB oraz cytokin prozapalnych, co także predysponuje do zaburzeń metabolicznych [52].

Pentraxyna 3

PTX3 jest glikoproteiną, wielofunkcyjnym białkiem ostrej fazy, kluczowym czynnikiem regulującym nieswoistą odpowiedź immunologiczną. PTX3 wiąże składową dopełniacza C1q i usuwa kompleksy immunologiczne aktywując klasyczną ścieżkę aktywacji dopełniacza. PTX3 uwalniana jest w wyniku uszkodzenia tkanek w wyniku reakcji zapalnej, bierze udział w modulowaniu aktywacji układu dopełniacza, a także pełni kluczową funkcje w odbudowie uszkodzonych tkanek, dzięki interakcji z fibryną i plazminogenem. Syntezowana jest w odpowiedzi na fragmenty patogenów (m.in.: lipopolisacharyd), agonistów receptora TLR oraz cytokiny prozapalne, m.in.: TNF-α oraz IL-1β [54,55]. Jej stężenie wzrasta znacząco także w wyniku destabilizacji i pęknięcia blaszki miażdżycowej, prowadząc do ostrego zespołu wieńcowego [56]. PTX3 uważania jest także za czynnik ograniczający rozwój infekcji o podłożu bakteryjnym, grzybiczym, wirusowym oraz wskaźnik prognostyczny w przebiegu infekcji, jej stężenie jest odwrotnie proporcjonalne do stopnia ciężkości infekcji [57,58]. Dotychczas brak jest danych literaturowych dotyczących zmian stężeń tego parametru w przebiegu HIV czy terapii antyretrowirusowej.

Czynnik pochodzenia stromalnego

SDF-1 syntezowany jest głównie przez komórki zrębu szpiku kostnego, a jego ekspresja występuje w wielu tkankach i komórkach, m.in.: wątrobie, mięśniu sercowym, śledzionie, trzustce. SDF-1 jest silnym czynnikiem chemotaktycznym dla limfocytów i odgrywa kluczową rolę w regulacji reakcji zapalnej, mielopoezie, a także patogenezie HIV, będąc ligandem dla CXCR4, który jest wykorzystywany przez niektóre szczepy wirusa jako koreceptor [59]. Poza

tym, odgrywa kluczową rolę w procesach angiogenezy. Działa także stabilizująco na blaszkę miażdżycową [59,60]. Znaczenie i dokładny mechanizm działania SDF-1 w zakażeniu HIV nie jest jednoznaczny w przebiegu całego cyklu życiowego wirusa. SDF-1 hamuje wnikanie do komórki wirusów X4 tropowych, a także prawdopodobnie zwiększa wirulencje szczepów CCR5 tropowych oraz stymuluje działanie białka Tat, zwiększając wydajność syntezy wirusowego DNA. Wykazano podwyższone stężenie SDF-1 u pacjentów zakażonych HIV. Wysokie poziomy SDF-1 są uważane za czynnik prognostyczny niewystarczającej odbudowy układu immunologicznego, mimo zastosowania skutecznego leczenia antyretrowirusowego. Jak pokazują dane z piśmiennictwa, u pacjentów, u których terapia okazała się nieskuteczna, stężenie SDF-1 jest znacząco wyższe, niż u pacjentów, u których zaobserwowano znaczące odbudowanie i większą ilość komórek populacji CD4+ [61]. Wciąż jednak nie ma wystarczających danych dotyczących zmian stężenia SDF-1 w wyniku infekcji wirusem HIV oraz w wyniku zastosowania cART.

Chemokina RANTES

RANTES (*ang. Regulated upon Activation, Normal T Cell Expressed and Presumably Secreted*, RANTES) jest chemokiną z podrodziny CC, syntezowana przez limfocyty T, będąca mediatorem procesu zapalnego oraz posiadająca właściwości chemotaktyczne względem komórek układu odpornościowego, tj. limfocytów T, monocytów, komórek dendrytycznych, do miejsca uszkodzenia lub infekcji. Wykazano ochronną role RANTES w przebiegu infekcji wirusowych, w mechanizmie zależnym od rekrutacji komórek T efektorowych i pamięci oraz komórek dendrytycznych. RANTES jest agonistą receptora CCR5, będącego punktem uchwytu dla leków z grupy inhibitorów wejścia, stosowanych w terapii antyretrowirusowej i uznany jest za czynnik ograniczający progresję wirusa HIV [62]. Wykazano także, że RANTES redukuje replikację HIV w fagocytach jednojądrzastych krwi i płuc, stanowiąc swoisty rezerwuar wirusa, co może mieć znaczenie terapeutyczne w zapobieganiu i/lub leczeniu HIV [63]. Wykazano, że podwyższone poziomy RANTES korelują z progresją miażdżycy i ostrego zespołu wieńcowego [64]. RANTES, który zostaje uwolniony z aktywowanych płytek krwi, sprzyja ich agregacji oraz powstawaniu zmian miażdżycowych. Reguluje również aktywność limfocytów T w zmianach miażdżycowych, przyczyniając się do progresji choroby [65].

Wyniki i ich omówienie

Zaobserwowano statystycznie istotne niższe stężenia następujących parametrów (przedstawione jako mediany): IRS (2,4-krotnie, p=0,02); MSTN (2-krotnie, p=0,02); PYY (1,2-krotnie, p=0,02); GLP-1 (2,9-krotnie, p=0,004); PTX3 (1,6-krotnie, p=0,04); RANTES (2,3-krotnie, p=0,02) w osoczu krwi mężczyzn zakażonych HIV przed wdrożeniem leczenia, w porównaniu do wartości otrzymanych w grupie kontrolnej. Zaobserwowane istotne statystyczne różnice dla tych parametrów świadczą o znacznym wpływie zakażenia HIV na spadek ich ekspresji. Największą, prawie 3-krotną różnicę zaobserwowano dla GLP-1. Stężenia

FETU-A i SDF-1 choć były niższe w porównaniu do wartości w grupie kontrolnej (odpowiednio 2,3- i 2-krotnie), to jednak nie różniły się istotnie statystycznie. Jedynie dla DDP-4 u mężczyzn zakażonych HIV przed leczeniem mediana była 1,4- krotnie wyższa w porównaniu do grupy kontrolnej, jednak również nieistotnie statystycznie. Natomiast w obserwacji jednorocznej, po zastosowanym leczeniu cART mediana DDP-4 obniżyła się 2,2-krotnie.

W grupie mężczyzn zakażonych HIV po rocznej terapii cART mediany stężeń wszystkich parametrów były niższe w porównaniu do grupy kontrolnej, ale nie wykazano różnic istotnych statystycznie. Dane te wskazują na konieczność prowadzenia dalszych badań w tym zakresie, z uwzględnieniem dłuższego okresu obserwacji.

W grupie mężczyzn zakażonych HIV po terapii cART stężenia IRS, MSTN, GLP-1 oraz FETU-A były wyższe w porównaniu do wartości uzyskanych przed leczeniem, choć nieistotne statystycznie. Może to świadczyć o wpływie terapii cART na wzrost ekspresji wymienionych białek. Stężenia pozostałych parametrów- PYY, DPP-4, PTX-3 oraz RANTES były niższe w porównaniu do uzyskanych przed terapią cART, a dla PYY wykazano różnicę istotną statystycznie (p=0,04), świadczące o hamowaniu ekspresji tych białek w wyniku leczenia antyretrowirusowego. Jedynie dla SDF-1 stężenia nie różniły się przed i po terapii cART wykluczając wpływ leczenia na to białko.

W analizie wyników uwzględniających miano replikacji wirusa HIV RNA, liczby limfocytów T CD4+ i CD8+ wykazano istotną zależność jedynie dla dwóch parametrów: FETU-A i PTX3. u mężczyzn zakażonych HIV z mianem limfocytów T CD8+ ≤1000 komórek/µl stężenia FETU-A i PTX3 były odpowiednio 3-krotnie, (p=0,03) i 2,6-krotnie (p=0,04) wyższe po leczeniu w obserwacji jednorocznej w porównaniu do wartości przed terapią cART, co wskazuje na zależność poziomu tych białek od odpowiedzi na leczenie antyretrowirusowe i parametrów układu immunologicznego.

Wykazano wyższe wartości stężeń dla wszystkich badanych parametrów wprowadzając schemat terapeutyczny oparty na INSTIs w porównaniu do wyników otrzymanych u mężczyzn zakażonych HIV leczonych PIs.

Podsumowanie

Uzyskane wyniki wskazują na istotne zmiany ekspresji wybranych parametrów w przebiegu zakażenia HIV oraz związane z wdrożeniem terapii cART, w stosunkowo krótkim okresie obserwacji wynoszącym jeden rok. w osoczu krwi mężczyzn zakażonych HIV aż sześć z dziewięciu badanych parametrów, tj. IRS, MSTN, PYY, GLP-1, PTX3, RANTES, wykazały istotnie statystycznie niższe mediany stężeń w porównaniu do osób zdrowych, co pokazuje znaczący wpływ zakażenia na obniżenie ich ekspresji. Po rocznej terapii cART nie wykazano różnic istotnych statystycznie między stężeniami badanych parametrów, a grupą kontrolną, co sugeruje wzrost ekspresji badanych białek po zastosowaniu leczenia antyretrowirusowego. Badania pokazały znaczący wpływ terapii z użyciem PIs na spadek stężeń wszystkich badanych

parametrów, co może wskazywać na znaczący udział tej grupy leków w rozwoju schorzeń towarzyszących. Potrzebne są dalsze badania nad przydatnością tych parametrów, które dostarczą nowych informacji na temat patogenezy i rozwoju chorób współistniejących związanych z HIV. Uzyskane dane mogą być przydatne w monitorowaniu przebiegu zakażenia HIV, skuteczności leczenia czy optymalizacji terapii.

7.4. Publikacja 4

Publikacja 4 pt.: ***Differences in expression of selected interleukins in HIV- infected subject undergoing antiretroviral therapy*** jest pracą oryginalną opublikowaną w czasopiśmie *Viruses* (IF 2020: 5,048 Pkt. MEiN:100). w artykule oceniono wpływ zakażenia HIV oraz zastosowanej terapii cART na stężenie trzech interleukin: IL-4, IL-7 i IL-15 w osoczu krwi mężczyzn zakażonych HIV, w odniesieniu do grupy kontrolnej złożonej z mężczyzn niezakażonych HIV, w obserwacji jednorocznej.

Charakterystyka badanych interleukin

Interleukina 4

IL-4 jest cytokiną reakcji zapalnej typu II, syntezowaną przez limfocyty Th2, bazofile i komórki tuczne. Odgrywa znaczącą rolę w odpowiedzi na reakcję zapalną wywołaną zarówno przez pasożyty, jak i alergeny. IL-4 jest także regulatorem różnicowania limfocytów B i limfocytów Th2 oraz przełączania przeciwciał klas IgG1 i IgE, co ma kluczowe znaczenie w rozwoju reakcji alergicznej [18]. IL-4 działa antagonistycznie do IFNγ, hamuje też uwalnianie cytokin prozapalnych [66]. w przebiegu infekcji wirusem HIV i dalszej jej progresji, dochodzi do deregulacji układu immunologicznego i znacznej przewagi odpowiedzi związanej z komórkami Th2, które produkują IL-4, IL-6, IL-10 i aktywują eozynofile oraz limfocyty T pomocnicze do produkcji przeciwciał: IgG1 i IgE, promując w ten sposób odporność humoralną i tłumienie odporności komórkowej. w rezultacie dochodzi do immunosupresji, predysponującej do wielu chorób oportunistycznych związanych z zakażeniem wirusem HIV. IL-4 wpływa także na przebieg zakażenia poprzez interakcję z receptorami CCR5, zmniejszając ich ekspresję, ograniczając wejście wirusa do komórki CCR5-tropowego. IL-4 zwiększa też ekspresję CXCL4 oraz nasila ekspresję HIV poprzez aktywacje transkrypcji wirusowej [67].

Interleukina 7

IL-7 jest cytokiną syntezowaną przez komórki zrębowe szpiku oraz grasicy. Jej główną funkcją jest regulacja rozwoju komórek odpornościowych, w tym limfocytów B, limfocytów T oraz komórek NK. Wytwarzana jest także przez neurony, keratynocyty, enterocyty, komórki śródbłonka oraz komórki dendrytyczne. IL-7 ma wiele aktywności biologicznych i wpływa na różne typy komórek poprzez wiązanie się ze swoim receptorem (*ang. interleukin 7 receptor*, IL-7R). Niedobory IL-7 lub IL-7R mogą prowadzić do poważnego upośledzenia rozwoju komórek odpornościowych. IL-7R ulega ekspresji w tymocytach, limfocytach T, komórkach pre-B, makrofagach i innych komórkach odpornościowych [68]. Szlaki sygnałowe IL-7 również prowadzą do zmiany w ekspresji modulatorów receptorów limfocytów T, a w rezultacie zwiększonego przeżycia oraz proliferacji limfocytów T oraz wzmocnienie sygnałów z receptorów limfocytów T. u osób zakażonych HIV obserwuje się podwyższone stężenia IL-7, które odwrotnie korelują z liczbą limfocytów T CD4+, co może świadczyć o działaniach rekompensa-

cyjnych, w celu przywrócenia populacji CD4+ [69]. Działanie IL-7 stało się celem badań klinicznych dotyczących analogów IL-7 i potencjalnym wykorzystaniem ich w przebiegu m.in. HIV. [70]. IL-7 może być także uznana za marker odbudowy układu immunologicznego podczas terapii antyretrowirusowej i może być przydatny w monitorowaniu postępów leczenia.

Interleukina15

IL-15 jest multifunkcyjną cytokiną, o zróżnicowanych efektach. Odgrywa kluczową funkcję w obronie przeciwwirusowej oraz przeciw niewirusowym patogenom wewnątrzkomórkowym. IL-15 jest także niezbędna do różnicowania i rozwoju komórek NK oraz do homeostatycznej ekspansji limfocytów T CD8+ i limfocytów NK. IL-15 jest czynnikiem warunkującym przeżycie i hamującym apoptozę limfocytów T, B oraz komórek NK, poprzez zwiększenie ekspresji białek antyapoptotycznych. Produkowana jest głównie przez makrofagi, monocyty i komórki dendrytyczne. Warunkuje cytotoksyczność oraz proliferację komórek NK, odgrywając kluczową rolę w obronie gospodarza poprzez bezpośrednie zabijanie komórek zakażonych wirusem oraz syntezę interferonu γ (ang. interferon gamma, INF-γ), a także aktywację makrofagów. Poza tym IL-15 bierze udział w różnicowaniu komórek dendrytycznych i neutrofili. Aktywacja genu IL-15, indukowana wirusem HIV powiązana jest z masową proliferacją limfocytów T CD8+ i utrzymywaniem limfocytów T pamięci, swoistych dla wirusa [71]. Prawdopodobnie IL-15 korzystnie wpływa na przebieg zakażenia wirusem HIV w mechanizmie zależnym od makrofagów i stymulowania ich do produkcji chemokin takich jak: IL-8 i MCP-1, zwiększając skuteczność reakcji immunologicznej w miejscu zapalenia. Wykazano obniżoną ekspresję IL-15 u pacjentów zakażonych wirusem HIV. Poza tym wytwarzanie IL-15 przez komórki jednojądrzaste krwi obwodowej było znacząco zmniejszone u pacjentów nieleczonych antyretrowirusowo lub nieskutecznie leczonych [72].

Wyniki i ich omówienie

W badaniach wykazano istotną statystycznie różnicę miedzy średnim stężeniem IL-4 w osoczu mężczyzn zakażonych HIV w momencie rozpoznania zakażenia, w porównaniu do wartości w grupie kontrolnej (2-krotnie wyższe, p=0,03) oraz w porównaniu do średniego stężenia tej interleukiny po rocznym leczeniu antyretrowirusowym (1,6-krotnie wyższe, p=0,04). Wykazano również istotnie wyższe stężenie IL-4 (p=0,001) w osoczu mężczyzn zakażonych z mianem HIV RNA >100 000 kopii/ml w porównaniu do miana HIV RNA ≤100 000 kopii/ml.

Statystycznie istotnie wyższe stężenie IL-7 zaobserwowano u mężczyzn zakażonych HIV przed terapią, w porównaniu do stosujących cART (p=0,02). Zaobserwowano także statystycznie istotne różnice między stężeniem IL-7 u mężczyzn zakażonych HIV przed cART, w zależności od liczby limfocytów T CD4+ oraz CD8+. Mediana IL-7 u mężczyzn zakażonych HIV z liczbą limfocytami T CD4+ ≤ 300 komórek/µl była 2,3 razy wyższa w porównaniu do stężenia u mężczyzn zakażonych wirusem HIV z liczbą limfocytów T CD4+ > 300 komórek/µl (p=0,026). Stężenie IL-7 u mężczyzn zakażonych HIV z liczbą limfocytów T CD8+ ≤

1000 komórek/µl była 6-krotnie wyższa w porównaniu do mężczyzn zakażonych wirusem HIV z liczbą limfocytów T CD8+ >1000 komórek/µl (p=0,027).

Zauważono tendencję spadkową stężenia IL-15 u mężczyzn zakażonych HIV w momencie rozpoznania choroby, w porównaniu do wyników otrzymanych u tych pacjentów po rocznej terapii cART, różnice jednak nie były istotne statystycznie.

Zastosowanie w terapii cART schematu leczniczego z użyciem PIs spowodowało spadek stężenia IL-4 i IL-7 w porównaniu do leczenia z użyciem INSTIs. IL-15 okazała się jedynym badanym parametrem, którego stężenie wzrosło po zastosowaniu PIs, jednak różnica ta nie była istotna statystycznie.

Podsumowanie

Wykazano istotne zmiany ekspresji badanych interleukin w przebiegu zakażenia wirusem HIV oraz zmianę ich stężenia po zastosowaniu terapii cART. Najbardziej wyraźne zmiany stwierdzono dla IL-4 oraz IL-7, co wskazuje na ich istotny udział w przebiegu zakażenia wirusem HIV. Wyższe stężenie IL-7 u pacjentów zakażonych HIV przed terapią, w porównaniu do pacjentów po rocznej terapii, może świadczyć o działaniach kompensacyjnych w celu przywrócenia populacji limfocytów T CD4+. Dane te wskazują, że IL-7 może być dobrym wskaźnikiem predykcyjnym odbudowy populacji limfocytów T CD4+ oraz CD8+ podczas terapii cART. Wzrost ekspresji IL-4 zauważony w przebiegu zakażenia HIV jest związany ze stymulacją produkcji limfocytów Th2 i nasiloną odpowiedzią immunologiczna typu humoralnego. Wprowadzenie terapii cART prowadzi do spadku miana HIV RNA i tym samym zmniejszenia syntezy IL-4, co potwierdzają otrzymane wyniki.

Konieczne są dalsze badania, które pomogłyby określić przydatność tych interleukin w monitorowaniu przebiegu infekcji, zwłaszcza jako parametrów o wartości prognostycznej dotyczącej odbudowy układu immunologicznego w wyniku stosowanej terapii.

8. WNIOSKI

1. Artykuł poglądowy dostarczył informacji o potencjalnym udziale sirtuin w przebiegu zakażenia HIV i ich współudziale w rozwoju chorób towarzyszących, wskazując na SIRT1, SIRT3, SIRT6, jako mające największe znaczenie w przebiegu zakażenia HIV i rozwoju chorób współistniejących

2. Zaobserwowano wpływ zakażenia HIV na wartość stężeń analizowanych parametrów. Stężenia sześciu z 15-stu badanych parametrów, tj. SIRT6, IRS, MSTN, PYY, GLP-1, PTX3, RANTES w osoczu krwi mężczyzn zakażonych HIV były istotnie statystycznie niższe, a stężenia IL-4 i IL-7 istotnie statystycznie wyższe w porównaniu do wyników w grupie kontrolnej - mężczyzn niezakażonych HIV.

3. Wykazano, że wdrożona terapia cART prowadzi do zmian stężenia większości badanych parametrów, ale istotne statystycznie różnice wykryto tylko dla czterech z nich: SIRT6, PYY, IL-4 i IL-7. Istotny wzrost stężenia odnotowano dla SIRT6, a istotny spadek stężenia dla PYY, IL-4 i IL-7 w osoczu krwi mężczyzn zakażonych HIV po rocznej terapii antyretrowirusowej

4. Wykazano istotną zależność pomiędzy liczbą limfocytów T CD4+, CD8+ oraz mianem HIV RNA, a stężeniem niektórych parametrów. Zaobserwowano różnice istotna statystycznie między ilością limfocytów T CD8+, a stężeniem SIRT6, FETU-A, PTX3 oraz IL-7. Dla IL-7 taką zależność wykazano również od ilości limfocytów T CD4+. Miano HIV RNA wpłynęło istotnie na zmiany stężenia IL-4.

5. Zauważono obniżone stężenia badanych parametrów (oprócz IL-15) u osób zakażonych HIV leczonych inhibitorami proteazy (PIs) w porównaniu do terapii z użyciem inhibitorów integrazy (INSTIs), ale jedynie dla SIRT1 różnica ta była istotna statystycznie.

6. Otrzymane wstępne wyniki, wskazują na konieczność kontynuacji badań dla określenia wartości predykcyjnej badanych parametrów w ocenie ryzyka rozwoju chorób współistniejących jako skutek uboczny stosowanej terapii u osób z HIV.

9. PODSUMOWANIE

Dzięki wysokiej skuteczności terapii cART, infekcja wirusem HIV stała się chorobą przewlekłą, a długość życia osób zakażonych jest obecnie zbliżona do średniej długości życia w populacji ogólnej. Jak wskazują badania, przewlekły charakter zakażenia HIV oraz stosowanie skutecznej i długotrwałej terapii cART, wpływa jednak na rozwój zaburzeń metabolicznych, w tym zaburzeń gospodarki węglowodanowej, lipidowej, chorób sercowo-naczyniowych, co związane jest też pośrednio z występowaniem przewlekłego stanu zapalnego w wyniku zakażenia. Dane literaturowe w tym zakresie są jednak znacząco ograniczone, co częściowo uniemożliwia optymalizację terapii lub wprowadzenie odpowiednich działań profilaktycznych lub leczniczych, zapobiegając rozwojowi schorzeń towarzyszących oraz pogorszeniu stanu klinicznego pacjentów.

W wyniku przeprowadzonych badań własnych, zaobserwowano zmiany w stężeniach badanych sirtuin, a zwłaszcza SIRT6, wskazując na ich potencjalną rolę w rozwoju zaburzeń metabolicznych u osób zakażonych HIV. Znacząco obniżone stężenie SIRT1 u zakażonych HIV stosujących leczenie oparte na PIs, wskazuje także na potencjalny udział SIRT1 w rozwoju schorzeń towarzyszących będących następstwem ich stosowania. Wciąż jednak potrzebne są dalsze badania, które dostarczą szczegółowych danych, dotyczących potencjalnych mechanizmów działania lub wspólnych ścieżek sygnalizacyjnych dla leków z grupy PIs i INSTIs oraz SIRT1.

Ponadto zaobserwowano, że stężenia pozostałych badanych parametrów, poza IL-15, były obniżone u osób stosujących schemat cART oparty na PIs, w porównaniu do schematu terapeutycznego opartego na INSTIs. Nie były jednak to różnice statystycznie istotne. Powyższe dane sugerują wpływ PIs na zmiany stężeń badanych parametrów, co może wskazywać na znaczny udział tych leków w rozwoju zaburzeń metabolicznych i schorzeń towarzyszących zakażeniu HIV w powiązaniu ze zmianami ekspresji SIRT1.

Podsumowując, przeprowadzone badania wskazują na możliwość wykorzystania powyższych parametrów w monitorowaniu przebiegu zakażenia HIV, optymalizacji terapii antyretrowirusowej oraz możliwych schorzeń współistniejących jako następstwo zastosowanego leczenia, tak, aby minimalizować ryzyko działań ubocznych terapii oraz zachować jej wysoką skuteczność. Wstępne wyniki przedstawione w pracy sugerują potrzebę prowadzenia dalszych badań w tym zakresie, na większej liczbie osób zakażonych HIV, a także z uwzględnieniem dłuższego okresu obserwacji prowadzonej terapii antyretrowirusowej, niż w przeprowadzonej w badaniu obserwacji 12-sto miesięcznej.

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11. WYKAZ STOSOWANYCH SKRÓTÓW

ADR - *ang. adverse drug effect* - skutki uboczne działania leków
AIDS - *ang. aquired Immunodeificiency Syndrome* - zespół nabytego niedoboru odporności
cART - *ang. combined antiretroviral therapy* - skojarzona terapia antyretrowirusowa
CD4 - ang. *cluster of differentation 4*
CVD - *ang. cardiovascular diseases* - choroby sercowo-naczyniowe
DPP-4 - *ang. dipeptidyl peptidase-4* - dipeptydylopeptydaza 4
EACS- *ang. European AIDS Clinical Society*- Europejskie Stowarzyszenie Badań nad AIDS
EMA - *ang. European Medicine Agency* - Europejska Agencja Leków
FDA - *ang. Food and Drug Administration* - Agencja Żywności i Leków
FETU-A - *ang. fetuin A* - fetuina A
FOXO - *ang. forkhead box o* - białka należące do rodziny forkhead box O
GIP - *ang. glucose-dependent insulinotropic peptide* - glukozozależny polipeptyd insulinotropowy
GLP-1 - *ang. glucagon-like peptide-1*- peptyd glukagonopodobny 1
GLUT-4 - *ang. glucose transporter type 4* - transporter glukozy typu 4
HIF-1α - *ang. hipoxia inducible factor 1 alpha* - czynnik indukowany hipoksją 1-alfa
HIV - *ang. human immunodeficiency virus* - ludzki wirus upośledzenia odporności
IEL - *ang. intraepithelial lymphocytes*- limfocyty śródnabłonkowe
IFNα - *ang. interferon alpha* - interferon α
IL-4, 7, 15 - *ang. interleukin 4,7,15* - interleukina 4, 7, 15
INSTIs - *ang. integrase Strand Transfer Inhibitors* - inhibitory integrazy
IRS - *ang. irisin* - iryzyna
LXR - *ang. liver X receptor* - receptor wątrobowy X
MAPK - *ang. mitogen-activated protein kinases* - kinazy aktywowane mitogenami
MSTN - *ang. myostatin* - miostatyna
NF-kB - *ang. nuclear factor kappa-light-chain-enhancer of activated B cells*- jądrowy czynnik transkrypcyjny NF-kB
NNRTIs - *ang. non-Nucleoside reverse transcriptase inhibitors* - nienukleozydowe inhibitory odwrotnej transkryptazy
NRTIs - *ang. nucleoside reverse transcriptase inhibitors* - nukleozydowe inhibitory odwrotnej transkryptazy
PGC-1α - *ang. peroxisome proliferator-activated receptor gamma coactivator 1*-alpha - koaktywator 1α receptora γ aktywowanego przez proliferatory peroksysomów
PIs - *ang. protease inhibitors* - inhibitory proteazy
PTX3 - *ang. pentraxin 3* - pentraksyna 3
PYY - *ang. peptide YY* - Peptyd YY
RANTES - *ang. regulated on activation, normal t expressed and secreted*- chemokina RANTES
rhIL-7 - *ang. human recombinant IL-7* - rekombinowana ludzka interleukina-7
ROS - *ang. reactive oxygen species* - reaktywne formy tlenu
SDF-1 - *ang. stromal cell-derived factor 1* - Czynnik pochodzenia stromalnego 1

SIRT1,3,6 - *ang. sirtuin 1,3,6,-* Sirtuina 1,3,6
SNP - *ang. single nucleotide polymorphism* - polimorfizmy pojedynczego nukleotydu
SREBP-1,2 - *ang. sterol regulatory element-binding transcription factor 1,2* - białko wiążące element regulatorowy steroli 1 ,2
Tat - *ang. trans-Activator of transcription*

12. SPIS ZAŁĄCZNIKÓW

Załącznik 1 Publikacja 1

Jurkowska K, Szymańska B, Knysz B, Kuźniarski A, Piwowar A. Sirtuins as Interesting Players in the Course of HIV Infection and Comorbidities. Cells. 2021; 10(10):2739. https://doi.org/10.3390/cells10102739

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Załącznik 2 Publikacja 2

Jurkowska K, Szymańska B, Knysz B, Piwowar A. The Effect of Antiretroviral Therapy on SIRT1, SIRT3 and SIRT6 Expression in HIV-Infected Patients. Molecules. 2022; 27(4):1358. https://doi.org/10.3390/molecules27041358

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Załącznik 3 Publikacja 3

Jurkowska K, Szymańska B, Knysz B, Piwowar A. Effect of Combined Antiretroviral Therapy on the Levels of Selected Parameters Reflecting Metabolic and Inflammatory Disturbances in HIV-Infected Patients. Journal of Clinical Medicine. 2022; 11(6):1713. https://doi.org/10.3390/jcm11061713

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Załącznik 4 Publikacja 4

Szymańska B, **Jurkowska K**, Knysz B, Piwowar A. Differences in Expression of Selected Interleukins in HIV-Infected Subjects Undergoing Antiretroviral Therapy. Viruses. 2022; 14(5):997. https://doi.org/10.3390/v14050997

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Załącznik 5 Całkowity dorobek naukowy

Załącznik 6 Zgoda Komisji Bioetycznej

Załącznik 7 Oświadczenia współautorów

Załącznik 1

cells

MDPI

Review

Sirtuins as Interesting Players in the Course of HIV Infection and Comorbidities

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Abstract: The sirtuins (SIRTs) are a family of enzymes from the group of NAD^+-dependent deacetylases. Through the reaction of splitting the acetyl group of various transcription factors and histones they regulate many processes in the organism. The activity of sirtuins is linked to metabolic control, oxidative stress, inflammation and apoptosis, and they also affect the course of viral infections. For this reason, they may participate in the pathogenesis and development of many diseases, but little is known about their role in the course of human immunodeficiency virus (HIV) infection, which is the subject of this review. In the course of HIV infection, comorbidities such as: neurodegenerative disorders, obesity, insulin resistance and diabetes, lipid disorders and cardiovascular diseases, renal and bone diseases developed more frequently and faster compared to the general population. The role of sirtuins in the development of accompanying diseases in the course of HIV infection may also be interesting. There is still a lack of detailed information on this subject. The role of sirtuins, especially SIRT1, SIRT3, SIRT6, are indicated to be of great importance in the course of HIV infection and the development of the abovementioned comorbidities.

Keywords: HIV; sirtuins; HAART; cART; comorbidities

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1. Introduction

The number of people infected with HIV and developed acquired immunodeficiency syndrome (AIDS) is still large, but thanks to the introduction of highly active antiretroviral therapy (HAART), the life expectancy of infected people has significantly increased. The incidence of AIDS-defining diseases has decreased, but the development of accompanying diseases is becoming the most important health and social problem among HIV-infected patients [1]. Cohort analysis conducted in the years 1999–2004, among patients in the USA living with HIV (n = 68.669), showed a significant increase in mortality (32.8%) due to non-AIDS-related comorbidities (NARCs) [2]. Morbidities, such as: diabetes, cardiovascular diseases, hypertension, hyperlipidemia, kidney diseases, osteoporosis, hepatitis C virus infection, occur much more often in infected people due to accelerated aging processes in HIV-positive cohorts [3,4]. A multicenter, cross-sectional study conducted by Serrão et al. [3] among HIV-infected patients over the age of 50 (n = 401) has shown that there is a positive relationship between the age of the patients and the occurrence of NARCs, which include: diabetes mellitus, hypercholesterolemia, arterial hypertension, acute myocardial infarction, stroke, renal failure, renal lithiasis, chronic hepatitis C (CHC), chronic hepatitis B, emphysema/bronchitis, osteoporosis and depression/chronic anxiety ($p < 0.001$), as well as between NARC and duration of infection ($p < 0.001$). It is also noteworthy that nearly 90% of patients had at least one NARC, and 35% had three or more. The most common were: hypercholesterolemia, hypertension, depression/chronic anxiety, chronic hepatitis C and diabetes mellitus, which occurred in over 50% of patients [3].

The selection of appropriate medication and optimization of therapy, also in terms of additionally used drugs, seems to be particularly important to avoid the risk of interaction or lowering the effectiveness of antiretroviral therapy. However, there is still little literature data on the detailed mechanisms of the increased risk of metabolic diseases or accelerated aging [1]. Research on signaling pathways that are involved in the abovementioned disorders is still needed to better understand the pathogenesis of the diseases. The latest literature data indicate that proteins from the sirtuin family play a role in the deepening of these comorbidities or increasing their occurrence in HIV-infected patients.

The aim of this review is to indicate the potential role for sirtuins in the course of HIV infection, as well as their involvement in the development of the abovementioned comorbidities and worsening of HIV patients' condition. In this review we describe our current understanding of the biological function of the sirtuin (SIRT 1–7) family in the course of HIV infection as well as the development of other diseases—comorbidities not directly induced by HIV infection. This research reviews the literature based on the MEDLINE database using the keywords: sirtuins, HIV, cardiovascular disease, insulin resistance, diabetes, osteoporosis, kidney diseases, neurodegenerative diseases in the years 2009–2020; 3209 records were found, of which 175 were selected for this review.

2. Sirtuins Family—The General Role in Organisms

Sirtuins (SIRTs) were first isolated from yeast Sacharomyces cerviesiae. They belong to distinct class III NAD^+-dependent histone deacetylases (HDACs). In humans, there are seven genes for sirtuins, where SIRT 1 belong to class Ia, SIRT 2 and SIRT3 3 to the class Ib, SIRT 4 to class II, SIRT 5 to class III and SIRT 6, 7 to class IV. This classification was created based on sequence homology to all proteins from the Sir2 (silent information regulator) protein family [5]. Their main function is the reaction of splitting the acetyl group from proteins, transcription factors, histones, etc., with cofactor NAD^+, and thus the covalent modification of target proteins. Equally importantly, though this is yet to be fully understood, in addition to their deacetylase activity, these proteins can have alternative enzymatic activities, including ADP ribosylation (SIRT1, SIRT4 and SIRT6), desuccinylation and demalonylation (SIRT5), delipoylation (SIRT4) and depalmitoylation and demyristoylation (SIRT6). This breadth of functions is partly derived from sirtuin's diverse intracellular localizations: nuclear (SIRT1, SIRT6 and SIRT7), cytoplasmic (SIRT2), mitochondrial (SIRT3, SIRT4, SIRT5) [6–9]. The enzymes of the sirtuin family have similar chemical structure in both prokaryotes and eukaryotes. Characteristic elements of their structure are: (1) a catalytic domain consisting of about 260 amino acid residues, (2) two key subdomains: Rossman's NAD^+ binding folder and zinc ions binding subdomain, (3) an enzyme active site formed between subdomains, (4) a NAD^+ binding pocket [10].

Detailed information about their localization, function, role and main molecular targets are well described in many scientific publications [6–9], so therefore, we will not describe this data in detail in this study, but we will only present the most important information in terms of potential grab points for the action of sirtuins, which are common in the pathomechanism of HIV infection and the development of accompanying diseases.

3. Role of Sirtuins during HIV and Other Viral Infections

Given the diverse localizations and activities described above, sirtuins are core regulators of metabolism and transcription. They have the ability to control numerous cellular pathways required throughout the viral life cycle, but the exact role of sirtuins in viral infections has not yet been fully understood [11].

3.1. Viral Targets and Sirtuin Action

Sirtuins can potentially affect viral factors and influence the course of infection. An example of this type of interaction is the deacetylation of the Tat HIV viral protein by SIRT1, which affects the efficiency of transcription of the integrated viral genetic material [12]. Viral Tat protein influences chromatin modifying factors in a manner that is favorable to

viral replication, including: recruiting histone acetyltransferase 1, increasing the level of Tat acetylation and increasing the efficiency of the RNA transcription process, contributing to an increase in viral load [13,14]. Deacetylation of Tat by SIRT1 causes its reconstitution and recruitment of appropriate host proteins, including cyclin T1 to TAR element at the 5′ end of viral RNA, preventing the termination of the transcription process at the elongation stage and starting the next transcription cycle. Without Tat protein, the elongation process is ineffective, resulting in significantly higher levels of interrupted and fewer full-length viral transcripts [15]. Tat is also a factor directly affecting SIRT1, binding to its catalytic domain and thereby blocking deacetylase activity relative to NF-kB, p53, p21 or BCL2-associated X protein (BAX), causing an HIV-specific status of chronic immune activation [16]. By inducing a state of chronic immune activation due to HIV infection, the production of transcription factors and pro-inflammatory interleukins, among others: NF-kB and IL-2, is intensified, which allows the integration of the newly formed viral DNA into the host genome [17,18].

The greatest number of studies on the relationship between SIRT1 and HIV Tat protein concern IL-2 and NF-kB, the activity of which is crucial in the course of infection. Activation of IL-2 gene expression occurs via the T cell receptor (TCR) and the CD28 coreceptor. The virus protein Tat is involved in the process activation of the IL-2 gene and regulates expression of host genes in infected T cells [17]. As a result, the activation of T cells via TCR receptors causes cleavage of the NF- kB from Inhibitor of NF- kB (IkB) inhibitory factor, its translocation to the nucleus and activation due to post-translational modifications, including lysine acetylation by SIRT1 [19]. In vitro studies have shown a reduction in NFkB-p65, a subunit deacetylation in the presence of Tat as well as in the presence of nicotinamide (NAM)-factor, limiting the activity of SIRT1. Jurkat T cells vectored with Tat showed a 1200-fold increase in IL-2 mRNA levels in Tat infected cells, compared with only 250-fold increase in control cells. Mouse $SIRT1^{-/-}$ and $SIRT1^{+/+}$ embryonic fibroblasts exposed to tumor necrosis factor α (TNFα) to activate NF-kB showed an increased copy number of mRNA IκBα and NF-kB responsive product in $SIRT1^{-/-}$ mice. The introduction of Tat protein increased expression of IκBα in $SIRT1^{+/+}$ mice (5–11 times) and in $SIRT1^{-/-}$ mice (2–3 times) [15].

Tat also negatively affects SIRT1 activity by affecting the NAD^+/NADH ratio, a key factor for the modulation of its activity. Zhang et al. [20] showed on Hela-CD4-β-gal (MAGI) cell lines that the Tat protein inhibited nicotinamide phosphoribosyltransferase (NAMPt), an enzyme converting NADH to NAD^+ [20]. The exposure of MAGI cells to resveratrol increased NAD^+ levels and SIRT1 expression. Exposure to the SIRT1 inhibitor NAM and silencing of the SIRT1 gene by siRNA enhanced the activating effect of Tat on HIV transcription [12].

3.2. Host Targets and Sirtuins Action in Viral Infections

In vitro studies have shown that silencing the SIRT1 gene in MRC5 cells by small interfering RNA (siRNA) leads to increased replication efficiency of viruses, such as: herpes simplex virus-1 (HSV-1), influenza virus H1N, human cytomegalovirus (HCMV), adenovirus type 5 (Ad5), whereas SIRT1 activators, e.g. resveratrol (RSV) or CAY10602 (more selective for SIRT1 synthetic agent) reduced viral replication efficiency [11,13]. Such a broad antiviral spectrum of SIRT1 may be associated with metabolic activity and influence a number of host processes, such as glycolysis, fatty acid synthesis and fatty acid oxidation. SIRT1 action can prevent changes in cell metabolism that accompany some viral infections, including: HSV-1, HCM, HCMV, and keep metabolic homeostasis [13].

SIRT1 can affect viral replication through its effect on host transcription factors, which can be used in the course of a viral infection. These include: nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB), forkhead box protein O1 (FOXO1) or p53 [13]. Bogoi et al. [14] showed that people infected with HIV had a significantly higher gene expression of SIRT1 in isolated CD4+ cells, compared to the control group of non-infected subjects. Expression of genes encoding chromatin-modifying enzymes such as: methyl-

transferases, acetylases and deacetylases, including SIRT1, are altered in HIV-infected individuals, in order to create an appropriate environment for virus replication and the progression of infection [14].

SIRT 1 is believed to be one of the key factors in maintaining the anergy state of T cells. Up-regulation of SIRT1 causes a reduction in the expression of induction factors of T cells: NF-kB, activator protein 1 (AP-1) and IL-2 [14,16]. Gao et al. [16] showed on naive CD4+ lymphocytes isolated from transgenic mice that lymphocytes showed a potential mechanism of switching to an anergic state through the regulation of SIRT1 expression. The authors noted that the highest expression of SIRT1 occurs in anergic (latent) T cells, compared to activated, naive and differentiated effector T cells. T cells become anergic by stimulation of the TCR receptor without costimulation of the CD28 receptor. This process is a natural mechanism in the prevention of autoimmune processes. After TCR stimulation by anti CD28 and anti CD3, a significant increase in SIRT1 expression was observed. The authors showed that FOXO3a binds to the promoter for SIRT1 via EGR2 and EGR3, activating SIRT1 transcription peripheral T cell tolerance. By the reverse process of activating T cells, IL-2 sequesters FOXO3a into the cytoplasm, preventing the enhanced expression of SIRT1, which causes reversion of T cell anergy [16]. In ex vivo studies using CD8-depleted mononuclear cells (PBMCs) isolated from peripheral blood of HIV-infected patients treated with antiretroviral drugs, without detectable viremia, Samer et al. [17] demonstrated the ability of sirtuins inhibitor NAM to reverse HIV-1 latency by inhibiting histone deacetylation and inhibiting chromatin condensation. As a result, the latency was reversed and HIV was actively expressed. NAM has been shown to be more effective in reversing HIV latency and achieving serological remission compared to the combination of methyltansferase inhibitors (MTI): chaetocin and BIX01294 [17]. The described results may indicate the potential activity of SIRT1 as a latency reversing agent, which may be an interesting area for further research. Figure 1 shows a scheme of the effect of SIRT1 on the viral Tat protein and HIV transcription.

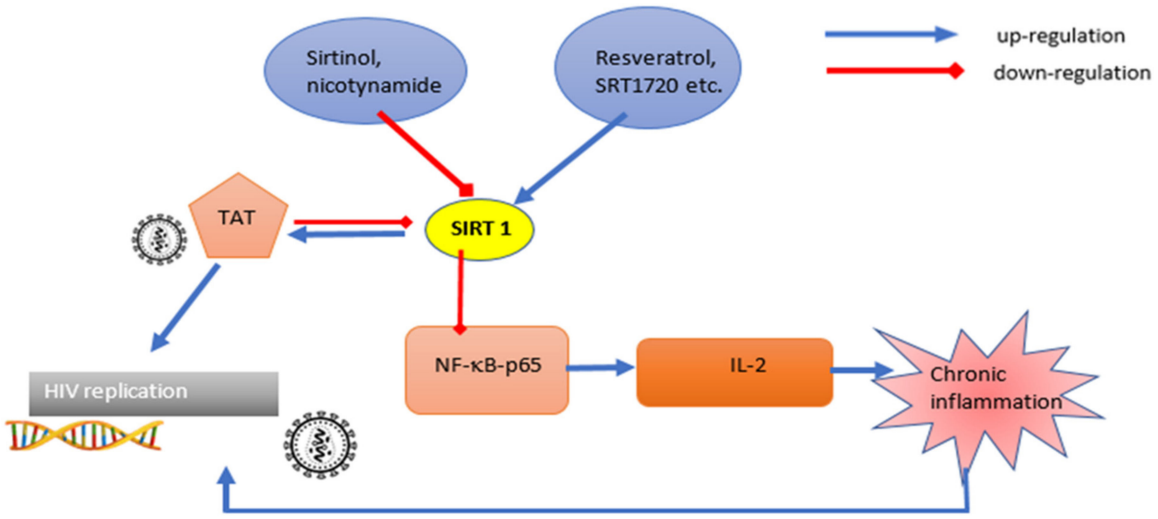

Figure 1. Scheme of interaction of SIRT1 1 with viral protein Tat [12]. Deacetylation of Tat by SIRT1 causes its reconstitution, preventing the termination of the transcription process at the elongation stage, starting the next HIV transcription cycle. Tat also directly affects SIRT1 by binding to its catalytic domain and thereby blocking deacetylase activity relative to NF-kB, causing production of transcription factors and pro-inflammatory interleukins, among others: IL-2, and as a result, HIV-specific status of chronic immune activation, which allows to integrate the newly formed viral DNA into the host genome. SIRT 1, sirtuin1; IL-2, interleukin 2; Tat, trans-sctivator of transcription; NF-κB, nuclear factor kappa-light-chain-enhancer of activated B cells.

4. Liver Disturbances in HIV and Sirtuin's Role

Literature data show that HIV patients are a group at risk of liver damage, hepatic inflammation and fibrosis development resulting from HIV infection itself, as well as the use of HAART [21]. In a Swiss cohort study (n = 2365) conducted between 2002–2008, 16% of participants developed an elevated alanine aminotransferase (ALT) level during the follow-up period, which was associated with HIV RNA above 100,000 copies/mL, Stavudine (d4T) and Zidovudine (AZT) treatment [22]. On the other hand, effective treatment significantly reduces the risk of severe liver diseases in HIV-infected patients [23]. The coreceptors of HIV: CC chemokine receptor 5 (CCR5) and C-X-C chemokine receptor type 4 (CXCR4), are present in hepatocytes, activated hepatic stellate cells (HSCs) and other liver cells, which explains the direct effect of the virus on the liver. Moreover, the presence of HIV RNA, proviral DNA and viral proteins has been demonstrated in all liver tissues [18,24]. Additionally, an almost twofold increase in the synthesis of collagen I, characteristic of fibrosis, and an 80-fold increase in MCP-1 levels in primary HSCs 48 h after HIV infection were shown, which indicate a direct pro-inflammatory and profibrogenic viral effect on HSCs. The profibrogenic effect in HSCs of the viral gp120 was also demonstrated by Bruno et al. [22], who observed increased expression of IL-6, chemoattractant protein-1 (MCP-1) and tissue inhibitor of metalloproteinase-1 (TIMP-1), which promotes liver fibrosis [22].

Among HIV-positive patients, hepatitis B virus (HBV) and hepatitis C virus (HCV) co-infection are common, which significantly increases the risk of liver cirrhosis and fibrosis. It is estimated that HCV co-infection occurs in approximately 5.8% of HIV-infected people [25]. Liver fibrosis is a reversible condition, caused by the activation of HSCs by platelet-derived growth factor (PDGF) or transforming growth factor β (TGF-β). As a result, HSCs increase the synthesis of collagen and extracellular matrix, which are the cause of liver fibrosis. HIV infection, even if successfully treated, accelerates HCV-induced liver fibrosis and leads to the earlier development of end-stage liver disease (ESLD) [26].

The influence of SIRT1 action in the development of CHC has been demonstrated. Li et al. [25] showed a significantly lower SIRT1 expression and increased levels of acetylated p53 in the liver tissues of CHC patients compared to the healthy controls. The decrease in SIRT1 expression was correlated with increased serum levels miR-34a and ac-p53, which suggests that SIRT1 may be inhibited by miR-34a, promoting apoptotic processes in hepatocytes [25].

Gupta et al. [26] noticed increased expression of profibrogenic factors, such as: TGF-β, matrix metalloproteinase-2 (MMP-2) and IL-6 in human hepatic stellate cells (LX-2) exposed to supernatants from HIV-infected PBMCs, compared to LX-2 treated uninfected PBMC supernatants. The authors also showed that LX-2 changed the expression of over 60 miRNAs, including those related to TGF-β signaling and the regulation of the expression of the profibrogenic genes: collagen type I alpha 1 chain (Col1a1), collagen type I alpha 2 chain (Col1a2), and collagen type III alpha 1 chain (Col3a1) and mothers against decapentaplegic homolog (SMADs) [26]. Research shows that the expression of the abovementioned genes and the activity of TGF-β is also regulated by SIRT6, which indicates its potential role in the development of hepatic disorders in HIV-infected people. The authors also showed that as a result of exposure to supernatants of HIV-infected PBMCs in the tested LX-2 cells, increased NF-κB p65 phosphorylation was observed, promoting pro-inflammatory and profibrotic effects [27].

Zhang et al. [27] showed that expression of SIRT6 was significantly reduced in HSCs isolated from mice fibrotic livers. Specific SIRT6 knockout in mouse primary HSCs resulted in activation of HSCs in a TGF-β dependent manner. Increased mRNA expression of fibrogenic genes: alpha-smooth muscle actin (α-SMA), Col1a1, Col1a2 and collagen type III alpha 1 chain Col3a1 was also noted. In contrast, the selective agonist of SIRT6- ML-800 inhibited the expression of the abovementioned genes, and the TGF-β-induced fibrosis in LX-2 cells. SIRT6 has also been shown to affect TGF-β in HEK293 cells by deacetylating lysine 54 of mothers against decapentaplegic homolog (SMAD) 2- profibrogenic transcription

factor, inhibiting its cytosol to nucleus translocation induced by TGF-β and its recruitment to the promoters of fibrogenesis genes; Col1a1 and Col1a2 acting protectively against liver fibrosis [27]. As shown by the above data, SIRT6, through down-regulation of TGF-β signaling and activation of related profibrogenic genes, indicates the participation of SIRT6 in the pathogenesis of liver fibrosis development in the course of HIV infection, eliminating the profibrotic effect of the virus.

It is mentioned that mitochondrial SIRT3 also has anti-fibrotic and anti-inflammatory activity. It deacetylates glycogen synthase kinase 3β (GSK-3β), increasing its activity, which in turn weakens TGF-β signaling in a mechanism dependent on β-catenin and SMADs. SIRT3 also contributes to the reduction in lipid accumulation in hepatocytes by activating AMPK signaling, weakening NF-κB inflammatory activity [28–30]. Li et al. [29] have shown that SIRT3 in NAFLD protects hepatocyte function by inhibiting OS and mitochondrial apoptosis via the ERK-CREB signaling pathway [29]. SIRT3 activation also increases the activities of antioxidant enzymes, such as CAT, SOD and GPx, showing a prevalent effect on liver fibrosis by reducing oxidative stress (OS) [31,32]. Increased oxidative stress is considered to be one of the causes of liver damage in HIV patients [31].

The literature data indicate the role of sirtuins as important regulators of hepatic metabolism, associated with, among others, carbohydrate and energy management. The effect of sirtuins on carbohydrate metabolism has been described earlier (point six). Calorie restriction increases NAD^+/NADH ratio, which activate SIRT1, resulting in activation of PPARα-, the main transcription factor regulating lipid metabolism during feeding and fasting periods. During fasting it intensifies beta oxidation, while during feeding causes de novo synthesis of lipids in the form of triglycerides, which are the reserve material in the liver [31]. In mice with NAFLD induced by a high fat diet, SIRT1 is a factor regulating the activity of QKI5-RNA-binding proteins—signal transduction and activation of RNA (STAR proteins)—which affect, among others, the synthesis of triglycerides in the liver. The exact function of QKI5 in the liver is not yet known. Western blot analysis and immunohistochemistry examination conducted by Zhang et al. [32] showed decreased liver expression of SIRT1, QKI5, FOXO1 and PPARα in the tested NAFLD mice when compared to the control group. Increased QKI5 acetylation in hepatocytes of the tested animals after the silencing of gene expression for SIRT1 with siRNA or after nicotinamide treatment was noted. An increase in FOXO1 and PPARα was also observed in SIRT1 agonist SRT1720-treated hepatocytes, and an inverse relationship has been demonstrated after exposure of hepatocytes to nicotinamide. It was also shown that the level of TG in hepatocytes was inversely proportional to the activity of SIRT1, significantly up-regulated by SRT170 and down-regulated by nicotinamide. The authors suggested that SIRT1 may be considered as a factor that regulates lipogenesis in hepatocytes by QKI5 via the FOXO1/PPARα signaling pathway. This was confirmed by studying the effect of the FOXO1 inhibitor AS1842856, which reduced FOXO1 and PPAR expression without affecting SIRT1 and QKI5. This effect was reversed under the influence of SRT1720 [32]. Studies on human fetal hepatocytes have shown that inhibition of SIRT1 increased de novo lipogenesis. Significantly increased expression of genes responsible for the synthesis of fatty acids was noted: acetyl-CoA carboxylase (ACC), stearoyl-CoA desaturase (SCD) and elongase of long chain fatty acids family 6 (ELOVL6) after exposure to the SIRT1 inhibitor, sirtinol (50 uM), compared to control [33].

As shown, about 30–70% of HIV-positive patients develop NAFLD without the involvement of HCV infection [34,35]. Studies in vpr transgenic mice (Vpr-Tg) showed a statistically significant higher fatty acid synthesis rate compared to WT mice. In addition, beta-oxidation of fatty acids was shown to be much less efficient in Vpr-Tg mice compared to WT mice. In the Vpr-Tg group, reduced expression of PPARα was also noted, compared to controls [36]. Sirtuin 1 regulates lipid metabolism directly via the SREBP-1, a key factor controlling the synthesis of fatty acids and triglycerides. During fasting, SIRT1 stimulates fat oxidation and inhibits their synthesis through SREBP-1c. SIRT1 deacetylates SREBP-1c in Lys-289 and Lys-309. Deacetylation reduces the stability of SREBP-1 and interaction

with promoters of lipogenesis enzymes genes. The interaction of SIRT1 and SREBP-1c has been proven both in vitro, in mouse hepatoma cells (Hepa1c1c7) and HepG2 cell model, and in vivo (8–10-week-old male BALB/c mice). An increase in the acetylation level of SREBP-1c in the livers of fasted animals with a silenced gene for SIRT1 (injected with adenoviral siSIRT1) compared to the control group (infected with adenovirus without siRNA for SIRT1) was shown. The SREBP-1c acetylation level was higher after treatment with glucose and insulin (to mimic feeding conditions) in HepG2 cells. After administration of 10 mM of the NAM-SIRT1 inhibitor, SREBP-1 acetylation was also significantly increased compared to control cells. High fat diet-induced obesity mice also showed increased levels of SREBP-1c, reduced hepatic levels of SIRT1, increased expression of the lipogenesis genes: SREBP-1c and Fas, and decreased expression of genes associated with fatty acid oxidation: ECI (3,2-trans enoyl-CoA-(Δ) isomerase) and MCAD (medium-chain acyl-coenzyme A dehydrogenase) compared to the control group [37]. Figure 2 shows schematically the participation of Sirtuin 1, Sirtuin 3 and Sirtuin 6 in the pathogenesis of liver disorders during HIV infection.

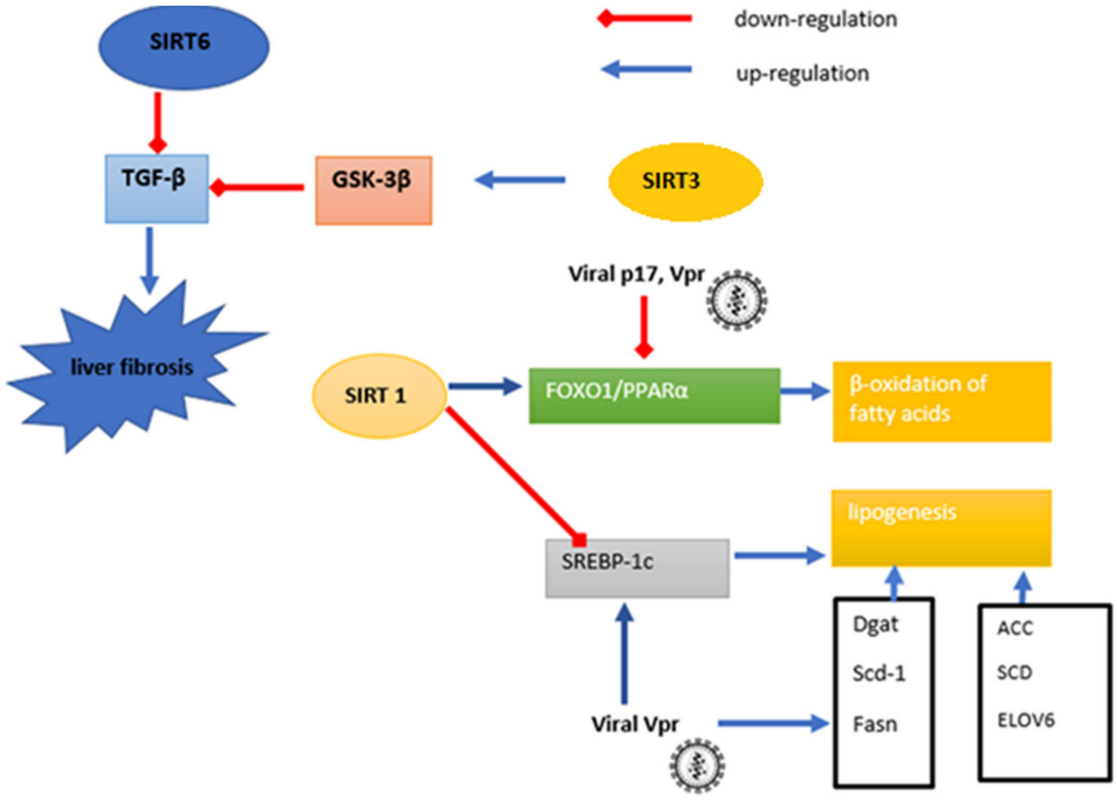

Figure 2. Scheme of connection between sirtuins, HIV and hepatic metabolism. SIRT3 deacetylates GSK-3β, increasing its activity, which in turn weakens TGF-β signaling. SIRT6, through down-regulation of TGF-β signaling reduces the expression of profibrogenic genes. SIRT1 up-regulates fatty acid oxidation in hepatocytes via the FOXO1/PPARα signaling pathway. Viral protein Vpr acts opposite to SIRT1 to increase expression of genes responsible for the synthesis of fatty acids by increasing SREBP1c activity. SREBP-1c, sterol regulatory element binding protein-1c; FOXO1, forkhead box protein O1; PPARα, peroxisome proliferator-activated receptor α; TGFβ, transforming growth factor β; GSK-3β, glycogen synthase kinase 3 beta.

5. Cardiovascular Risk in HIV-Infected Patients and Sirtuin Participation

An increased risk of cardiovascular disease is observed in HIV-positive patients. This is connected with the prevalence of metabolic disturbances and HIV-accompanying diseases: insulin resistance, dyslipidemia or hepatitis C, and changes in the immune system connected with HIV infection [38]. As epidemiological data show, increased mortality from

cardiovascular disease in such patients is observed, and it is one of the most common causes of non AIDS-related deaths in HIV-positive patients successfully treated with antiretroviral therapy [39]. According to the latest conducted meta-analysis, covering 32 original papers published in the period 1999–2008, the risk of myocardial infarction (MI) in HIV-positive people is significantly higher compared to the uninfected (relative risk: 1.73). An increased risk of MI has also been demonstrated depending on: CD4+ cell count, plasma viral load and long-term use of HAART, especially protease inhibitors (PIs), lopinavir/ritonavir (LPV/r) or indinavir (IDV) [40]. An increased risk in HIV patients of cardiovascular events, such as coronary heart disease, coronary calcification, silent myocardial ischemia, myocardial infarction and elevation of intima-media thickness has also been shown [41]. In a meta-analysis covering nine studies with 1229 HIV-positive patients and 1029 controls, the increased prevalence of non-calcified coronary plaques (NCP) was shown in HIV-positive patients on an average of 58% vs 17% compared to controls, and the risk of NCP was inversely correlated with CD4+ counts [42].

Excessive reactive oxygen species (ROS) production is also one of the key risk factors for CVD. Excessive free radical (hydroxyl, superoxide, hydrogen peroxide, lipid peroxyl) production causes a reduction in nitric oxide (NO) release as well as disorders in post-translational modification processes endothelial nitric oxide synthase (eNOS), caveolin-1 and increased production of eNOS inhibitor, asymetric dimethylarginine (ADMA), due to increased enzymatic activity of arginine-1 protein transferase (PRMT1). There are literature data showing the involvement of SIRT1 in the prevention of endothelial disorders as a result of OS [43].

In response to oxidative stress, caveolin-1 is phosphorylated in Tyr-14; this form sequesters SIRT1 to caveolae, reduces its amount in the cytosol and causes loss of its deacetylation capacity. Therefore, caveoline-1 can be considered as a potential inhibitor of SIRT1, by the binding of the caveolin-1 scaffolding domain (CSD) to the caveolin-binding domain (CBD) of SIRT1, leading to reduced NO production, and, as a result, endothelial dysfunction [44]. As a result of the absence or decreased SIRT1 activity, hyperacetylated PRMT1 causes a greater level of ADMA-eNOS inhibitor. SIRT1 is also responsible for deacetylation of eNOS at Lys-496 and Lys-506. This process increases enzyme activity and the production of NO, which prevents imbalance between endothelium-derived vasoconstrictors and relaxing factors, considered as a predictor of cardiac events [45]. Endothelial SIRT1 activity against eNOS is promoted by apurinic/apyrmidinic endonuclease 1/redox factor-1 (APE1/Ref-1)—an endonuclease that affects vascular homeostasis. It prevents inactivation of SIRT1 by free radical oxidation, through keeping sulfhydryl groups of cysteine in SIRT1 in a reduced form [46].

The decreased activity of SIRT1 also causes an increase in the amount of acetylated p53 in starting the process of apoptosis in endothelial cells (ECs) [47]. Inhibition of apoptosis in endothelial cells has been shown for viral gp120, which activates caspase 3, Bax and p38 MAP kinase signaling [41]. SIRT1 also deacetylates FOXO1, which has a positive effect on the regulation of apoptosis and cell cycle regulation. The FOXO1/SIRT1 signaling pathway also regulates the redox status of endothelium and maintains vascular homeostasis through an impact on vascular smooth muscle cells. Acetylated FOXO1 reduces the concentration of SIRT1 as well as catalase antioxidant enzymes (CAT) and manganese superoxide dismutase (MnSOD). SIRT1 also deacetylates Lys-81 of the p66Shc adapter protein, inhibiting its transcription by blocking its interaction of acetyl histone 3 with the promoter region for this protein, thereby preventing OS induction by p66Shc through the following mechanisms: down-regulation of GPx, MnSOD and APE1/Ref-1 and activation of membrane-associated NADPH oxidases [48].

Intercellular adhesion molecule 1 (ICAM-1) and vascular cell adhesion molecule 1 (VCAM-1) are also important factors in the development of endothelial dysfunction and atherosclerotic plaque formation, by facilitating the transmission of leukocytes through the epithelium and their adhesion [49]. Jiang et al. [50] demonstrated in human coronary artery endothelial cells (HCAECs) and porcine coronary artery rings treated for 16 h with recom-

binant gp120 (1 μg/mL) with or without the pretreatment of TNFα (as ICAM-1 inductor) that eNOS expression significantly decreased in both cell types with TNFα pretreatment compared to unexposed cells, and increased the expression of ICAM-1 in both cell types was shown: 5-fold under TNFα and about 80% after exposure to gp120, compared to controls. The use of ICAM-1 siRNA or specifically anti gp120 antibody inhibited conversion to eNOS expression caused by gp120 and TNFα. The authors concluded that TNFα and gp120 act synergistically and induce negative changes in endothelium, which may indicate a new mechanism for inducing endothelial disorder in the course of HIV infection [50]. Liu et al. [51] revealed the reversal of the viral gp120 effect of resveratrol (SIRT1 activator) on human umbilical vein endothelial cells (HUVECs). A dose-dependent (treatment 35, 40, 45 or 50 μM of resveratrol) decrease in ICAM-1 expression was noted. This study also showed that inhibition of ICAM-1 in TNFα treated cells was mediated by the inhibitory effect of resveratrol on the phosphorylation of p65 and IκB, activating NF-κB, thereby inhibiting the inflammatory process. The authors suggest that the effect of resveratrol on ICAM-1 can be mediated via the AMPK/p38/NF-κB signaling pathway [52]. The above data suggest that the mechanism of action of resveratrol is SIRT1 dependent. SIRT1 deacetylates Lys-310 in the RelA/p65 complex in NF-κB, suppressing its pro-inflammatory activity in response to OS, and inhibits the synthesis of pro-inflammatory cytokines or thrombotic factors (ICAM-1, VCAM-1) [52,53]. Pan et al. [53] have shown that resveratrol reverses the inhibitory effect of TNFα on SIRT1 expression. HUVECs treated with resveratrol (0, 5, 10 and 20 μM) showed a significantly decreased expression of SIRT1 after stimulation with 10 μg/L TNFα cells compared to controls. In addition, exposure of HUVECs to SIRT1 siRNA resulted in significantly increased expression of p65 and CD40, which was down-regulated after exposure to resveratrol. Silencing of SIRT1 expression also compensated the inhibitory effect of resveratrol on ROS production, which indicates that the antioxidant and anti-inflammatory effects of resveratrol are mainly related to SIRT1 activation. The authors point to a potential mechanism of SIRT1's action in protection against OS, by inhibiting TNFα-induced NFkB expression by attenuating p38 MAPK phosphorylation, which in turn promotes the nuclear translocation of p65 [53].

The viral Tat protein, which interacts with SIRT1, as described earlier (point four), also affects the risk of CVD through its effect on EC apoptosis and the development of inflammation. This occurs mainly in an NF-κB-dependent manner, increasing the expression of pro-inflammatory factors: ICAM-1, IL-1β, MCP-1, VCAM-1 and E-selectin, which has been shown on HUVECs and animal models [54]. Tat protein contributes to the apoptosis of ECs through its effect on the secretion of the TNFα and activation of the Fas–FasL-dependent pathway. Tat also induces ROS production through the impact on NF-kB, NADPH oxidase, MnSOD and glutathione GSH levels [55,56]. Endothelial dysfunction is also caused by another viral protein, Nef. Wang et al. [55] showed that Nef is present in the vascular cells, initiating atherosclerotic lesions. The authors showed that the induction of changes in endothelium results from two independent mechanisms: NF-kB and the production of MCP-1, and through the endothelial cell apoptosis via NADPH oxidase-dependent mechanism [55].

It has been shown that SIRT6 can affect the IGF-1/Akt signaling pathway, which regulates systemic aging processes and induces hypertrophy of myocardial cells in the myocardium, increasing predisposition to heart failure by deacetylating histone H3K9 in regions of promoters of IGF-1 up-regulating genes. SIRT6 influences the down-regulation of IGF-1/Akt through the c-Jun transcription factor. Studies in animal models have shown that mice with SIRT6 knockout developed concentric cardiac hypertrophy at about 8–12 weeks of age. Changes in the area of cardiomyocytes have also been noted, including their increased size, mitochondrial regression and vacuolization and interstitial fibrosis. Significantly higher levels of apoptotic proteins have also been demonstrated: caspase 3, Bax, TNF-related apoptosis-inducing ligand (TRAIL), Bcl-2-like protein 1 (Bim), Fas ligand (FasL) and cyclin-dependent kinase inhibitor 1B (CDKN1B) [56].

Some literature data have shown that mitochondrial sirtuins (SIRT 3, 4 and 5) also affect the cardiovascular system. The protective role of SIRT3 is primarily associated with antioxidant protection through the activation of SOD2 and CAT through deacetylation of the FOXO3a and protection against cardiomyocyte apoptosis [57,58]. SIRT3 in endothelial cells regulates the glycolysis process. ECs have the ability to perform metabolic reprogramming (so-called "metabolic flexibility"), which allows them to switch from oxidative phosphorylation to the use of glycolysis as the main source of energy. This mechanism allows the maintainance of tissue homeostasis and protects cells against the increased demand for energy during OS, hypoxia or tissue damage. In cultured ECs derived from SIRT3 knockout mice, damage to the glycolysis and glycolytic capacity process as well as reduced expression of 6-phosphofructo-2-kinase/fructose-2,6-biphosphatase 3 (FKFB3)—a factor that participates in the synthesis of the allosteric activator of PFK-1, fructose 2,6-bisphosphate—was observed. The above data indicate the participation of SIRT3 in the regulation of metabolic flexibility in a PFKFB3-dependent manner, by maintaining high glycolytic activity in the ECs [59].

The role of SIRT4 in CVD is also indicated, but the exact mechanism of action is unknown. Data indicate its high expression in the heart, and it is believed to be a regulating factor for heart hypertrophy in an angiotensin II (Ang-II)-dependent mechanism. Luo et al. [60] showed significantly less Ang-II -induced cell growth as well as decreased production of atrial natriuretic peptide (ANP), inhibiting the renin-angiotensin-aldosterone system (RAS) in cultured SIRT4 knockdown neonatal rat cardiomyocytes (NRCMs). This is a key mechanism in the regulation of not only cardiac hypertrophy but also blood pressure and electrolyte balance, which may indicate an important role of SIRT4 in the pathogenesis of CVD. After chronic Ang-II infusion for 4 weeks, SIRT4 knockout mice showed a significantly lower increase in heart weight to body weight ratio (HW/BW) compared to WT mice (8.9% vs 28.3%), which suggests the key role of SIRT4 in this process. In addition, SIRT4 knockout mice showed significantly greater heart ROS production compared to WT during cardiac hypertrophy, which is an unfavorable factor in the development of cardiac disorders [61]. In contrast to SIRT3, SIRT4 decreases mitochondrial MnSOD activity. SIRT4 overexpression in NRCMs decreased SIRT3-mediated MnSOD deacetylation and decreased its activity, promoting ROS accumulation during hypertrophic stress-inducing cardiovascular disorders [51].

Mitochondrial SIRT5 is highly expressed in the heart, as shown in both animal and human studies, although its function is not fully understood [60]. In the SIRT5 knockout mice, hearts with hypertrophy induced by transverse aortic constriction animal model showed impaired processes of oxidative phosphorylation, TCA, fatty acid oxidation and hypersuccinylation of proteins involved in these processes. It has been shown to decrease NAD^+/NADH ratio as well as the oxidation of glucose and fatty acids [62,63]. The role of SIRT5 in the regulation of platelet function and the formation of arterial thrombus, which is the cause of acute cardiovascular events, is also indicated. In studies on HAECs treated with SIRT5 si-mRNA, decreased expression levels of the fibrinolysis inhibitor PAI-1 were noted in response to TNFα, through activation of AMPK and reduced phosphorylation of (ERK)1/2 MAP kinase, which indicates the role of SIRT5 in the regulation of fibrinolysis. The SIRT5 knockout mice showed faster thrombus formation in the photochemically injured endothelium without altering platelet function and the clotting cascade. Increased levels of circulating D-dimers and an increased incidence of thrombus embolization have been noted. The above results were confirmed in peripheral blood mononuclear cells of patients with acute coronary syndrome (ACS), which showed significantly higher expression of SIRT5 and PAI-1 compared to the control group without ACS [64]. Figure 3 schematically shows the participation of sirtuins and HIV infection in the development of cardiovascular disorders.

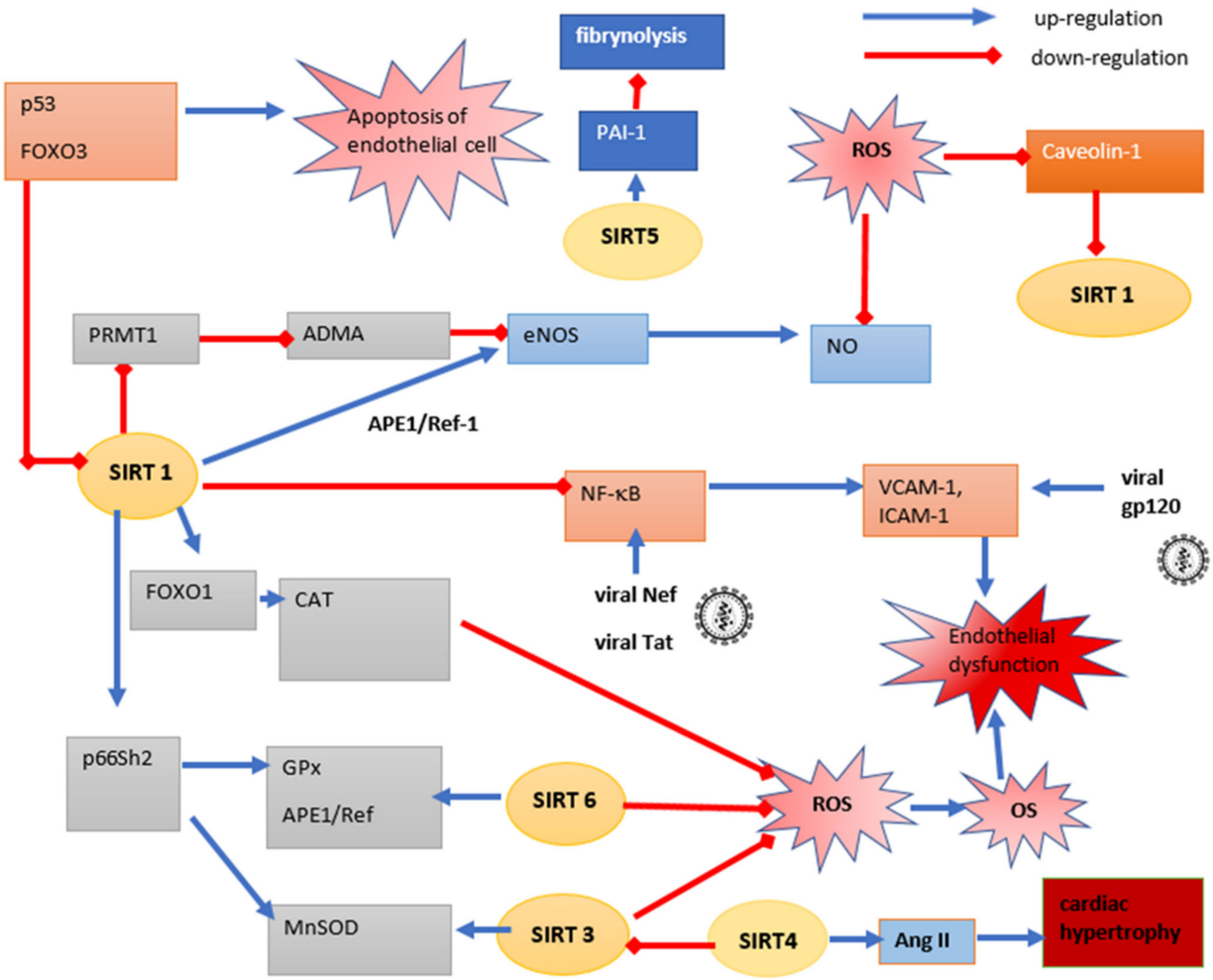

Figure 3. Scheme of connection between cardiovascular disorders, sirtuins and HIV infection. SIRT1 deacetylate PRMT1 and decrease levels of ADMA—eNOS inhibitor. SIRT1 is also responsible for deacetylation of eNOS, which increases enzyme activity and the production of NO. Endothelial SIRT1 activity against eNOS is promoted by APE1/Ref-1. SIRT1 decrease activity of p53, preventing process of apoptosis in endothelial cells. SIRT1 also deacetylates FOXO1, which has a positive effect on the regulation of apoptosis and cell cycle regulation. Decetylated FOXO1 increases the concentration of CAT and MnSOD. SIRT1 deacetylates Lys-310 in the RelA/p65 complex in NF-κB, suppressing its pro-inflammatory activity in response to OS, and inhibits the synthesis of pro-inflammatory cytokines or thrombotic factors (ICAM-1, VCAM-1). The protective role of SIRT3 is primarily associated with antioxidant protection through the activation of SOD2 and CAT through deacetylation of the FOXO3a and protection against cardiomyocyte apoptosis. In contrast to SIRT3, SIRT4 decreases mitochondrial MnSOD activity. By interacting with AngII, SIRT4 is also a contributing factor to cardiac hypertrophy. SIRT4 also regulates platelet function and the formation of arterial thrombus, by increasing the expression levels of the fibrinolysis inhibitor PAI-1. ROS, reactive oxygen species; OS, oxidative stress; SIRT1, 3, 4, 5, 6, sirtuin 1, 3, 4, 5, 6; eNOS, nitric oxide synthase 3; ADMA, asymetric dimethylarginine; PRMT1, arginine-1 protein transferase; APE1/Ref-1, purinic/apyrmidinic endonuclease 1/redox factor-1; FOXO3, forkhead box O3; VCAM-1, vascular cell adhesion molecule 1; ICAM-1, intercellular adhesion molecule 1; CAT, catalase; MnSOD, manganese-dependent superoxide dismutase; GPx, glutathione peroxidase; NF-κB, nuclear factor kappa-light-chain-enhancer of activated B cells; Ang II, angiotensin II; PAI-1, plasminogen activator inhibitor-1.

6. Insulin Resistance and Diabetes in HIV-Infected Patients and Sirtuins Participation

Type 2 diabetes mellitus (T2DM) is one of the most common concomitant diseases in HIV-infected people, and its occurrence significantly reduces the life expectancy of these patients [65,66]. Data analysis from National Health and Nutrition Examination Survey (NHANES) and Medical Monitoring Project (MMP) from 2009–2010 show a significantly higher risk of developing diabetes in HIV-infected people (n = 8610), which was 10.3% vs 8.3%, compared to the general population (n = 5604). Among the surveyed people from the HIV group, 52.3% had T2DM, while 3.9% had type 1 diabetes. In addition, compared to the general population, HIV-infected people suffer from diabetes at a younger age and in the absence of obesity. Moreover, the PIs and nucleoside reverse transcriptase inhibitors (NRTIs): zidovudine (AZT), stavudine (D4T), didanosine (DDI) have been indicated as antiretroviral drugs associated with an increased risk of carbohydrate metabolism disorders [67]. In addition, metabolic syndrome (MS), which is a set of risk factors contributing to the development of T2DM and cardiovascular diseases (CVD), including disturbances such as: abdominal obesity, hypertension, insulin resistance, elevated fasting plasma glucose, low high-density lipoprotein (HDL) cholesterol level and high serum triglycerides (TG) is diagnosed more often in HIV-infected people compared to the general population. Lipodystrophy syndrome, characterized by impaired fat metabolism, is also common in HIV-infected patients. This disturbance significantly increases the use of drugs, such as NRTIs and PIs, especially the older generation drugs [68]. PIs inhibit the reversible, uncompetitive glucose transporter type 4 (GLUT-4) in peripheral tissues, resulting in the disruption of insulin secretion and contributing to the development of insulin resistance. PIs also have an indirect effect on GLUT-4 through the up-regulation of heme oxygenase 1 (HO-1), and, as a result, the secretion of pro-inflammatory cytokines, such as IL-8, TNFα, CCL5 and MCP-1, inhibiting GLUT-4 functions and insulin receptor substrate 1 (IRS-1) by activation of c-Jun N-terminal kinase (JNK) and The IκB kinase (IKKβ). Another cause of the development of insulin resistance and T2DM are disorders of the adipocyte differentiation function as a result of PI use, which reduce the activity of phosphatidylinositol 3 kinase (PI3-K) and the intensify β cell apoptosis [69]. In a study of male patients treated with HAART in a regimen containing PIs indinavir (65.2%), nelfinavir (22%), ritonavir (7.6%) or ritonavir+saquinavir (4.6%), Vigouroux et al. [70] reveled that serum adiponectin levels were significantly negatively correlated with body mass index (BMI), waist-hip ratio (WHR), oral glucose tolerance test glycemia and insulinemia, triglycerides, apoB/A1 and hs-CRP levels, and positively correlated with quantitative insulin sensitivity check index (QUICKI) value. Patients treated with the NRTI stavudine had significantly lower serum adipokine levels [70]. This indicates an important role of the applied treatment in the development of these disorders.

Literature data showed that sirtuins affect glucose metabolism in the liver, pancreas, skeletal muscles or adipose tissue through various mechanisms, as described in detail in a number of studies [30,71,72]. The effect of sirtuins activity includes: increased gluconeogenesis, reduced glycolysis, increased lipolysis or increased insulin secretion from β cells, as will be detailed below [31,73,74]. Most of the literature data cover this area and most information is available on SIRT1, SIRT2, SIRT3 and SIRT6.

A main player from the sirtuin family in the modulation of glucose homeostasis is SIRT1, which affects insulin secretion in the pancreatic β cells. One of the target factors influenced by SIRT1 are transcription factors from the FOXO family, which affect the expression of genes related to carbohydrate and lipid metabolism, which will be discussed in detail in the following paragraphs and chapters. SIRT1, deacetylating FOXO1, allows the activation of transcription factors MafA and NeuroD, promoting insulin gene expression in pancreatic β cells [31]. SIRT1 is also the major factor regulating CREB-regulated transcription coactivator 2 (CRTC2) and FOXO1 activity influencing the gluconeogenesis process, depending on the energy supply. It was shown that the overexpression of SIRT1 in mice hepatocytes significantly reduced the amount of CRTC2 following glucagon exposure after 8 h fasting. Exposure of tested mice hepatocytes to SIRT1 inhibitors (sirtinol and nicoti-

namide) significantly increased CRTC2 activity, intensifying the process of gluconeogenesis. Mice with a hepatic-specific knockdown of SIRT1 gene showed significantly higher levels of CRTC2 and gluconeogenic gene expression compared to wild-type mice during prolonged exposure to glucagon. Deacetylation of CRTC2 by SIRT1 during an extended fasting period (over 18 h fasting) promotes ubiquitin-dependent CRTC2 degradation via COP1 (E3 ubiquitin-protein ligase). The authors concluded that SIRT1 is therefore a factor that regulates the energy balance by influencing the glucose metabolism [75]. Deacetylation of FOXO1 and its co-activator peroxisome proliferator activates receptor gamma coactivator 1 alpha (PGC-1α) by SIRT1, and also increases the transcription of gluconeogenesis genes during longer fasting periods. Deacetylation of PGC-1α by SIRT1 increases its transcriptional activity, increasing the expression of phosphoenolpyruvate carboxykinase (Pck) and glucose-6-phosphatase (G6pc) genes [31].

Another mechanism regulating glucose metabolism by SIRT1 is its effect on protein tyrosine phosphatase (PTP-1B) [69]. PTP-1B is a key phosphatase for the insulin receptor, reverses the action of tyrosine kinases and down-regulates insulin signal transduction. It catalyzes dephosphorylation of the insulin receptor, which in turn protects glucose uptake and blocks further steps in the insulin transduction pathway. SIRT1 modifies the activity of PTP-1B at the chromatin level by deacetylating the Lys9 of histone H3, and reduces its binding in the promoter region for PTP-1B, as shown in an in vitro study of C2C12 murine myotubes by Sun et al. [75] after recombinant HSV encoding SIRT1 infection and induction of insulin resistance by palmitate. The authors noted an increase in SIRT1 expression, insulin receptor (InsR) phosphorylation and glucose uptake after insulin stimulation, and this effect was inhibited by PTP-1B. A decrease in PTP-1B activity in in vivo studies with an increase in SIRT1 expression in the liver of the tested mice during fasting periods was also observed [75].

As mentioned earlier, HAART may negatively affect glucose metabolism, which may contribute to the development of T2DM and insulin resistance. One potential mechanism is the effect on mitochondrial uncoupling protein 2 (UCP2) by PIs. UCP2 is the protein responsible for controlled leakage of protons into the mitochondrial matrix; it lowers the electrochemical gradient of protons, thereby reducing ATP synthesis and glucose stimulated insulin release from β cells. SIRT1, by binding to the promoter for UCP2, inhibits this process, restoring insulin release from pancreatic β cells. The protease inhibitors used in HAART also interact with UCP2. It has been shown that rat insulinoma cells exposed to NFV (5–10 μM) significantly reduce ATP levels, while UCP2 levels are increased [76]. Studies on male Wistar rats orally treated with SQV (333 mg/kg/day) or ATV (133 mg/kg/day) showed significantly reduced levels of pancreatic ATP compared to the control group. Pancreatic UCP2 expression was instead increased in the ATV- and SQV-treated group compared to the control [77]. The above data may indicate the existence of a potential mechanism involving SIRT1, which can be considered to prevent drug-induced negative changes in HIV-infected patients.

Sirtuin 1 also deacetylates the HIF-1 by reducing its transcriptional activity, thereby affecting the glycolysis process by modifying transcription of lactate dehydrogenase (LDH), glucose-6-phosphate isomerase (PGI), phosphofructokinase-1 (PFK-1) or phosphoglycerate kinase 1 (PGK-1). Under hypoxia condition, as a result of lower NAD^+ concentration, HIF-1 acetylation does not occur, which activates glycolysis. SIRT1 also affects glycolysis processes independent of HIF-1 through deacetylation of phosphoglyceromutase-1 (PGAM-1), which catalyzes the conversion of 3-phosphoglycerate (3-PG) into 2-phosphoglycerate (2-PG) in glycolysis process, and thus its deactivation. In in vitro studies, Hallows et al. [78] showed increased levels of PGAM-1 acetylation in HEK293 cells under the influence of an SIRT1 inhibitor (10 mM sirtinol) [78].

Sirtuin 2 is also indicated as an important modulator of insulin resistance development. SIRT2 stabilizes and deacetylates PEPCK1 by inhibiting the action of E3 ubiquitin-protein ligase component N-recognin 5 (UBR5). PEPCK1 converts oxaloacetate into phosphoenolpyruvate in the gluconeogenesis process. Deacetylation of PEPCK1 prevents degra-

dation by ubiquitin-proteasome pathway, promoted by acetylation of Lys70, Lys71 and Lys594 by p300 acetyltransferase. In an animal study, it was found that SIRT2 knockdown decreased PEPCK1 level by 70% without changing the amount of its mRNA, and there was a 35% decrease in glucose levels in mice with SIRT2 knockdown [72]. SIRT2 has been shown to be down-regulated in mice with insulin resistant hepatocytes and accompanied by increased ROS accumulation and mitochondrial dysfunction. SIRT2 increases the expression of fusion-related protein mitofusin 2 (Mfn2) and downregulates mitochondrial-associated dynamin-1-like protein (Drp1), which improves mitochondrial function by enhancing the mitochondrial fragmentation process, and also enhances the expression of mitochondrial transcription factor A (TFAM), resulting in increased mitochondrial mass. Moreover, SIRT2 expression was negatively correlated with OS, obesity and insulin resistance in human PBMCs [79]. SIRT2 also regulates redox homeostasis by deacetylating FOXO3a, which increases MnSOD expression. Deacetylation of Lys403 of G6PD by SIRT2 also increases the production of NADPH and the reduced form of glutathione (GSH), which points to the antioxidant action of SIRT2 [71]. In adipocytes, down-regulation of SIRT2 reduces the degree of PGC-1α deacetylation and fatty acid catabolism. In visceral adipose biopsies of obese humans (BMI> 30 kg/m^2), increased expression of HIF1-α and decreased expression of SIRT2 were observed. An inverse relationship was observed in lean individuals (BMI <25 kg/m^2) [80]. SIRT2 is also considered to inhibit adipogenesis through deacetylation of FOXO1; as a result, it promotes its binding to peroxisome proliferator-activated receptor α (PPARα), reducing its transcriptional activity. After 24 h of fasting, mice showed increased expression of SIRT2 in white adipose tissue. Mice exposed to 5 °C (temperature as an inducer) for 12 h showed increased expression of SIRT2 in brown adipose tissue. Increased expression of SIRT2 was also noted in 3T3-L1 cells treated with β-adrenergic agonist, isoproterenol [81]. The above data indicate the key role of SIRT2 in the development of obesity, which is one of the main causes of the development of T2DM and other metabolic disorders connected with the course of HIV infection.

Sirtuin 3 is a factor that affects energy homeostasis primarily in skeletal muscles. It is the major mitochondrial deacetylase. The source of energy for skeletal muscles can be both glucose and lipids. They are characterized by metabolic flexibility and have the ability to change the energy substrate and obtain energy from glucose or lipid oxidation. A reduced amount of CREB, PGC-1α and CS and AMPK was found in SIRT3 deleted mice [82,83]. Jing et al. [84] showed that SIRT3 also deacetylates ATP synthase, affecting glucose homeostasis in skeletal muscle. This indicates an important role of this sirtuin in the development of metabolic disorders in the course of HIV. The pyruvate dehydrogenase (PDH) enzyme, which controls glucose use as a main energy source in skeletal muscles, is also affected by SIRT3 action. PDH is considered as a decisive factor in a complex mechanism of carbohydrate saving in the period of low glucose supply, and during the switch to use fats as the main source of energy. It catalyzes the oxidative decarboxylation of pyruvate in acetyl-CoA, allowing the incorporation of the glycolysis-pyruvate product in TCA. It has been shown that mitochondrial SIRT3 regulates the process of post-translational acetylation of the pyruvate dehydrogenase E1α subunit and decreases enzyme activity, which leads to a reduction in glucose oxidation and the use of fats as the main source of energy [85]. In SIRT3 knockout mice muscle mitochondrial lysates, PDH activity was significantly lower compared to WT mice. The increased amount of lactate and pyruvate in the muscles from fed SIRT3 KO mice, along with decreased PDH activity and a decreased amount of acylcarnitines and amino acids compared to WT suggests that SIRT3 deficiency causes changes in the metabolic profile of muscles towards the use of fatty acids and amino acids, which emphasizes the important role of SIRT3 in maintaining muscle energy homeostasis [83,86]. SIRT3 also deacetylates Lys 42 of a key enzyme responsible for liver β-oxidation of fatty acids-long-chain specific acyl-CoA dehydrogenase (LCAD), increasing its activity. Mice with SIRT3 knockdown starved for 48 h showed a reduction in fatty acid oxidation in brown adipose tissue and liver compared to the control group (WT mice). It was also shown that the SIRT3 knockdown mice showed an intolerance to cold exposure,

which is associated with impaired oxidation of fatty acids and metabolic stress [35,87]. Agrawal et al. [36] demonstrated that WT mice injected with Vpr viral protein showed reduced β-oxidation of fatty acids in the liver by about 45%, associated with decreased expression of enzymes regulated by PPARα among others, with lower expression of LCAD mRNA, the activity of which also regulates SIRT3. This indicates a possible interaction of this sirtuin with viral Vpr protein [36]. The effect of PPARα and SIRT3 on fat metabolism and the mechanistic action of Vpr on metabolism will be described in detail later in this article.

The literature data also indicate the role of SIRT6 in glucose metabolism disturbances, which may also be associated with the development of diabetes, which, as we know, is associated with HIV course. Xiong et al. [86] observed a reduction in glucose-stimulated insulin secretion (GSIS) in mice with knockdown of the SIRT6 gene by nearly 50% compared to the control group. Changes in mitochondria function and lower ATP levels were also observed, which resulted in disturbances in the transport of electrons in the respiratory chain; disturbances in plasma membrane depolarization and post-depolarization as well as calcium flux in β-cells were also observed. The effect of SIRT6 on glucose metabolism is associated primarily with the inhibition of gluconeogenesis. This process takes place via the PGC-1α, indirectly, by the activation of general control non-repressed protein 5 (GCN5), which subsequently acetylates PGC-1α, and in turn inhibits the expression of phosphoenolpyruvate carboxykinase C (PEPCK-C) genes and glucose 6-phosphatase, associated with gluconeogenesis. SIRT6 also acts by deacylating FOXO1-inhibiting gluconeogenesis, as described above [31,87]. Sirtuin 6 has been shown to influence the IRS/PI3K/AKT signaling pathway. As a result, inhibition of IRS phosphorylation leads to down-regulation of AKT (which also reduces expression of FOXO1, GSK3, mTORC1), insulin signaling and decreased glucose uptake. SIRT6 also inhibits GLUT-1 and -4 expression, which prevents hypoglycemia [88]. Old generation protease inhibitors (mainly ritonavir and indinavir) also interact with GLUT-4, impairing glucose transport, as mentioned above, which is one of the causes of disorders of carbohydrate metabolism in patients with HIV [89]. Bresciani et al. [89] have shown in the 3T3-L1 adipocyte cell model that exposure to lopinavir/ritonavir caused overexpression of miRNA-218 and the associated lower expression of lipin-1, the main factor determining GLUT-4 expression [89]. SIRT6 regulates glycolytic enzymes (PDK1, PFK1 and LDH) and HIF-1α activity in skeletal muscle, which also leads to the suppression of GLUT1 and GLUT3 [89,90]. Glycolysis is another mechanism regulated by SIRT6. Studies have shown that SIRT6, similarly to SIRT1, deacetylates and thereby inhibits HIF-1 and H3K9, which increases the expression of the genes responsible for glycolysis [86,90]. Figure 4 shows schematically the role of sirtuins (SIRT1, SIRT2, SIRT3 and SIRT6) in the development of carbohydrate disorders, taking into account the impact of HIV infection and antiretroviral therapy.

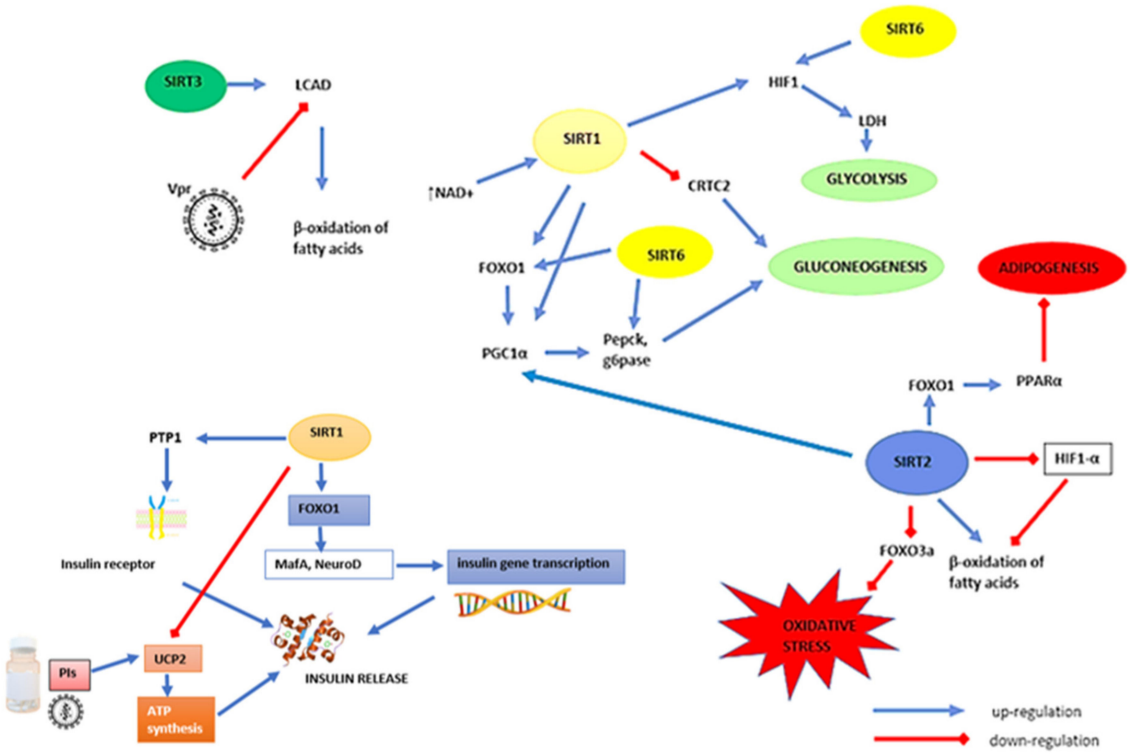

Figure 4. Scheme of connection between sirtuins, carbohydrate metabolism and HIV infection. SIRT1, by deacetylation of FOXO1, activates the transcription factors MafA and NeuroD and increases the expression of the insulin gene in pancreatic β cells. By deacetylation of PT-1B, SIRT1 reduces its insulin signal transduction and insulin release inhibitory activity. SIRT1 binds to the promoter for UCP2, inhibits the process of reducing ATP synthesis and restores insulin release from pancreatic β cells. Protease inhibitors act the opposite of SIRT1, they increase the activity of UCP2. SIRT1 down-regulates CRTC2, inhibiting gluconeogenesis during an extended fasting period. Deacetylation of FOXO1 and its co-activator PGC-1α by SIRT1, increases the transcription of gluconeogenesis genes. SIRT1 and SIRT6 deacetylates the HIF-1 by reducing its transcriptional activity, thereby intensifying glycolysis. SIRT2 regulates redox homeostasis by deacetylation of FOXO3a, which increases MnSOD expression and the reduced form of glutathione. In adipocytes, SIRT2 deacetylates PGC-1α, intensifying fatty acid catabolism and gluconeogenesis. SIRT2 also inhibits adipogenesis through deacetylation of FOXO1, promoting its binding to PPARα and reducing its transcriptional activity. SIRT3 deacetylates a key enzyme responsible for liver β-oxidation of fatty acids, LCAD, increasing its activity. Vpr viral protein reduce β-oxidation of fatty acids in the liver and decreases expression of LCAD. The effect of SIRT6 on glucose metabolism is associated with the inhibition of gluconeogenesis via the PGC-1α, which in turn inhibits the expression of PEPCK and glucose 6-phosphatase, associated with gluconeogenesis. SIRT6 also acts by deacylating FOXO1-inhibiting gluconeogenesis. SIRT1,2,3,6, sirtuin 1,2,3,6; HIF1, hypoxia inducible factor-1; LDH, lactate dehydrogenase; CRTC2, CREB regulated transcription coactivator 2; FOXO1, forkhead box protein O1; FOXO3a, forkhead box protein O3a; IL-6, interleukin 6; LCAD, long-chain specific acyl-CoA dehydrogenase; PC1α, peroxisome proliferator activated receptor gamma coactivator 1 alpha; STAT3, signal transducer and activator of transcription 3; UCP2, mtochondrial uncoupling protein 2; PTP1, protein tyrosine phosphatase 1; PPAR-α, peroxisome proliferator-activated receptor alpha; HIF1-α, hypoxia-inducible factor 1-alpha.

7. Bone Metabolism Disturbances in HIV-Infected Patients and the Role of Sirtuins

Negative changes associated with bone metabolism are common in HIV-infected patients. An open prospective cohort study (n = 5826) conducted between 2000–2006 as part of the HIV outpatient study (HOPS) showed that fracture rates and relative proportion of fragility fractures were significantly higher in HOPS patients compared to the general population [91]. Gibellini et al. [92] showed that in plasma of HIV-infected men, levels of markers associated with bone formation, such as osteoprotegerin (OPG), receptor activator of NF-kappa b-ligand (RANKL), and the TNF-related apoptosis-inducing ligand (TRAIL) were significantly higher in the HIV-infected group compared to the control group [93]. The

problem of reduced bone density is primarily caused by the negative impact of the Vpr on RANKL. Up-regulation of RANKL causes increased osteoclast formation and lower levels of OPG (RANKL inhibitor), the main factors determining bone metabolism and the degree of bone resorption [91,94]. In a cross-sectional study, statistically significant lower OPG production by T cells and higher T cell RANKL production was shown in HIV-infected patients, compared to healthy individuals [94]. Titanji et al. [95] showed significantly lower OPG expression and higher RANKL expression in B cells of HIV-positive patients, compared to negative controls. The authors also found a correlation between RANKL/OPG ratio in B cells and T-score (difference between the current bone density value and the theoretical mean peak bone mass), Z-score (difference between the current bone density value and the age-appropriate theoretical mean normal value) and total bone mineral density (BMD) in areas with the highest fracture risk: hip and femoral neck [95].

A higher risk of osteoporosis is associated not only with the HIV infection itself, but HAART also has an impact. In a cohort study (n = 40.115), Womack et al. [93] found significant positive correlations between increased fragility fracture risk and current PI use among HIV-infected patients [93]. The decline in BMD is independent of the type of HAART, however, PIs were considered to be the most osteotoxic agents. Some studies show that PIs in particular contribute to bone metabolism disorders. In patients treated with PIs, bone density in the lumbar spine after one year decreased significantly compared to other therapeutic regimens [96,97]. In vitro studies on human osteoblast cultures have shown that PIs change the expression of some genes responsible for calcium deposition in bones, they reduce alkaline phosphatase (ALP) and runt-related transcription factor- 2 (Runx- 2). Down-regulation in the expression of tissue inhibitors of metalloproteinases-3 (TIMP-3), responsible for osteoblast differentiation and matrix development processes, has been noticed in NFV and IDV [98]. Cozzolino et al. [99] have shown that among the analyzed HIV cases, a decrease in BMD by more than 5% was observed in 31% of patients after 4 years of virologic suppression, as a result of combined antiretroviral therapy (cART). In addition, in the National Health and Nutrition Examination Survey (NHANES), a significantly higher incidence of lower BMD in the femoral neck was demonstrated compared to controls (47% vs. 29%). PIs probably have a negative effect on vitamin D3 metabolism, as they can inhibit 25-α-hydroxylase 1a, which converts 25-hydroxyvitamin D into its active form, 1,25-dihydroxyvitamin D [99]. Another mechanism may be increased bone resorption caused by proximal renal tubular damage by tenofovir (TDF), leading to hypophosphatemia and increased parathyroid hormone release. In addition, tenofovir phosphate compounds interact directly with osteoblasts and osteoclasts, contributing to increased bone resorption [98,100]. Negredo et al. [100] showed significantly reduced BMD in HIV-infected patients, which was connected with changes in TDF to abacavir (ABC) after a 48-week period, and was manifested by a statistically significant lower level of some serum bone markers: collagen type 1 cross-linked C-telopeptide (CTX), type I procollagen N-terminal propeptide (P1NP), osteocalcin and higher sclerostin levels [100].

The influence of sirtuins on bone metabolism is increasingly indicated in HIV-infected patients. Choi et al. [101] have shown that SIRT1 is a deacetylating agent of SRY-Box transcription factor (SOX2), the main factor maintaining the self-renewal and ability to differentiate mesenchymal stem cells (MSCs), that is also observed in osteoblasts. SOX2 maintains stem cells by regulating the expression of Dickkopf-related protein 1 (DKK1). The SIRT1/SOX-2 axis has been shown to regulate the differentiation or regeneration of MSCs. Deacetylation of SOX2 by SIRT1 inhibits its transport from the nucleus to cytosol, degradation by proteasomes and ubiquitination, as demonstrated in in vitro studies on bone marrow mesenchymal stem cells (BM-MSCs) [101]. A positive effect of resveratrol (RSV), an SIRT1 activator, on maintaining SOX2 activity has also been demonstrated in cultured MSCs, which retained their ability to regenerate and proliferate under exposure to RSV (1 μM), compared to unexposed cells [102]. The positive effect of RSV was also shown in an animal model, where it was administered in three doses (5, 25 and 45 mg/kg/day) in female rats after ovariectomy. Statistically significant higher BMD was shown, compared to

the post-ovariectomy group that did not receive RSV at medium and high doses [103]. The same authors, in an in vitro study on BMSCs, confirmed that RSV significantly increased the level of SIRT1 expression, while reducing NF-κB p65 and p-IκBα compared to controls (untreated with resveratrol). As confirmed by siRNA-SIRT1 transfection, the beneficial effect of resveratrol is due to a modification of the SIRT1/NF-κB signaling pathway activity. The authors also showed that SIRT1 activation contributed to the induction of BMSC differentiation towards osteoblasts [104]. Similar results were found by Zainabadi et al. [103] in mice treated with SIRT1 synthetic agonist, SRT1720 (100 mg/kg/day). There was an almost 30% increase in femoral bone mass in SRT1720-treated mice, compared to only vehicle treated mice [103]. Expression of SIRT1 was confirmed in BM-MSC and primary bone marrow cultures. Cohen-Kfir et al. [104] in an animal model (SIRT1+/− and wild type (WT) mice) as well as a BM-MSC cell model showed significantly reduced bone formation in SIRT1+/− mice compared to WT. The authors revealed a statistically significantly lower mRNA expression of sialoprotein, osteocalcin and type 1 collagen in SIRT1 +/− BM-MSC. The study additionally showed that Sirt +/− mice significantly increased the expression of the sclerostin (SOST) gene, one of the major osteoblastogenesis inhibitors [104]. Similar results were obtained in vitro when examining human osteoarthritis subchondral osteoblasts, which also showed increased expression of SOST and decreased SIRT1 expression compared to non-osteoarthritic subchondral osteoblasts [105]. In studies conducted by Mora et al. [106], statistically significant lower serum concentration of Wnt antagonists sclerostin and DKK-1 were found in HIV-infected patients compared to healthy people [106]. The impact of HAART on sclerostin levels was also confirmed by studies of Erlandson et al., who showed that the median value of sclerostin was higher before HAART implementation compared to post-HAART in HIV-infected patients [107].

The other enzyme from the sirtuin family that is important in bone remodeling disturbances is SIRT6. Zhang et al. [108] showed abnormal bone remodeling formation and resorption processes in mice with SIRT6 knockdown. Genes in osteoblasts are indicated as potential mechanism changes in the expression of runt-related transcription factor 2 (Runx2) and Osterix (Osx). In the absence of SIRT6, there is increased acetylation of histone H3K9 in the promoter region for Runx2 and Osx, responsible for inhibiting blastogenesis and the transition of osteoblasts to osteocytes. The authors suggest that SIRT6 may be considered as an important determinant of osteoblastogenesis. Increased expression of OPG and DKK1 has also been observed in SIRT6 deficiency, which causes disturbances in osteoblast and osteoclast differentiation. Studies also show that SIRT6 is a positive regulator of osteogenic differentiation. SIRT6 regulates osteogenic differentiation via one bone morphogenetic protein (BMP) signaling in a 300/CBP-associated factor (PCAF)-dependent manner, which, when activated, binds to the bone morphogenetic protein 2 (BMP2) and bone morphogenetic protein 4 (BMP4) promoters, which in turn activate Runx2, a key osteogenic differentiation factor [108]. Sirtuin 6 also controls IGF-1-mediated bone resorption, affecting hypoxia-induced osteoblast apoptosis. Under hypoxia, higher pro-inflammatory cytokine production and increased OS and glycolysis can be observed. Studies conducted on human osteoblast cells (HOB) have shown that SIRT6 inhibits the above processes. In a culture under hypoxia cells, increased expression of lactate dehydrogenase (LDH) and lactate as well as increased reactive oxygen species (ROS) generation was observed. Overexpression of SIRT6 reduced the above changes and decreased expression of pro-inflammatory factors IL-6, IL-1β, TNFα, confirming the role of SIRT6 in preventing inflammatory bone resorption [109]. Figure 5 schematically shows the effect of sirtuins (SIRT1 and SIRT6) on bone metabolism during HIV infection.

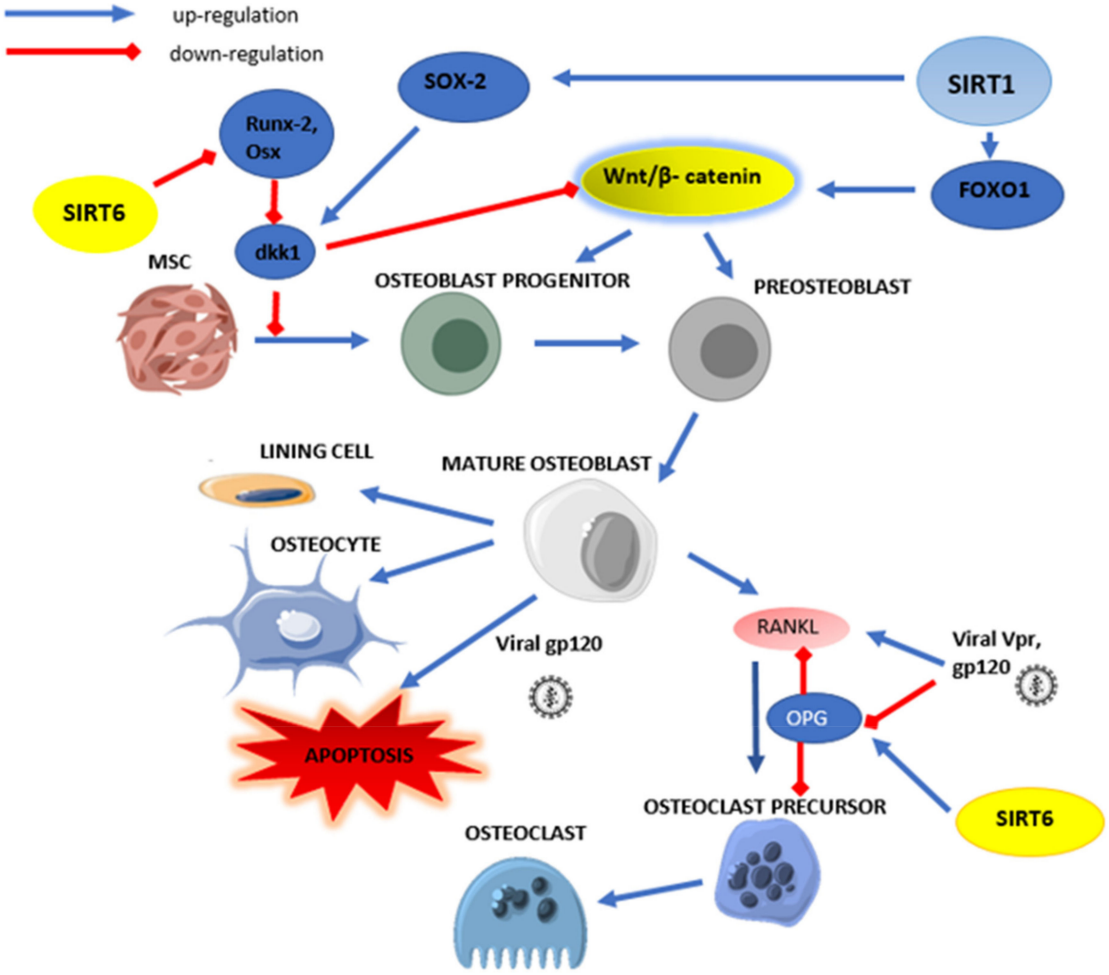

Figure 5. Scheme of connection between sirtuins, HIV infection and bone metabolism. SIRT1 deacetylates and up-regulates SOX2, the main factors maintaining the self-renewal and ability to differentiate mesenchymal stem cells in osteoblasts. FOXO1 deacetylation by SIRT1, up-regulates the differentiation of preosteoblast and osteoblast progenitor into osteoblasts. SIRT6 down-regulates Runx2 and Osx genes, responsible for inhibiting blastogenesis and the transition of osteoblasts to osteocytes. SIRT6 up-regulates OPG-RANKL inhibitor, decreasing bone resorption. RANKL, receptor activator for nuclear factor κ B ligand; OPG, osteoprotegerin; FOXO1, forkhead box protein O1; SOX-2, SRY-Box transcription factor 2; Runx2, runt-related transcription factor 2; DKK-1, Dickkopf-related protein 1; MSC, mesenchymal stem cells.

8. Kidney Diseases in HIV-Infected Patients and the Role of Sirtuins

Kidney diseases are diagnosed more often in HIV patients compared to the general population. These diseases include acute kidney injury (AKI), HIV-associated nephropathy (HIVAN), comorbid chronic kidney disease (CKD), HIV immune complex kidney disease (HIVICK), thrombotic microangiopathy and treatment-related kidney toxicity predisposed to CVD, heart failure and end-stage renal disease (ESRD) [110,111]. In the years 1996–2006, CKD became one of the most common causes of death in people infected with HIV and one of the most common comorbidities in HIV-infected patients, affecting 2–10% of infected patients in European and American countries [111]. CKD is also a risk factor for the development of AKI in HIV-positive patients. Other risk factors include glomerular filtration rate (eGFR) < 60 mL/min/1.73m^2, proteinuria > 30 mg/dL, diabetes, hypertension, HCV-co infection [112]. Proximal renal tubular dysfunction, albuminuria and renal impairment are more prevalent in HIV-positive people compared to uninfected controls [113]. In a cross-sectional study conducted on 2339 HIV-positive patients, CKD was diagnosed in 13.3% of them, 12.6% had albuminuria and 4.6% had eGFR < 60 mL/min/1.73m^2 [114]. Additional risk factors for kidney disease are age, hypertension, diabetes mellitus, nadir CD4+ cell

count <200 cells/μL, use of tenofovir disoproxyl fumarate (TDF) and use of TDF with ritonavir-boosted PIs [111,115]. Mitochondrial dysfunction and depletion of mitochondrial DNA are considered to be potential mechanisms of TDF toxicity, as a result of drug accumulation caused by dysfunction or inhibition of the multidrug resistance-associated protein-4 (MRP4) transporter. It has been shown that incidence of CKD significantly increases with the next year of exposure to TDF (incidence rate ratio [IRR]: 1.14), ritonavir-boosted atazanavir (IRR: 1.20) and ritonavir-boosted lopinavir (IRR: 1.11), but not other ritonavir-boosted PIs or abacavir [116]. The virus utilizes lipid rafts, and through their mediation enters the cell [117]. HIVAN, the most common kidney disease in infected patients, is characterized by podocyte collapse, hyperplasia, tubulointerstitial and glomerular damage, focal glomerular sclerosis and proteinuria. Mitochondrial dysfunction and changes in their morphology have been shown to be the cause of HIVAN, which is associated with the expression of viral proteins Nef, Vpr and Tat, ROS generation and apoptosis of renal cells. Tg26 mice kidneys showed significantly lower expression of PGC-1α-promoting mitochondrial biogenesis; mitofusin-2 (MFN2), a mitochondrial membrane protein responsible for mitochondria fusion; and PARKIN-E3 ubiquitin ligase, which induces the autophagic degradation of mitochondria [118,119].

It was shown that renal tubular cells express HIV proviral genes, even with undetectable viremia in the blood. Podocytes are also a reservoir of the virus. The suggested mechanism for the entry of the virus into renal cells is via its interaction with the DEC-205 (CD205) receptor secreted at the tubular cells. The binding of viral gp120 to DEC-205 has been demonstrated. Expression of C-X-C motif chemokine receptor 4 (CXCR4) was demonstrated in podocytes. In podocytes, endothelial and tubular cells, SIRT1 is indicated as an important agent in kidney disturbances. Deacetylation of PGC-1α by SIRT1 increases its activity as a cofactor for the transcriptional repressor protein YY1, promoting mTOR mediated growth and division of mitochondria. PGC-1α also activates PPARα and ERRs that regulate the processes of e.g., β-oxidation of fatty acids, affecting mitochondrial processes. Lempiäinen et al. [120] found in Wistar rats kept under calorie restriction (energy intake 70%) with ischemia/reperfusion (I/R) kidney injury showed improved renal function by attenuating the nitrative stress caused by I/R, which resulted in preventing tubular necrosis compared to control group (rats with I/R without calorie restriction). The authors indicated that during kidney injury, the expression of PGC-1α, AMPK, SIRT1 and eNOS was reduced. Calorie restriction partially inhibited this effect by increasing SIRT1 expression [120]. Rats treated with SIRT1 activator SRT1720 had significantly increased renal ATP levels, which prevented mitochondrial mass decline, increased PGC-1α expression, decreased apoptosis and prevented renal histologic architecture damage, compared to vehicle treated animals [121]. Mice with AKI induced by cisplatin showed mitochondrial abnormalities in tubular cells with a reduced SIRT3 mRNA expression and protein level. Treatment of the tested mice with AMPK activator, AIKAR (5-aminoimidazole-4-carboxamide-1-β-D-ribofuranoside) and antioxidant ALKAR (agent acetyl-l-carnitine) improved renal function through restoring SIRT3 activity. Furthermore, in vitro studies on renal proximal tubular epithelial cells (RPTECs) damaged by cisplatin showed reduced SIRT3 expression compared to controls' mitochondrial fission. It has been shown that SIRT3 prevents the recruitment of dynamin-related protein 1 (DRP-1) and the up-regulation of mitochondrial fission factor (MFF) and PTEN-induced kinase 1 (PINK1) in mitochondria, preventing mitophage processes and preserving their integrity. In addition, SIRT3 has a positive effect on the potential of mitochondrial membranes and prevents depolarization because it increases the activity of optic atrophy 1 (OPA1) profusion protein, thus preventing the fragmentation and cleavage of mitochondria as well as the loss of organelles in tubular epithelial cells, which suggests the renoprotective effect of SIRT3 [122].

SIRT1 has been shown to regulate the functioning of the RAS system with a positive effect on blood pressure and kidney function. SIRT1 binds to the promoter for angiotensin-converting enzyme 2 (ACE2) and increases its expression, promoting the conversion of Ang to AngII, preventing vasoconstriction, sodium reasorption and decreased hemodynamics of

the kidneys [123]. The activation of the RAS is also considered a factor in the development of HIVAN. Reducing the production of AngII has been shown to significantly slow the progression of HIVAN [124]. In studies on Tg26 mice, the phenotype of HIVAN (sclerosed or collapsed) has been shown to be associated with RAS activation. After 4 weeks of subcutaneous administration of normal saline (control group), ACE inhibitor captopril (5 mg/kg/day), tekturna-renin inhibitor (50 mg/kg/day) and angiotensin receptor (AT1) antagonist telmisartan (0.3 mg/kg/day) to tested animals, significantly lower amounts of sclerosted glomeruli and significantly lower levels of blood urea nitrogen were observed in all study groups, indicating improved renal function compared to the control group. The amount of collapsed glomeruli remained unchanged [124]. The above data may indicate the renoprotective effect of SIRT1, also in the course of HIVAN. Other studies have shown a protective effect of SIRT1 on podocytes, which is crucial for the proper functioning of the glomeruli by preventing excessive OS and apoptosis, as well as the subsequent damage due to deacetylation and a decrease in the activity of the previously described transcription factors: NF-κB, STAT3, FOXO4, p53 and PGC-1α. Deacetylation of FOXO4 by SIRT1 prevents activation of BCL-2 gene transcription and the induction of apoptosis in podocytes and tubular cells. Increased accumulation of acetylated FOXO4 has been demonstrated as a result of decreased SIRT1 activity in podocytes in the course of hyperglycemia in diabetic kidney disease. Weakened expression of SIRT1 in cultured podocytes isolated from diabetic mice was caused by an increased amount of advanced glycation end products (AGEs) characteristic of long-term hyperglycemia [125]. Increased OS and DNA damage have been demonstrated in HIV-infected podocytes as a result of the activation of the p66ShcA/FOXO3a pathaway. Activation of p66ShcA creates a complex with FOXO3a and causes its sequestration to the cytosol, which inhibits the expression of antioxidant genes of MnSOD or genes regulating the cell cycle, including cyclin-dependent kinase inhibitor (CDKI) [126].

It has been shown that the up-regulation of p66Shc protein is correlated with renal damage both in vivo and in vitro [127]. Presumably, SIRT1 deacetylates p66ShcA in Lys 81, preventing its pro-oxidative effects, as demonstrated in aortic endothelial cells of diabetic mice [128]. The existence of a similar mechanism in the kidneys has been indicated by studies conducted by Yang et al. [127], who analyzed renal tissue samples of renal biopsies from diabetic nephropathy patients and controls (non-diabetic renal diseases patients). The expression of p66Shc increased by about 50% and SIRT1 expression decreased by approximately 30% in glomeruli and renal tubules of patients compared to controls. The authors also showed decreased p-AMPK and SIRT1 expression compared to controls and increased expression of p66Shc in the kidneys of diabetic mice. After administration of probucol—an antioxidant drug—the above changes were reversed. In vitro studies using human proximal tubular cells (HRCE) exposed to high glucose conditions (30 mM, for 1–6 h) showed that probucol activates AMPK/Sirt1 pathway and inhibits p66Shc expression and ROS generation by phosphorylation of AMPK in diabetic nephropathy [127]. SIRT1 is also an activating factor of FOXO3, which confirms its antioxidant effect. Attenuated renal function and glomerulosclerosis has been demonstrated in rats with diabetic nephropathy via activation of SIRT1 through RSV treatment [129].

Another pathomechanism in podocytes is the reorganization of the cytoskeleton through the influence of SIRT1 on cortactin. Deacetylated cortactin is transported from the nucleus to the cytosol and is responsible for maintaining the cytoskeletom integrity and the proper functioning of the split membrane. It was noted that in SIRT1 ($pod^{-/-}$ mice (mice with podocyte-specific SIRT1 knockout), podocyte damage (induced by protamine sulfate) was more severe compared to podocyte injury in WT mice. Analysis of isolated glomerules showed significantly reduced expression of podocyte-specific proteins, WT-1 (Wilms' tumor 1 protein), nephrin, synaptopodin, in $SIRT1^{-/-}$ mice compared to WT. Moreover, $SIRT1^{-/-}$ mice podocytes showed increased F-aktin accumulation and albuminuria in comparison with WT mice with glomerular disease, which indicates an increased damage to the cytoskeleton in the absence of SIRT1 [130]. Increased NF-Kb p65 and STAT3

acetylation in HIVAN has been demonstrated in parallel with decreased SIRT1 expression in the glomeruli of mouse and human HIVAN kidneys. In vivo studies on Tg-26 mice, following administration of BF175, a SIRT1 agonist, decreased albuminuria and expression of inflammatory markers. In the course of HIV infection, miR-34 leads to a decrease in SIRT1 expression in the kidney, which contributes to the development of inflammation following kidney injury [131]. Figure 6 shows schematically the participation of sirtuins in the development of kidney diseases in HIV-infected patients.

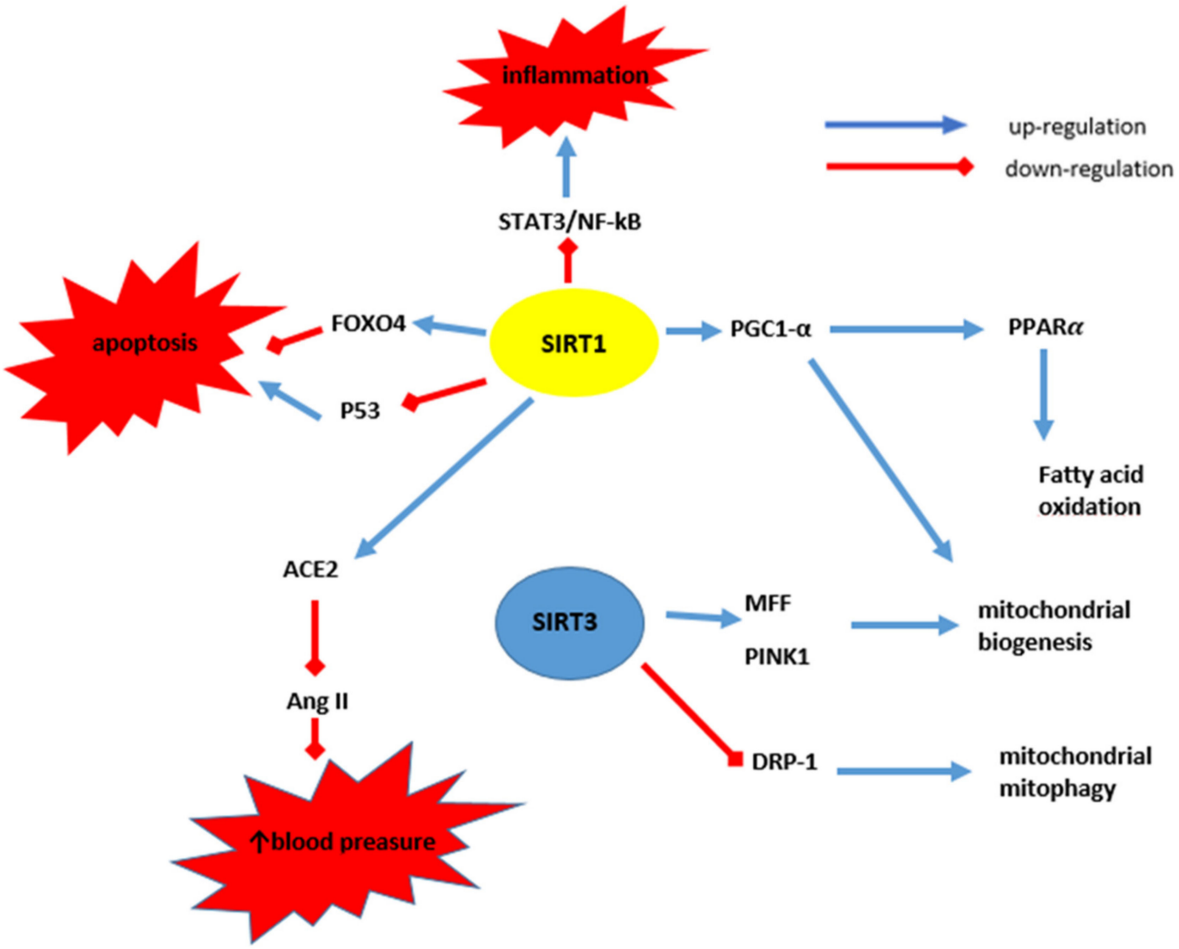

Figure 6. Scheme of connection between sirtuins, kidney disturbances and HIV infection. SIRT1 decreases the activity of NF-κB, STAT3, FOXO4, p53 and PGC-1α in podocytes, preventing excessive OS, inflammation and apoptosis. Deacetylation of PGC-1α by SIRT1 increases its activity, promoting growth and division of mitochondria. PGC-1α also activates PPARα and regulates the processes of β-oxidation of fatty acids, affecting mitochondrial processes. SIRT1 binds to the promoter for angiotensin-converting enzyme 2 (ACE2) and increases its expression, promoting the conversion of Ang to AngII, and regulates the functioning of the RAS system with a positive effect on blood pressure and kidney function. Deacetylation of FOXO4 by SIRT1 prevents activation of BCL-2 gene transcription and the induction of apoptosis in podocytes and tubular cells. SIRT3 prevents mitophage processes and preserves mitochondria integrity by the down-regulation of DRP-1 and the up-regulation of MFF and PINK1. SIRT 1, 3, sirtuin 1, 3; FOXO4, forkhead box O4; NF-κB, nuclear factor kappa-light-chain-enhancer of activated B cells; Ang II, angiotensin II; ACE2, angiotensin-converting enzyme 2; PPARα, peroxisome proliferator-activated receptor alpha; PGC1-α, peroxisome proliferator-activated receptor gamma coactivator 1-alpha; STAT3, signal transducer and activator of transcription 3; MFF, mitochondrial fission factor; PINK1, PTEN-induced kinase 1; DRP-1, dynamin-related protein; OS, oxidative stress.

9. NeuroAIDS in Aging HIV Population and Sirtuin Participation

As shown by the data, the number of people infected with HIV over the last 50 years is over 10%, and in more developed regions it reaches up to 50%, and it is estimated that it will grow [132]. Accelerated aging in HIV-positive patients is associated with the induction of oxidative stress; mitochondrial dysfunction; cell cycle arrest and induction of the state of chronic immune activation in the course of infection; viral proteins, such as Nef or Tat; or chronic antiretroviral therapy [133]. One of the characteristics of accelerated cellular aging is shortening telomere length. Decreased absolute length of peripheral blood leukocytes' telomere length has been demonstrated in HIV-positive patients compared to uninfected controls. Telomere length was inversely correlated with the CD4 nadir + cell count and duration of infection [134]. It has also been shown that sirtuins affect telomere length; prevent their abrasion and promote DNA damage repair; preserve genome integrity; and stabilize the chromatin structure [135]. In liver specific telomerase knockout mice, telomere shortening results in repression of all sirtuins in a p53-dependent manner: SIRT1, SIRT2, SIRT6 by miRNA-34a, 26a and 145a, and SIRT3, SIRT4, SIRT5 by peroxisome proliferator-activated receptor gamma coactivator 1-alpha and beta (PGC-1α/β). On the other hand, NAD + supplementation caused telomere stabilization through overexpression of sirtuins [136]. SIRT6 is considered a critical regulator of DNA repair in telomeric regions, and is a positive regulator of longevity through its influence on the metabolic and telomere functions [137]. Sirtuins, by regulating many signaling pathways, such as FOXOs, AMP-activated protein kinase, insulin/IGF-1 signaling and others, are considered to be key lifespan regulators delaying cellular aging [8,31]. In the aging HIV population, as stated in the preceding paragraphs, there is a greater risk of age-related morbidities, such as cardiovascular diseases or metabolic disorders, including neurodegenerative diseases [1].

NeuroAIDS, which is a collection of cognitive, motor and autonomic disorders associated with HIV infection, is still a serious problem in medical practice among HIV-positive patients. The central nervous system (CNS) is the viral reservoir that is occupied in the initial stage of infection, leading to HIV-associated neurocognitive disorders (HAND) [138]. Current treatment regimens have good CNS penetration, and rapid implementation of cART effectively prevents more severe forms of HAND [139]. As a result of the introduction of cART, the incidence of diseases such as AIDS dementia (AD), myelopathy or sensory neuropathy has significantly decreased [140]. However, about 50% of HIV-infected patients suffer from one of the milder forms of HAND, the most common being asymptomatic neurocognitive impairment (ANI) and mild neurocognitive disorder (MND) [132]. HIV RNA is still detected in the cerebrospinal fluid (CSF) of virologically suppressed patients [141].

Neurotoxicity as a result of HIV is mainly related to the infiltration of infected macrophages, lymphocytes and multinucleated giant cells. As a result of infiltration of virus-activated cells of the immune system into endothelial cerebral vessels, the integrity of the blood-brain barrier (BBB) is damaged, facilitating further migration of infected cells. The damage is perpetuated by the release of pro-inflammatory cytokines and toxins causing neuroinflammation of macrophages. Significantly increased concentrations of neopterin—a macrophage activation marker—have been shown in patients virologically suppressed by cART [142]. Moreover, inflammatory changes in CSF are present in HIV-positive patients, even in asymptomatic stages of infection [139]. Despite effective treatment and undetectable viremia, viral proteins (Tat, Gp120 and Vpr) are active in the CNS and interact with CXCR4 and CCL5 coreceptors, causing activation of microglia, astrocytes and macrophages. The viral proteins Tat, gp120 and Vpr induce apoptosis of neurons through increased expression of TNF-α, IL-6 and IL-1 and ROS production. The presence of Tat protein has been demonstrated in CSF in HIV-infected people successfully treated with antiretroviral therapy [143].

All seven sirtuins are widely distributed in the CNS structures. SIRT1 and SIRT2 are most commonly expressed, mitochondrial sirtuins (SIRT 3, SIRT4, SIRT5) are expressed to a lesser extent and SIRT6 at the lowest degree. SIRT1 expressions are observed mostly in

the cerebellum, hippocampus and hypothalamus neurons, SIRT2 in the spinal cord and brainstem, cerebellum cortex, striatum and hippocampus [144].

SIRT1 is considered to be one of the essential factors in stabilizing the genome in neurons. In vitro studies on cultured mice SIRT1 knockout neurons showed elevated levels of DNA damage as well as decreased Ser/ Thr kinase ATM and NBS1 protein activity after double strand break (DSB) induction by intron-encoded endonuclease (I-PpoI), which indicates significant involvement of SIRT1 in the first steps of DSB detection. SIRT1 also deacetylates histone deacetylase 1 (HDAC1), supporting its neuroprotective effect through the nonhomologous end-joining (NHEJ) signaling pathway [145]. The Tat protein induces DSB of the DNA strand, leading to apoptosis if the damage is not repaired [138]. The data above show the potential neuroprotective role of SIRT1 also in the course of HIV neuroinfection.

Another factor in neuroAIDS pathogenesis is mitochondrial toxicity. Postmortem brain specimens from HIV+ patients with documented HAND have been shown to alter mitochondrial functions in neurons and astroglia associated with reduced PGC-1α, the main factor affecting mitochondrial biogenesis by enhancing TFAM transcription, participating in mtDNA replication and transcription. In the tested samples, it was shown that the expression of TFAM and PGC-1α was reduced by 40% in NCI samples and by 50% in HAND samples, compared to non-HAND samples [146]. Research shows that SIRT1 is a factor in promoting mitochondrial biogenesis, activating acetyl-CoA synthetase 2 (AceCS2), then AMPK, which in turn phosphorylates and activates PGC-1α [147]. It has been shown that the Tat protein induces an increase in the potential of the mitochondrial membrane, causing changes in synaptic activity [148]. Mouse primary microglial cells (mPMs) exposed to viral Tat protein showed elevated expression of senescence markers, p21 and p16 proteins; augmented cell-cycle arrest; decreased telomerase activity, increased release of pro-inflammatory cytokines; and down-regulation of SIRT3. The authors indicated that the reduction in SIRT3 activity was mediated by miR-505 and up-regulated by HIV Tat. The authors also demonstrated decreased expression levels of SIRT3 in the striatum of HIV-1 transgenic rats compared to WT rats in an in vivo study, as well as decreased expression levels of antioxidant enzymes, Gpx, SOD2 and CAT, in the prefrontal cortex and in the striatum of HIV Tg rats. The expression of p16, p21 and IL1β were also increased in the samples of frontal cortical brain HIV-infected patients compared to age-matched controls. These studies indicate a protective role of SIRT3 in senescence of microglia as a result of the activity of the Tat protein [149]. Changes in mitochondrial morphology (elongated and broken combs) have been noticed in studies on mice exposed to gp120. The tested animals showed neuropathological changes analogous to HAND. The potential mechanism of this type of change is increased mitofusin 1 (MFN1) and OPA1, which was confirmed by the authors in an in vitro model for rat cortical neurons and SH-SY5Y rat neuroblastoma cells exposed to gp120 [150]. Analogous disturbances in the functioning of the mitochondria are also present in the course of Alzheimer's disease (AD). SIRT3 in AD has been shown to prevent OS induction through deacetylation of SOD2; disruption of mitochondrial membrane potential; and activation of dynamin-related protein 1 (DRP1) and mitochondrial fission 1 protein (FIS1) factors promoting excessive mitochondrial cleavage, leading to neuronal death due to disrupted ability of mitochondria movement to the synapse and disrupted ATP supply [151]. In addition, SIRT3 prevents the excessive division of mitochondria through deacetylation and activation of OPA1 and MFN1, eliminating the excessive activity of DRP and FIS1 [152]. The viral Tat protein causes neuroinflammation and microglia activation also by promoting mitochondrial dysfunction and disturbing mitophagic processes. In mouse primary microglial cells exposed to HIV-1 Tat, a significantly increased expression of mitochondrial kinase PINK1 was shown. Similar results were obtained in vivo in the frontal cortex of HIV-1 Tg rats through a significantly higher expression of PINK1 being observed compared to WT animals [153]. As research shows, SIRT3, mediated by FOXO3, regulates the expression of genes responsible for the normal processes of mitochondrial

fusion and decay (MF2, MRP1, FIS1, TFAM, PCG1α), regulating adequate mitochondrial quantity and quality [151].

Another mechanism of HAND and accelerated aging of infected patients seems to be the activation of astrocytes. It has been shown that miRNA-34a and -138 in the brains of HIV-infected rats were up-regulated compared to age-matched WT rats. These differences were not observed in the younger animals. It was also shown that the abovementioned up-regulation of the miRNAs was inversely correlated with the expression of SIRT1. The authors point to the anti-aging role of SIRT1, which counteracts the deleterious effect of Tat protein, causing up-regulation of glial fibrillar acid protein (GFAP) in a NF-kB-dependent manner, which in turn causes astrogliosis [154].

A relationship with occludin has also been demonstrated. Occludin is one of the BBB proteins in pericytes. It affects the expression of SIRT1 and is a factor that limits HIV transcription. Primary human brain capillary pericytes infected with HIV showed an inverse correlation of HIV replication with occludin expression levels. Both HIV infection and depletion of occludin in the tested cells decreased SIRT1 expression and increased NFκB-p65 acetylation. The authors concluded that occludin, via NFκB/SIRT-1 pathway, modulates HIV-1 transcription, pointing to a significant role of SIRT1 in the pathogenesis of neurological disorders in HIV-infected patients [155]. Chronic activation of miR-142 is also the cause of neuropathology in HAND. In primary human neuron culture and neuroblastoma cells expressing miR-142, decreased monoamine oxidase A (MAO-A) expression was observed compared to controls not expressing miR-142, which is one of the causes of neurotransmitter imbalance and neuronal dysfunction related to HAND. As indicated by the authors, the down-regulation of the neurotransmitter-metabolizing enzyme, MAO-A, is associated with SIRT1-dependent action and the loss of its protective functions as a result of miR-142 [156].

Disturbances in the functioning of dopaminergic neurons (DA) and disturbances in the dopamine mechanism, which are a characteristic feature of Parkinson's disease (PD), have also been demonstrated in the course of HIV infection [157]. The study of the substantia nigra of HIV-infected individuals showed the expression of neural α-synuclein and β-amyloid deposits, which were not noticed in age-matched control samples [158]. As research shows, the level of SIRT2, increasing with age, is associated with accumulation of a decreased amount of acetylated α-synuclein, and, as a result, increased neurotoxicity and loss of DA neurons. As shown in the PD model, inhibition of SIRT2 by adenylate kinase 1 (AK1) reduced α-synuclein toxicity by reducing its aggregation [159]. As shown by research on mouse hippocampal neurons, by deacetylation of reticulon 4B protein (RTN4B), SIRT2 supports its ubiquitination and disintegration, which in turn inhibits the activity of β-secretase 1 (BACE1) and inhibits the formation of β-amyloid [160]. Figure 7 schematically shows sirtuin's participation in the developlemt of neurodegenerative diseases in HIV-infected patients.

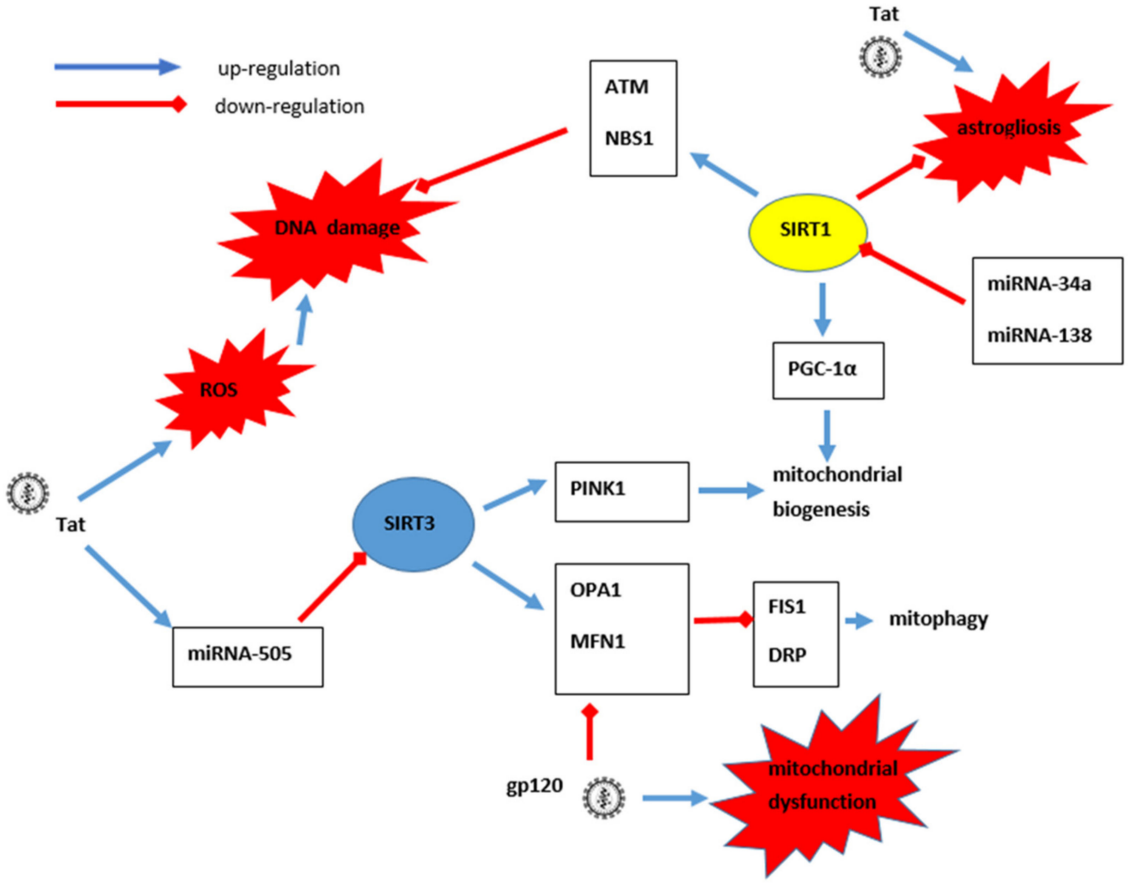

Figure 7. Scheme of connection between sirtuins, neurocognitive disturbances and HIV infection. The Tat protein induces DSB of the DNA strand, leading to OS and apoptosis if the damage is not repaired. SIRT1 activates Ser/ Thr kinase ATM and NBS1 after DSB induction, stabilizing the genome in neurons. SIRT1 is a factor promoting mitochondrial biogenesis through activating PGC-1α. SIRT1 also counteracts the deleterious effect of Tat protein, causing up-regulation of glial fibrillar acid protein in a NF-kB-dependent manner, which in turn causes astrogliosis. SIRT3 prevents disruption of mitochondrial membrane potential and activation of DRP1 and FIS1 factors, promoting excessive mitochondrial cleavage, leading to neuronal death. SIRT3 prevents the excessive division of mitochondria through deacetylation and activation of OPA1 and MFN1, eliminating the excessive activity of DRP and FIS1. SIRT 1, 3, sirtuin 1, 3; PGC-1α, peroxisome proliferator-activated receptor gamma coactivator 1-alpha; PINK1, PTEN-induced kinase 1; DRP-1, dynamin-related protein; OPA1, optic atrophy 1 protein 1; MFN1, mitochondrial fission 1 protein; OS, oxidative stress.

10. Other Disturbances

During the course of HIV infection, different oral disorders occur. It is estimated that HIV-associated oral abnormalities occur in 30–80% of the HIV patient population. Moreover, oral lesions are indicated as early signs of HIV infection and can predict its progression to AIDS [161,162]. It is mentioned that for persons living with HIV who have not yet implemented therapy, the presence of oral manifestations may predict AIDS progression [163]. Furthermore, the presence of oral manifestations in HAART treated patients can be a surrogate marker for HAART efficacy [164]. Although the prevalence of oral lesions like hairy leukoplakia, candidiasis or Kaposi's sarcoma has been proven to be lower among patients on HAART [165], other conditions such as oral wartsand salivary gland disease [166–168] are more prevalent in antiretroviral treated population as part of immune reconstitution. It is indicated that the junction of tooth and gingiva provides a potentially weak barrier for virulence factors of bacteria. Oral lesions are associated with HIV infection, as well as diverse medication used in the management of patients with HIV disease (e.g., didanosine, indinavir, zidovudine, indinavir). Lately it has been indicated that the introduction of HAART appears to have reduced the incidence of HIV-associated

oral lesions [169,170]. There is only little literature to date concerning the role and participation of sirtuins in the development of dental diseases in HIV-infected patients. As shown by Jang et al. [171] in human dental pulp cells (HDPCs) isolated from freshly extracted molar teeth, examining sirtuin gene expression in odontoblastic differentiation (reflected by osteocalcin (OCN) and dentin sialophosphoprotein (DSPP) expression) revealed that all seven sirtuin genes (SIRT1-7 genes) were expressed, and especially the expression of SIRT4 was increased in a time-dependent manner. The authors suggested that SIRT4 could influence the odontoblast differentiation process; however, further research is needed to determine the potential effects of other sirtuins on the odontogenic potential of HDPCs [171]. Kim et al. [172] showed that overexpression of SIRT1 increased mineralized nodule formation, and up-regulated the levels of odontoblastic markers (OCN, ALP, dentin matrix protein-1 and DSPP) as well as angiogenic markers (fibroblast growth factor-2, vascular endothelial cadherin and platelet endothelial cell adhesion molecule 1), while SIRT1 inhibition of SIRT1 down-regulated the expression of those genes. Down-regulation of the SIRT6 decreased the mineralization effect in HDPCs, and overexpression of SIRT6 increased mineralization effects, such as calcium nodule formation, ALP activity and odontoblast differentiation marker genes [172]. Some literature data indicated the role of SIRT1 in the regulation of precancerous oral lesions and oral cancer. However, its biological role in the regulation of oral cancer is not yet fully understood [173]. This seems to be important in light of increased development of such disorders in HIV-infected patients and due to the fact that the biological role of sirtuins in cancer is constantly being recognized and growing [8,10]. SIRT1 has been widely studied and yet there are conflicting results regarding the association between the two, as SIRT1 is known to suppress or promote cancer depending on its cellular content or type [173,174]. The expression level of SIRT1 has been shown to play an important role in the pathogenesis of oral squamous cell carcinoma (OSCC). A statistically significantly lower expression of SIRT1 in resected specimens from patients with OSCC has been demonstrated compared to the control group [175]. The influence of SIRT1 on epithelial e-cadherin activity is indicated as a potential mechanism, which allows the preservation of the integrity of the epithelium and preventing of metastases in oral cancer. SIRT1 also prevents further neoplastic transformation in fibroblasts through its suppressive effect on TGF-β [173].

11. Conclusions

The introduction of HAART has significantly increased the effectiveness of HIV treatment, as demonstrated by statistical data on the decreasing mortality from AIDS-defining diseases. Currently, the challenge is to develop appropriate procedures, therapeutic schemes and preventive measures to reduce the risk of or delay the occurrence of non-HIV associated conditions. Therefore, it is important to know the detailed mechanisms in the development of the abovementioned diseases. Data discussed in this review indicate the potential involvement of sirtuins in cellular signaling pathways shared with some viral proteins, leading to osteoporosis (SIRT1, SIRT6), T2DM and insulin resistance (SIRT1, SIRT2, SIRT3, SIRT6), liver diseases and lipid metabolism disorders (SIRT1, SIRT3, SIRT6), cardiovascular diseases (SIRT1, SIRT2, SIRT4, SIRT5, SIRT6), kidney diseases (SIRT1, SIRT3), neurocognitive disorders (SIRT1, SIRT2, SIRT3) and oral diseases (SIRT1, SIRT4). Therefore, it seems that further examination of sirtuin expression in HIV-positive patients and possible regulations of their action to avoid comorbidities are required.

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Załącznik 2

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Article

The Effect of Antiretroviral Therapy on SIRT1, SIRT3 and SIRT6 Expression in HIV-Infected Patients

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Abstract: Human Immunodeficiency Virus (HIV) infection and the chronic use of combined antiretroviral therapy (cART) may affect the occurrence of certain disturbances in the body. There is growing interest in sirtuins–enzymes involved in the regulation of many metabolic processes in the organism and in the pathogenesis of many diseases which also exhibit potential antiviral activity. The aim of the study was to investigate the connection of cART to the expression of Sirtuin 1 (SIRT1), Sirtuin 3 (SIRT3) and Sirtuin 6 (SIRT6) in HIV-infected men. The plasma levels of sirtuins were measured before and one year after cART, and related to HIV viral load, lymphocytes T CD4+ and CD8+ count as well as the applied cART. The levels of sirtuins in plasma were measured in HIV-infected patients (n = 53) and the control group (n = 35) by immunoassay methods. There were statistically significant ($p < 0.05$) differences between SIRT6 in the HIV-infected patients before therapy and in the subgroups, depending on the count of lymphocytes T CD8+. There were significant differences in the levels of SIRT1 depending on the applied treatment regimen. The obtained results indicate the most significant changes in the expression of SIRT6 in the course of HIV infection and suggest an influence of the type of cART on the level of SIRT1, which indicates its important role in the course of HIV.

Keywords: SIRT1; SIRT3; SIRT6; cART; HIV; comorbidities; sirtuins

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1. Introduction

About 37.9 million people worldwide are currently infected with the Human Immunodeficiency Virus (HIV) [1]. Due to the introduction of Highly Active Antiretroviral Therapy (HAART), the life expectancy of people infected with HIV comes close to that of the general population [2]. Despite the use of an effective method of treatment–combined antiretroviral therapy (cART)–and the resulting significant reduction in mortality from Acquired Immunodeficiency Syndrome (AIDS), it is still a serious socioeconomic and health problem [3,4].

The introduction of cART has proved to be a breakthrough in the treatment of HIV infection. This therapy involves the use of at least three drugs from different available pharmacological groups, ensuring the inhibition of viral replication to levels undetectable by the most sensitive analytical methods; it prevents the development of drug resistance and enables the restoration of immune system function as well as preventing or delaying the occurrence of AIDS [5]. There are different therapeutic regimens based on the application of at least two nucleoside reverse transcriptase inhibitors (NRTIs) in combination with non-nucleoside reverse transcriptase inhibitors (NNRTIs), integrase transfer inhibitors (INSTIs), protease inhibitors (PIs), fusion inhibitors and C-C Chemokine Receptor 5 (CCR5) antagonists. In addition to two NRTIs, recommended regimens include protease inhibitors (PIs) or integrase transfer inhibitors (INSTIs) [6–8].

PIs act at a late stage in the replication cycle of the virus, inhibiting the activity of the protease enzyme, which in turn prevents the breakdown of structural and enzymatic protein precursors of the gag and gag-pol virus proteins, leading to the formation of immature, non-infectious virions, unable to initiate another replication cycle. PIs are characterized by a low risk of drug resistance (high genetic barrier) and high effectiveness [5]. Almost all PIs are inhibitors of the Cytochrome P450 3A4 (CYP3A4) isoenzyme, resulting in a high risk of drug interactions. Ritonavir is a particularly potent inhibitor of CYP3A4, which is used to potentiate the effects of the remaining PIs (booster), allowing for a simplified dosing schedule and lower toxicity. Cobicistat is also used to boost PIs [9]. However, PIs are burdened with numerous side effects, mainly related to disorders of adipose tissue and glucose metabolism (dyslipidemia, impaired glucose tolerance, insulin resistance etc.) [10].

INSTIs are a new class of antiretroviral drugs with a high safety profile and efficacy. The first INSTI was raltegravir, registered by the Food and Drug Administration (FDA) in 2007 [5]. INSTIs block the integrase enzyme, which catalyzes the formation of covalent bonds between the viral and host DNA, preventing the incorporation of viral DNA into the host genome. Compared to PIs, they are burdened with a lower risk of metabolic disorders; however, they are characterized by a lower genetic barrier (except dolutegravir and bictegravir) [11].

Since the approval of the first antiretroviral drug zidovudine in 1987, significant progress has been made in the treatment of HIV infection. Currently, more than 20 different active substances are available. Older drugs are gradually being replaced by new, much less toxic derivatives. For example, the currently used NNRTIs abacavir, tenofovir disoproxil fumarate, and tenofovir alafenamide, compared to the old generation zidovudine and didanosine, are less frequently associated with the occurrence of serious side effects such as mitochondrial toxicity, lipodystrophy, lipoatrophy, hepatotoxicity and hematological disorders [7,9]. Clinical trials on the use of a regimen other than cART are also being conducted in order to maintain high effectiveness and minimize the risk of treatment complications [12]. The first (since 2017) two-drug therapy was a combination of dolutegravir and rilpivirine [13,14]. Another new preparation is a combination of dolutegravir and lamivudine [15].

At present, patient care in terms of comorbidities, especially those associated with accelerated aging, long-term therapy and a chronic inflammation state, are equally important as effective antiretroviral treatment. The quick identification of comorbidities and the implementation of appropriate prophylactic or therapeutic procedures seems particularly crucial [1,2]. In recent years, there have been many reports on enzymes from the sirtuin family that have indicated their participation in the modulation of many metabolic processes [16,17]. Sirtuins (SIRT 1–7) are evolutionarily conserved, NAD+ dependent class III deacetylases which regulate gene expression mainly by deacetylation of histones and other enzymatic, structural or transcription factors [18]. They are also characterized by other enzymatic activities: ADP ribosylation (SIRT1, SIRT4 and SIRT6), desuccinylation and demalonylation (SIRT5), delipoylation (SIRT4), demyristoylation and depalmitoylation (SIRT6), and they have different cell localization: nuclear (SIRT1, SIRT6, SIRT7), cytoplasmic (SIRT2), and mitochondrial (SIRT3, SIRT4, SIRT5). So far, in terms of the development and course of many diseases, the best known are SIRT1, SIRT3, and SIRT6 [19]. Numerous studies on the use of activators or inhibitors of sirtuin activity in the treatment of neoplastic or metabolic diseases, mainly type 2 diabetes (T2DM), are currently being conducted [20,21]. Data on sirtuins in HIV-infected individuals are rare and mainly relate to SIRT1 [22,23]. To the best of the authors' knowledge, there are no data available on other sirtuins in the course of HIV infection.

SIRT1 expression has been demonstrated in most tissues, including skeletal muscles, liver, adipocytes, kidneys etc. SIRT1 has been shown to participate in multiple signaling pathways related to gluconeogenesis, glycolysis, insulin secretion, DNA repair, aging, and lipid metabolism [16]. It regulates the activity of many transcription factors, including forkhead box transcription factors (FOXOs), hypoxia-inducible factor 1-alpha (HIF-1α),

liver X receptor (LXR), sterol regulatory element-binding protein 1 (SREBP-1), peroxisome proliferator-activated receptor gamma co-activator 1alpha (PGC-1α), p53, and nuclear factor kappa-light-chain-enhancer of activated B cells (NFkB) [22]. The interaction of SIRT1 with the HIV viral protein Trans-Activator of Transcription (Tat), which is a transcriptional Trans activator of integrated proviral mRNA, has also been described [24]. Deacetylation of Tat increases the efficiency of HIV transcription by continuing the elongation of viral mRNA. Tat also blocks SIRT1, thereby reducing NFkB deacetylation, leading to the activation of inflammatory processes. SIRT1 may have a significant and direct impact on the process of HIV infection and chronic immune activation–one of the causes of accompanying comorbidities [23,24].

SIRT3 is the major mitochondrial deacetylase responsible for maintaining proper ATP levels in cells. It regulates the activity of pyruvate dehydrogenase (PDH) and is responsible for the maintenance of energy homeostasis in skeletal muscles [25]. In the liver, it also regulates the processes of beta fatty acid oxidation through long-chain acyl-CoA dehydrogenase (LCAD) deacetylation. In addition, SIRT3 increases the expression of PGC-1α and the uncoupling of Protein 1 (UCP1) in brown adipose tissue (BAT), thus enhancing thermogenesis under the influence of calorie restriction. SIRT3 also affects antioxidant processes through deacetylation and increasing the activity of the antioxidant enzymes superoxide dismutase 2 (SOD2) and catalase (CAT) in a forkhead box protein O3a (FOXO3a) dependent manner [26].

SIRT6, located in the cell nucleus, mainly catalyzes the reactions of mono- ADP-ribosylation and the deacetylation of histone 9 proteins (H3K9ac, H3K56ac and H3K18ac) as well as transcriptional factors responsible for aging processes, metabolism and inflammatory processes, e.g., NF-κB, hypoxia-inducible factor 1 (HIF-1) and cellular transcription factor (c-Myc) [27]. SIRT6 is a critical regulator of DNA repair in telomeric regions and a positive regulator of longevity through its influence on the metabolism and telomere functions [28]. SIRT6 participates in the processes of DNA repair in various mechanisms, including the up-regulation of a double-strand break (DSB) repair factor–DNA-dependent protein kinase (DNA-PK) and SNF2H, a chromatin-remodeling factor, ensuring genome stability and acting as a tumor suppressor [16]. SIRT6 regulates the process of glycolysis by deacetylation of HIF-1α and, consequently, reducing the expression of glycolytic genes, including Glucose transporter-1 (GLUT1), lactate dehydrogenase (LDH), phosphofructokinase-1 (PFK1) and pyruvate dehydrogenasekinase-1 (PDK1). [18,29] It also influences the process of gluconeogenesis through deacetylation of PGC-1α, increasing the expression of gluconeogenic genes as a result. However, SIRT6 also regulates the expression of gluconeogenesis in a FOXO1-dependent mechanism by reducing the expression of these genes. Moreover, SIRT6 is a positive regulator of beta oxidation of fatty acids in the liver through the deacetylation of Lys780 of Nuclear Receptor Coactivator 2 (NCOA2) and the activation of Peroxisome proliferator-activated receptor alpha (PPARα) [16,27]. By deacetylating H3K9 in the NFkB promoter region and Lys310 of the p65 subunit (RelA) of NFkB, SIRT6 inhibits proinflammatory activity [28].

Detailed data on the mechanisms of the development of co-morbidities resulting from HIV infection as well as detailed data on the effects of cART are still limited. Due to the various functions performed by sirtuins in the regulation of many physiological processes, abundant data indicating their participation in the pathogenesis of different diseases, growing scientific interest in the role of these enzymes, and the lack of, or insufficient data on, the role of selected sirtuins–SIRT1, SIRT3 and SIRT6–in the course of HIV infection, the aim of the study was to show possible changes in the expression of these selected sirtuins during antiretroviral therapy. The plasma levels of SIRT1, SIRT3, and SIRT6 were measured before and one year after cART, and related to HIV viral load, lymphocytes T CD4+ and CD8+ count, and the applied treatment regimen. The obtained data will allow for a preliminary assessment of the influence of selected sirtuins on the course of HIV infection and cART therapy.

2. Results

The study group consisted of HIV-infected men before cART (group A) and one year after the implementation of cART (group B, HIV-infected men treated with the two therapeutic regimens: INSTIs or PIs), and the control group (group C) consisted of non-HIV-infected men.

All demographic and clinical data of the study and control group are presented in Table 1, and immunological data concerning the patient group are provided in Table 2.

Table 1. Demographic and biochemical data of HIV-infected patients before (**A**) and after cART (**B**) and control group (**C**) with statistical analysis.

Groups Characteristic	A (n = 53)	B (n = 53)	C (n = 34)	p *
	Me (IQR)	Me (IQR)	Me (IQR)	
Age (Y)	33 (28–40)	34 (29–41)	36 (30–43)	NS
BMI [kg/m2]	24.15 (21.55–24.80)	24.00 (21.56–24.81)	22.30 (18.00–26.80)	NS
FBG [mg/dL]	96.00 (91.90–98.10)	98.90 (92.40–103.90)	95.00 (87.20–99.20)	NS
TC [mg/dL]	174.90 (156.00–187.00)	174.00 (155.00–189.00)	180.00 (165.00–195.00)	NS
LDL [mg/dL]	96.00 (85.00–103.00)	99.06 (80.00–110.00)	100.00 (96.00–115.00)	NS
HDL [mg/dL]	76.60 (63.00–84.00)	77.15 (67.00–82.00)	75.00 (59.00–101.00)	NS
TG [mg/dL]	149.00 (119.00–160.00)	157.20 (100.00–162.00)	163.00 (151.00–175.00)	NS

Abbreviations: cART–combined antiretroviral therapy; A–HIV-infected patients before cART; B–HIV-infected patients after cART; C–control group; BMI–Body Mass Index; FBG–fasting blood glucose; TC–total cholesterol; LDL–low-density lipoprotein; HDL–high-density lipoprotein; TG–triglycerides; Me–median; IQR–interquartile range; N–number of participants; NS–not statistically significant; * Kruskal–Wallis test.

Table 2. Immunological data of HIV-infected men groups before (**A**) and after cART (**B**) with statistical analysis.

Groups Characteristic	A	B	p *
	Me (IQR)	Me (IQR)	
HIV RNA [copies/mL]	148,000 (5190–245,000)	20 (15–34)	<0.001
CD4+ cell count [cells/μL]	340 (234–386)	570 (398–762)	<0.001
CD8+ cell count [cells μL]	999 (717–1190)	855 (706–1062)	0.004

Abbreviations: cART–combined antiretroviral therapy; A–HIV-infected patients before cART; B–HIV-infected patients after cART; C–control group; Me–median; IQR–interquartile range; NS–not statistically significant; * Wilcoxon test.

There were no statistically significant differences ($p > 0.05$) in age, BMI (Body Mass Index), or values of basic biochemical parameters such as TC (total cholesterol), LDL (low-density lipoprotein), HDL (high-density lipoprotein), TG (triglycerides) and FBG (fasting blood glucose) between groups A, B and C. The differences between median HIV viral load, lymphocytes T (LT) CD4+ and LT CD8+ count in patients before and after treatment were statistically significant.

Median levels, interquartile ranges and statistical analysis for SIRT1, SIRT3 and SIRT6 in the pre-treatment (A) and post-treatment (B) of HIV-infected men and the control group (C) are provided in Table 3.

Table 3. Results for SIRT1, SIRT3 and SIRT6 in the plasma of HIV-infected men before (**A**) and after cART (**B**) and in the control group (**C**) with statistical analysis.

Groups	A	B	C	*p* *	post-hoc
	Me (IQR)	Me (IQR)	Me (IQR)		
SIRT1 [ng/mL]	7.20 (4.00–21.70)	4.70 (2.20–64.00)	8.50 (2.70–24.10)	0.305	A:C = NS B:C = NS A:B = NS
SIRT3 [ng/mL]	5.80 (4.00–21.10)	5.70 (2.00–29.10)	8.00 (2.70–21.10)	0.131	A:C = NS B:C = NS A:B = NS
SIRT6 [ng/mL]	2.80 (0.80–10.50)	4.40 (2.20–23.20)	7.30 (2.30–19.30)	0.003	A:C = 0.007 B:C = NS A:B = 0.022

Abbreviations: cART–combined antiretroviral therapy; SIRT1, SIRT3, SIRT6–sirtuin 1, 3, 6, respectively; A–HIV-infected patients before cART; B–HIV-infected patients after cART; C–control group; Me–median; IQR–interquartile range; NS–not statistically significant; * Kruskal–Wallis test.

The median level of SIRT1 in the group of HIV-infected men before treatment (A) was over 1.5-fold higher compared to the group after treatment (B), and almost 1.2-fold lower than in the control group (C). The median level of SIRT1 in the group after cART was 1.8-fold lower compared to the median level of SIRT1 in the control group. However, these differences were not statistically significant. Median levels of SIRT3 were similar in both groups of HIV-infected men (A and B) and were approximately 1.4-fold lower compared to the control group but without statistical significance. There were statistically significant ($p < 0.05$) differences in plasma levels of SIRT6 in pre-treatment (A), post-treatment (B) and control (C) groups ($p = 0.003$). Post hoc analysis showed a statistically significant difference in the SIRT6 plasma level in the pre-treatment group (A) compared to the post-treatment group (B) (almost 1.6-fold lower; $p = 0.022$) and statistically significant differences between the median level of SIRT6 in the pre-treatment group (A) compared to the control group (almost 2.6-fold lower; $p = 0.007$), (Table 2).

The median levels and interquartile ranges with statistical analysis of examined sirtuins in the plasma of HIV-infected men before cART (A) and after cART (B) are presented in Table 4 and Table S1 and divided into subgroups according to LT CD4+ count $\leq$300 cells/μL or >300 cells/μL.

In group A, before cART, 18 (34%) patients had an LT CD4+ count $\leq$ 300 cells/μL and 35 (66%) an LT CD4+ count > 300 cells/μL. In the group after cART (B), 10 (19%) patients had an LT CD4+ count $\leq$ 300 cells/μL and 43 (81%) an LT CD4+ count > 300 cells/μL. There was a downward trend in SIRT1, SIRT3 and SIRT6 levels with an increase in LT CD4+ count >300 cells/μL by 53%, 66% and 53%, respectively, in the pre-treatment group (A), and by 32%, 16% and 24% in the post-cART group (B). There was no statistically significant difference between SIRT1, SIRT3 and SIRT6 levels in subgroups with LT CD4+ count $\leq$300 cells /μL before cART therapy and the levels of those sirtuins in the subgroup with LT CD4+ count $\leq$ 300 cells /μL after treatment. Similar results were obtained in subgroups with LT CD4+ count > 300 cells /μL before and after treatment (Table 4).

The median levels and interquartile ranges with statistical analysis of examined sirtuins in the plasma of HIV-infected men before cART (A) and after cART (B), divided into subgroups according to LT CD8+ count $\leq$ 1000 cells/μL and >1000 cells/μL, are presented in Table 5 and Table S2.

Table 4. Results for SIRT1, SIRT3 and SIRT6 in the plasma of HIV-infected men before cART (**A**) and after cART (**B**) subgrouped according to LT CD4+ count.

	CD4+ Count ≤ 300 [Cells/μL]		
	Me (IQR)	**Me (IQR)**	***p* ***
	A	**B**	
SIRT1 [ng/mL]	12.00 (4.10–24.80)	6.90 (2.50–19.80)	NS
SIRT3 [ng/mL]	15.45 (4.50–42.00)	6.80 (1.90–61.50)	NS
SIRT6 [ng/mL]	3.80 (1.50–25.50)	5.80 (1.90–79.00)	NS
	CD4+ count > 300 [cells/μL]		
	Me (IQR)	**Me (IQR)**	
SIRT1 [ng/mL]	5.70 (3.60–16.60)	4.70 (2.20–72.20)	NS
SIRT3 [ng/mL]	5.30 (3.50–11.40)	5.70 (2.00–47.80)	NS
SIRT6 [ng/mL]	1.80 (0.70–8.30)	4.40 (2.10–37.70)	NS

Abbreviations: cART–combined antiretroviral therapy; SIRT1, SIRT3, SIRT6–sirtuin 1, 3, 6, respectively; A–HIV-infected patients before cART; B–HIV-infected patients after cART; Me–median; IQR–Interquartile range; NS–not statistically significant; * Wilcoxon test.

Table 5. Results for SIRT1, SIRT3 and SIRT6 in the plasma of HIV-infected men before cART (**A**) and after cART (**B**) in the subgroup with LT CD8+ count ≤ 1000 cells/μL and LT CD8+ and in the subgroup with LT CD8+ count >1000 cells/μL with statistical analysis.

	CD8+ Count ≤ 1000 [Cells/μL]		
	Me (IQR)	**Me (IQR)**	***p* ***
	A	**B**	
SIRT1 [ng/mL]	10.50 (4.30–24.80)	5.90 (2.20–68.55)	NS
SIRT3 [ng/mL]	9.10 (4.40–42.00)	6.45 (2.05–43.65)	NS
SIRT6 [ng/mL]	3.55 (1.20–25.50)	6.00 (2.20–37.20)	0.04
	CD8+ count > 1000 [cells/μL]		
	Me (IQR)	**Me (IQR)**	
SIRT1 [ng/mL]	4.60 (3.60–16.60)	3.60 (2.20–22.60)	NS
SIRT3 [ng/mL]	4.80 (3.40–11.40)	2.50 (1.90–15.80)	NS
SIRT6 [ng/mL]	1.65 (0.60–5.90)	2.80 (2.10–16.50)	0.01

Abbreviations: cART–combined antiretroviral therapy; SIRT1, SIRT3, SIRT6–sirtuin 1, 3, 6, respectively; A–HIV-infected patients before cART; B–HIV-infected patients after cART; Me–median; IQR–Interquartile range; N–number of participants; NS–not statistically significant; * Wilcoxon test.

In group A, before cART, 26 (49%) HIV-infected men had an LT CD8+ count ≤1000 cells/μL and 27 (51%) an LT CD8+ count > 1000 cells/μL. In the group after cART (B), 36 (68%) patients had an LT CD8+ count ≤ 1000 cells/μL and 17 (32%) an LT CD8+ count > 1000 cells/μL.

There was a downward trend in the level of SIRT1 and SIRT3 with an increase in LT CD8 + count > 1000 cells/μL by 56% and 47%, respectively, in the pre-cART group (A) and by 39% and 61% in the post-cART group (B). A statistically significant difference was demonstrated between SIRT6 levels in the subgroup with LT CD8+ count ≤ 1000 cells/μL before cART and SIRT6 in the subgroup with LT CD8+ count ≤ 1000 cells/μL after treatment (p = 0.04). A statistically significant difference was also demonstrated between SIRT6 levels in the subgroup with LT CD8+ count > 1000 cells/μL before cART treatment and SIRT6 in the subgroup with LT CD8+ count > 1000 cells/μL after treatment (p = 0.01). Such a relationship was not detected for SIRT1 and SIRT3 (Table 5).

Due to low HIV viral load after antiretroviral treatment (mean 20 copies/mL in group B), sirtuins in the subgroups with HIV RNA ≤100,000 copies/mL and HIV RNA >100,000 copies/mL were compared only in HIV-infected men prior to cART (A), as shown in Table 6.

Table 6. Results for SIRT1, SIRT3 and SIRT6 in the plasma of HIV-infected men before cART (**A**) in the subgroup with HIV RNA ≤ 100,000 copies/mL, and in the subgroup with HIV RNA > 100,000 copies/mL.

Group A	**HIV RNA ≤ 100,000 [Copies/mL] (n = 22)**	**HIV RNA > 100,000 [Copies/mL] (n = 31)**	p *
	Me (IQR)	**Me (IQR)**	
SIRT1 [ng/mL]	5.25 (2.90–16.30)	8.90 (4.00–68.90)	NS
SIRT3 [ng/mL]	5.15 (4.10–16.20)	7.80 (3.60–48.90)	NS
SIRT6 [ng/mL]	1.80 (0.60–5.90)	3.60 (0.90–25.50)	NS

Abbreviations: cART–combined antiretroviral therapy; SIRT1, SIRT3, SIRT6–sirtuin 1, 3, 6, respectively; group A–HIV-infected patients before cART; Me–median; N–number of participants; IQR–Interquartile range; NS–not statistically significant; * Mann–Whitney *U* test.

In group A, before cART, 22 (42%) of HIV-infected men had ≤100,000 HIV RNA copies/mL and 31 (58%) had >100,000 copies/mL. It was not possible to create subgroups depending on HIV RNA ≤ 100,000 copies/mL and HIV RNA > 100,000 copies/mL in group B, because all patients in this group had a viral load below 100,000 copies/mL. Therefore, the sirtuin levels for this group coincide with the data shown in Table 3. There were no statistically significant differences between the levels of SIRT1 and SIRT3 in the subgroup of HIV-infected men with HIV RNA ≤ 100,000 copies/mL and in the HIV-infected men with HIV RNA > 100,000 copies/mL in the pre-treatment group (A). There was an upward trend in SIRT1 and SIRT3 levels with an increase in HIV RNA >100,000 copies/mL by 41% and 34%, respectively. The median level for SIRT6 in the subgroup with HIV RNA ≤ 100,000 copies/mL was twofold lower than the median level in the subgroup with >100,000 HIV RNA copies/mL, but the difference was not statistically significant.

Medians and interquartile ranges for SIRT1, SIRT3 and SIRT6 in the plasma of HIV-infected men treated with Protease inhibitors (PIs) and Integrase transfer inhibitors (INSTIs) are presented in Table 7.

Interesting results were obtained in the analysis of sirtuins expression depending on the treatment regimen used–PIs or INSTIs. The median levels of all sirtuins were lower in HIV-infected men treated with PIs compared to the median levels shown in HIV-infected men treated with INSTIs: 4-, 3.3-, and 3.4-fold, respectively. A significant difference between SIRT1 levels was found in the subgroups of HIV-infected men receiving PIs and INSTIs therapy (p = 0.025) and not demonstrated for SIRT3 and SIRT6 (Table 7).

Table 7. Results for SIRT1, SIRT3 and SIRT6 in the plasma of HIV-infected men after cART (**B**) in the subgroup treated with Protease inhibitors (PIs) and the subgroup treated with Integrase transfer inhibitors (INSTIs) with statistical analysis.

Group B	Pis (n = 25)	INSTIs (n = 26)	p *
	Me (IQR)	Me (IQR)	
SIRT1 [ng/mL]	3.05 (2.10–19.80)	12.40 (1.40–79.40)	0.025
SIRT3 [ng/mL]	2.95 (1.90–15.80)	9.70 (2.10–51.60)	NS
SIRT6 [ng/mL]	3.20 (2.20–13.70)	10.90 (2.20–37.70)	NS

Abbreviations: cART–combined antiretroviral therapy; SIRT1, SIRT3, SIRT6–sirtuin 1, 3, 6, respectively; B–HIV-infected patients after cART; a–subgroup treated with Protease inhibitors (PIs); b–subgroup treated with Integrase transfer inhibitors (INSTIs); Me–median; IQR–Interquartile range; N–number of participants; NS–not statistically significant; * Mann–Whitney *U* test.

3. Discussion

Due to the increasing amount of scientific data on the participation of sirtuins in the pathomechanism and course of many diseases, there is a growing interest in the role of these enzymes in viral diseases, including HIV infection [19,30]. Due to their broad spectrum of activity and their regulation of many life processes or metabolic processes in the organism, their participation in viral infections is highly probable [31,32]. Additionally, there is a growing interest in the use of modifiers of sirtuins activity in different types of therapies, including antiviral therapies [17,30].

The authors' own study showed that, as a result of one-year cART therapy, the level of SIRT1 in HIV-infected men was decreased when compared to SIRT1 levels in HIV-infected men before treatment. The obtained results may suggest a decrease in SIRT1 expression caused by both HIV infection and the antiretroviral therapy used.

Wang 2020 et al. showed increased NF-kB p65 subunit and signal transducer and activator of transcription 3 (STAT3) acetylation in HIV-associated nephropathy (HIVAN) and decreased SIRT1 expression in the glomeruli of mouse and human HIVAN kidneys. The authors also showed that, in the course of HIVAN, the reduction of SIRT1 expression occurs through a mechanism dependent on miRNA-34a [33]. Zhan et al. showed increased miRNA-34a exposure in human vascular endothelial cells (ECs) and arteries isolated from HIV-positive patients treated with antiretroviral therapy (lopinavir and ritonavir) and cART-naïve (pre-treatment) patients. MiRNA-34 expression was significantly elevated by HIV antiretroviral therapy and promoted miRNA-induced senescence of ECs. The authors indicate that p53 protein is the key factor up-regulating miRNA34a and simultaneously acting as a down-regulator of SIRT1, as demonstrated in in vitro studies, where expression of p53 was significantly increased in ECs treated with Tat and lopinavir with ritonavir. The authors also confirmed that miRNA-34a directly affects SIRT1, and its level is reduced by the Tat protein and antiretroviral drugs (lopinavir, ritonavir) in ECs [34].

The authors' studies have shown that SIRT1 expression is significantly influenced by the cART treatment regimen. SIRT1 levels were higher in HIV-infected men treated with INSTIs compared to HIV-infected men treated with PIs, which may be interesting for further research.

Di Rosa et al. showed that HeLa cells exposed to HIV-based lentivirus and newly synthesized inhibitors of SIRT3 and SIRT2 reduced the rate of viral DNA integration into the host genome to an extent similar to raltegravir. In contrast, exposure to resveratrol significantly increased HIV DNA integration into the host genome. The potential involvement of sirtuins with DNA-fixing proteins such as Ku70 (SIRT3 and SIRT1), ATM/Nsb complex (SIRT1), and Poly (ADP-ribose) polymerase (SIRT6) may indicate a beneficial antiretroviral effect of sirtuin inhibitors as well as their beneficial influence on the post-integration repair

process necessary for the incorporation of the viral DNA into the host genome, especially in the case of treatment with INSTIs [35]. Further research is required to confirm the existence of such a relationship.

However, it should be taken into account that sirtuins may participate in many other antagonistic signaling pathways, and therefore the role of sirtuins in the course of HIV cannot be clearly assessed. PIs have a significant impact on the metabolic process, which is one of the causes of side effects during cART [36]. The authors' study showed that PIs (lopinavir and ritonavir or darunavir and cobicistat) significantly reduced the expression of SIRT1.

So far, little data is available on the involvement of SIRT3 in viral infections. Single data refer to the effect of SIRT3 on Hepatitis B Virus (HBV) progression. Ren et al. showed that silencing the SIRT3 gene enhanced HBV transcription and replication in primarily human hepatocytes and HepG2 cells. The authors point out that the repression of HBV transcription by SIRT3 is related to the decreased binding of host RNA polymerase II and Yin Yang transcription factor 1 (YY1) to covalently closed circular DNA (cccDNA) of HBV. SIRT3 is also considered a limiting factor for oxidative stress caused by HBV X protein (HBx), and thereby it also limits the replication of the virus [37]. SIRT3 influences antioxidant processes through deacetylation and increasing the activity of the antioxidant enzymes–SOD2 and CAT [26].

Yu et al. observed increased reactive oxygen species (ROS) production in CD8+ and CD4+ T cells in the course of HIV infection, as well as changes in mitochondrial membrane potential and mitochondrial mass compared to cells isolated from HIV–uninfected individuals [38]. The above data may indicate a potential role of SIRT3 in the course of HIV infection through its antioxidant properties and its regulation of mitochondrial homeostasis. In the authors' own study, no significant differences were found in the level of SIRT3 in the plasma of HIV-infected men compared to the control group. The cellular localization of SIRT3 in mitochondria may prevent the ability to assess its changes in the course of HIV infection. More research is needed to confirm the obtained results.

Among the three examined sirtuins, the greatest changes were found in SIRT6 expression in the plasma of HIV-infected men. The data obtained may suggest a beneficial effect of antiretroviral therapy on the level of SIRT6 in HIV positive patients. However, no significant differences between types of therapy were found.

The authors' own study demonstrated a significantly lower SIRT6 level in HIV-infected men compared to the control group. The exact mechanism explaining the influence of SIRT6 on the course of HIV is still unknown. One of the suggested mechanisms is the activation of SIRT6 by Interferon type 1 (IFN-1) signaling as a result of HIV infection. Hardy et al. showed that IFN-1 activity was significantly higher in HIV-infected patients compared to healthy controls. Moreover, plasma levels of IFN-1 were inversely correlated with CD4+ cell count and positively correlated with HIV RNA [39].

The obtained differences in the level of SIRT6 before and after treatment may also be related to the improvement of parameters such as: LT CD4+, CD8+ count, HIV RNA and the patients' improved clinical condition as a result of antiretroviral therapy. Such a relationship was observed in the case of SIRT6 and LT CD8+ count.

A significant increase in the level of SIRT6 was associated with an increase in LT CD8+ count after one year of antiretroviral therapy, demonstrating the beneficial effect of cART not only on the increase of SIRT6 expression but also on the response of the immune system. HIV-specific CD8+ T cells are prone to apoptosis, which may affect their ability to control HIV infection. CD8+ lymphocyte-mediated immune responses play a key role in controlling infection, increasing the survival and effector function of HIV-specific CD8+ T cells as well as their ability to control HIV [40].

4. Materials and Methods

4.1. Patient Characteristics

The study group consisted of 53 HIV-infected men with a mean age of 34 years who were patients at the Center for Preventive and Therapeutic Infectious Diseases and Addiction Therapy in Wroclaw as well as in the Department of Infectious Diseases, Liver Diseases and Acquired Immune Deficiencies of the Medical University of Wroclaw. The control group consisted of 35 healthy HIV-negative males with a mean age of 36 years and without any chronic or inflammatory diseases such as diabetes mellitus, cardiovascular diseases or hepatitis B or C virus infection. In the group of HIV-infected patients, inclusion criteria were the patient's consent for tests, confirmation of the presence of HIV infection, and cART use. Exclusion criteria were diseases such as diabetes, cancer, hypertension, neurocognitive diseases, and especially urinary tract diseases as well as concomitant use of drugs other than cART.

In the case of the HIV-infected men, blood was drawn twice: before and one year after antiretroviral therapy. Whole human blood (5 mL) was collected from both groups (patients and control) in a fasting state. Blood samples were taken into EDTA-treated tubes (Sarstedt, Warsaw, Poland). Tubes were centrifuged by MPW-350 laboratory centrifuge (MPW Instruments, Poland) at 1500× *g* for 10 min to separate the plasma. Plasma was removed and placed in Eppendorf tubes and stored at −80 °C for further investigation.

HIV-infected men were treated with two therapeutic regimens, which included two NRTIs (emtricitabine and tenofovir alafenamide) in combination with PIs (ritonavir-boosted lopinavir or cobicistat-boosted darunavir) or INSTIs (dolutegravir).

Data on lymphocytes T CD4+ and CD8+ count, HIV viral load and biochemical parameters such as TC, LDL, HDL, TG, FBG and BMI were obtained from medical records.

4.2. Determination of SIRT1, SIRT3, SIRT6 Levels in Plasma of HIV-Infected Men and Healthy Controls

The measurement of sirtuins concentrations was performed by the enzyme-linked immunoassay (ELISA) method using Human Sirtuin 1 ELISA Kit (Cat.No E2557Hu), Human Sirtuin 3 ELISA Kit (Cat.No E2559Hu), and Human Sirtuin 6 ELISA Kit (Cat.No E2562Hu) Bioassay Technology Laboratory (BT Lab; Shanghai Korain Biotech Co Ltd., Shanghai, China) according to the manufacturers' instructions. Standards and serum samples were added into a 96-well plate. After adding the biotin-conjugated anti-SIRT1/SIRT3/SIRT6 antibody and streptavidin-horseradish peroxidase, the plate was incubated for 60 min at 37 °C. The wells were then washed five times with wash buffer. Substrate solutions A and B were added, and the plate was incubated for 10 min at 37 °C for color development. Finally, the reaction was stopped by the stop solution. The intensity of color in each well was measured at 450 nm with a microplate reader (STAT FAX 2100, Palm City, FL, USA).

4.3. Statistical Analysis

Statistical analysis was performed using the Statistica 13.3 PL program (StatSoft, Cracow, Poland). For measurable variables, medians and the range of variability (minimum and maximum values) were calculated. For qualitative variables, the frequency of their occurrence (percentage) was calculated. All investigated quantitative variables were checked with the Shapiro-Wilk test to establish the type of distribution. Variables with abnormal distribution were presented as the median and interquartile range (IQR) 25-75%. Results with normal distribution were presented as the mean ± standard deviation (SD). The comparison of qualitative variables between the groups was made using the chi-square test (χ^2). Since the obtained results did not have the characteristics of a normal distribution, non-parametric tests were used. The Mann–Whitney U test was used for two independent samples (HIV-infected men, control). For dependent samples (HIV-infected men before and after cART), the non-parametric Wilcoxon test was used. The Kruskal–Wallis test was used to compare multiple independent samples. The obtained results were also analyzed in patient subgroups, divided according to: CD4+ count (below and above 300 cells/μL), CD8+

count (below and above 1000 cells/μL), HIV RNA (below and above 100,000 copies/mL) and the type of therapeutic regimen (INSTIs or PIs). For all analyses, $p < 0.05$ was accepted as a significant value.

5. Conclusions

This is the first study of the expression of SIRT1, SIRT3 and SIRT6 in HIV-infected men. It is also the first study using clinical material (plasma). The levels of all examined sirtuins were reduced in the plasma of HIV-infected men compared to non-HIV infected men, suggesting a negative effect of HIV infection on their expression in one year of observation. There was no significant effect on the level of SIRT1 and SIRT3 after the implementation of one year of antiretroviral therapy, which depended on HIV viral load and the CD4+ and CD8+ T lymphocytes count. The greatest changes in expression were demonstrated in the case of SIRT6, the levels of which increased significantly after the use of cART, thus proving the beneficial effect of the implemented antiretroviral therapy on the level of this enzyme as well as the therapy's relationship with HIV viral load, LT CD8+ count, and plasma levels of SIRT6, which may, in turn, reflect the body's immune response to HIV infection. Interesting results were obtained by analyzing the expression of sirtuins depending on the cART treatment regimen. The expression of all examined sirtuins (SIRT1, SIRT3, and SIRT6) was higher after cART with INSTIs; in the case of SIRT1, the difference was statistically significant. Protease inhibitors (PIs) significantly lowered the level of all sirtuins, which may indicate a significant role of sirtuins in response to antiretroviral therapy. However, the mechanism of these processes is still unknown.

Future Perspectives

The obtained data suggest the influence of SIRT6 and SIRT1 in the course of HIV infection and cART therapy. The obtained results indicate a need for further research on sirtuin expression, which could potentially create new perspectives in the treatment or optimization of therapy as well as enable better monitoring of the course of HIV infection. The provided explanation of changes in sirtuin-regulated pathways will enable a more detailed understanding of their importance for the treatment of HIV patients in the future. Although the regulation of sirtuin activity can be identified as a potential target of therapy, the above data indicate the need for further research on the role of sirtuins in the course of HIV infection, especially of SIRT6, for which the greatest changes were observed. The exact molecular mechanisms involved are yet to be understood, and a specification of the viral or host genes regulated by SIRT6, or vice versa, is still required.

Supplementary Materials: The following supporting information can be downloaded at online, Table S1: Results for SIRT1, SIRT3 and SIRT6 in the plasma of HIV-infected men before cART (A) and after cART (B) subgrouped according to LT CD4+ count.; Table S2: Results for SIRT1, SIRT3 and SIRT6 in the plasma of HIV-infected men before cART (A) and after cART (B) in the subgroup with LT CD8+ count $\leq$ 1000 cells/μL and LT CD8+ and in the subgroup with LT CD8+ count > 1000 cells/μL with statistical analysis.

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Załącznik 3

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Effect of Combined Antiretroviral Therapy on the Levels of Selected Parameters Reflecting Metabolic and Inflammatory Disturbances in HIV-Infected Patients

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Abstract: Subjects infected with human immunodeficiency virus (HIV) treated with combined antiretroviral therapy (cART) show a greater predisposition to metabolic disturbances compared to the general population. The aim of the study was to assess the effect of cART on the level of selected parameters related to carbohydrate and lipid metabolism, cardiovascular diseases and inflammation in the plasma of HIV-infected patients against the uninfected. The levels of irisin (IRS), myostatin (MSTN), peptide YY (PYY), glucagon-like peptide-1 (GLP-1), dipeptidyl peptidase IV (DPP-4), fetuin A (FETU-A), pentraxin 3 (PTX 3), chemokine stromal cell-derived factor 1 (SDF-1), and regulated on activation normal T cell expressed and secreted (RANTES) in the plasma of HIV-infected patients and the control group were measured by immunoassay methods. HIV-infected patients were analyzed in terms of CD4+ T cells and CD8+ T cell count, HIV RNA viral load, and the type of therapeutic regimen containing either protease inhibitors (PIs) or integrase transfer inhibitors (INSTIs). The analysis of HIV-infected patients before and after cART against the control group showed statistically significant differences for the following parameters: IRS ($p = 0.02$), MSTN ($p = 0.03$), PYY ($p = 0.03$), GLP-1 ($p = 0.03$), PTX3 ($p = 0.03$), and RANTES ($p = 0.02$), but no significant differences were found for DPP-4, FETU-A, and SDF-1. Comparing the two applied therapeutic regimens, higher levels of all tested parameters were shown in HIV-infected patients treated with INSTIs compared to HIV-infected patients treated with PIs, but the differences were not statistically significant. The obtained results indicated significant changes in the expression of selected parameters in the course of HIV infection and cART. There is need for further research on the clinical usefulness of the selected parameters and for new information on the pathogenesis of HIV-related comorbidities to be provided. The obtained data may allow for better monitoring of the course of HIV infection and optimization of therapy in order to prevent the development of comorbidities as a result of long-term use of cART.

Keywords: HIV; combined antiretroviral therapy; panel parameters; carbohydrate; lipid metabolism; cardiovascular diseases; inflammation

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1. Introduction

About 37.7 million people worldwide are currently infected with the human immunodeficiency virus (HIV) [1]. The introduction of combined antiretroviral therapy (cART) has proved to be a breakthrough in the treatment of HIV infection [2]. This therapy involves the use of at least three drugs from different available pharmacological groups, ensuring the inhibition of viral replication to levels undetectable by the most sensitive analytical methods; it prevents the development of drug resistance and enables the restoration of immune system function as well as preventing or delaying the occurrence of acquired immunodeficiency syndrome (AIDS). There are different therapeutic regimens based on

the application of at least two nucleoside reverse transcriptase inhibitors (NRTIs) in combination with non-nucleoside reverse transcriptase inhibitors (NNRTIs), integrase transfer inhibitors (INSTIs), protease inhibitors (PIs), fusion inhibitors, and C-C Chemokine Receptor 5 (CCR5) antagonists. In addition to two NRTIs, recommended regimens include protease inhibitors (PIs) or integrase transfer inhibitors (INSTIs) [3–5].

Antiretroviral treatment effectively controls HIV infection and guarantees a good quality of life for many years. Despite its obvious benefits, HIV-infected people treated with antiretroviral therapy show a greater predisposition to metabolic disorders compared to the general population [4,5]. The prevalence of metabolic syndrome in people living with HIV (PLWH) is estimated at approximately 20–33% [6]. One of the causes of metabolic disorders in addition to lifestyle and the use of cART is the state of chronic inflammation. Increased expression of cytokines and other proinflammatory factors (e.g., soluble CD163, CD40, CD27, interleukin-6, CRP, D-dimer, cystatin C) especially in the area of adipose tissue, liver, skeletal muscles, and the digestive system causes changes in metabolism and increased storage of adipose tissue, predisposing to type two diabetes mellitus (T2DM) and cardiovascular diseases (CVD) [7]. However, the exact mechanisms behind the development of metabolic disorders in HIV-infected people are still not fully understood.

IRS is an adipomyokine, which is a fragment of fibronectin type III domain-containing protein 5 (FNDC5/FRCP2/PeP) in the cell membrane, secreted mainly by skeletal muscles and visceral and subcutaneous adipose tissue [8]. Irisin up-regulates the metabolism of adipose tissue and thermogenesis and reduces the formation of new adipocytes [8,9].

MSTN, otherwise known as growth differentiation factor-8 (GDF-8), is a negative regulator of muscle growth and a member of the transforming growth factor β (TGF-β) superfamily. It is expressed in skeletal muscles, the heart muscle, and adipose tissue [10]. In vitro studies also indicated its role in adipogenesis, neuronal control in insulin resistance, and communication between muscles and adipose tissue [11].

GLP-1 is an incretin peptide whose main action is to increase the release of insulin from pancreatic β cells and inhibit the release of glucagon, and it has a hypoglycemic effect [12]. It also inhibits gastric secretion and intestinal motility, regulating appetite in a manner similar to PYY. GLP-1 analogues are widely used in the treatment of T2DM and obesity [13].

PYY is a neuropeptide that regulates the gut-brain axis and acts as a satiety factor, inhibiting gut motility, appetite, and further food intake. It also regulates carbohydrate metabolism. It is secreted by endocrine L cells of the gastrointestinal tract after ingestion, together with GLP-1 incretin, and by pancreatic endocrine cells and gastric intestinal neurons [14].

DPP-4 is a serine exopeptidase that cleaves GLP-1 shortly after it is secreted. It is a widespread enzyme present in many tissues including the liver, gut, placenta, lung, and kidney [15]. DPP-4 inhibitors are also widely used in the treatment of T2DM and show a number of other beneficial pleiotropic effects apart from their hypoglycemic effect, e.g., anti-inflammatory [16].

FETU-A is a multifunctional glycoprotein mainly synthesized in the liver and released into the bloodstream. Significant amounts of FETU-A are also synthesized in adipose tissue [17].

PTX 3, like the C-reactive protein, is an acute phase protein and a member of the pentraxin family that is released within damaged tissue in dendritic cells (DCs), monocytes, macrophages, fibroblasts, chondrocytes, adipocytes, epithelial cells, vascular endothelial cells, smooth muscle cells, mesangial cells, granulosa cells by pro-inflammatory cytokines (such as interleukin-1β and tumor necrosis factor α), and microorganisms. Elevated levels of PTX3 have been found in the course of sepsis and bacteraemia, cardiovascular diseases, and coronary artery disease [18,19].

SDF-1, or chemokine CXCL12, is a multifunctional protein and an endogenous ligand for the C-X-C motif chemokine receptor four (CXCR4) receptor, which is also a coreceptor

for some HIV-1 strains present in many tissues including fibroblasts, osteoblasts, and endothelial cells [20].

RANTES, or CCL5, is a chemokine from the CC subfamily that is a strong inflammatory mediator with chemotactic properties for immune cells in the place of injury or infection. RANTES which is released from activated platelets mediates the retention of monocytes in the inflamed site of the epithelium and promotes platelet aggregation as well as the formation of atherosclerotic lesions. It also regulates the activity of T lymphocytes in atherosclerotic lesions, contributing to the progression of atherosclerosis [20,21].

The aim of the study was to assess the effect of cART on the level of selected parameters characterizing carbohydrate and lipid metabolism, cardiovascular diseases, and inflammation (IRS, MSTN, PYY, GLP-1, DPP-4, FETU-A, PTX3, SDF-1, and RANTES) in the plasma of HIV-infected subjects before and one year after the implementation of cART. The analyses took into account the influence of parameters characterizing the state of the immune system such as CD4+ T cells and CD8+ T cells count, HIV RNA viral load, and the type of antiretroviral treatment regimen used: PIs or INSTIs. The obtained data may enable the clinical usefulness of selected parameters and provide new information on the development of disorders accompanying HIV infection.

2. Materials and Methods

2.1. Institutional Review Board Statement

The study was conducted in accordance with the Declaration of Helsinki, and the protocol was approved by Ethics Committee of Wroclaw Medical University (KB-597/2019). Written informed consent was obtained from all participants.

2.2. Patient Characteristics

The study group consisted of HIV-infected men (N = 53) at a mean age of 34 years treated in the Center for Preventive and Therapeutic Infectious Diseases and Addiction Therapy in Wroclaw and in the Department of Infectious Diseases, Liver Diseases and Acquired Immune Deficiencies of the Medical University of Wroclaw. All patients were infected with the HIV-1 strain. Inclusion criteria for the study group were confirmation of the presence of HIV infection and intake of cART drugs. Exclusion criteria were diseases such as: diabetes mellitus, cancer, hypertension, urinary tract diseases, and concomitant use of drugs other than cART.

The control group consisted of 35 healthy HIV-negative men at a mean age of 36 years without any chronic or inflammatory diseases such as: diabetes mellitus, renal diseases, cardiovascular diseases, or hepatitis B or C virus infection.

HIV-infected men were treated with two therapeutic regimens which included two NRTIs (emtricitabine and tenofovir alafenamide) in combination with PIs (ritonavir-boosted lopinavir or cobicistat-boosted darunavir) or INSTIs (dolutegravir).

Data on CD4+ T cells and CD8+ T cells count, HIV RNA viral load, and biochemical parameters such as TC (total cholesterol), LDL-C (LDL cholesterol), HDL-C (HDL cholesterol), TG (triglycerides), FBG (fasting blood glucose), and BMI (body mass index) were obtained from medical records of patients.

2.3. Determination of Selected Parameters Levels in Plasma of HIV-Infected Men and Healthy Controls

In HIV-infected men, blood was drawn twice: before and one year after starting to take cART. Whole human blood was collected from HIV-infected men and the control in a fasting state. Blood samples were taken into EDTA-treated tubes (5 mL blood, containing 1.6 mg/mL EDTA, Sarstedt, Poland). Tubes were centrifuged by MPW-350 laboratory centrifuge (MPW Instruments, Warszawa, Poland) at 1500× g for 10 min to separate the plasma. Plasma was removed and placed in Eppendorf tubes (Eppendorf AG, Hamburg, Germany) and stored at −80 °C for further investigation.

The measurement of concentrations of selected parameters was performed by enzyme-linked immunoassay (ELISA) method using: human irisin ELISA kit (Cat.No E3253Hu), human growth differentiation factor 8 ELISA kit (Cat.No E3058Hu), human peptide YY ELISA kit (Cat.No E1369Hu), human glucagon-like peptide 1 ELISA kit (Cat.No E0022Hu), human dipeptidyl peptidase 4 ELISA kit (Cat.No E6631Hu), human fetuin A ELISA kit (Cat.No E1386Hu), human pentraxin 3 ELISA kit (Cat.No E1938Hu), human stromal cell derived factor 1 ELISA kit (Cat.No E3353Hu), and human regulated on activation in normal T cell expressed and secreted or C-C motif chemokine 5 ELISA kit (Cat.No E3663Hu), Bioassay Technology Laboratory (BT Lab; Shanghai Korain Biotech Co., Ltd., Shanghai, China) according to the manufacturer's instructions. Standards and serum samples were added into a 96-well plate. After adding biotin-conjugated antibodies and streptavidin–horseradish peroxidase, the plate was incubated for 60 min at 37 °C. The wells were then washed five times with wash buffer. Substrate solutions A and B were added and the plate was incubated for 10 min at 37 °C for color development. Finally, the reaction was stopped by the stop solution. The intensity of the color in each well was measured in a microplate reader. Absorbance was read at 450 nm with a microplate reader STAT FAX 2100 (Awareness Technology Inc., Palm City, FL, USA).

2.4. Statistical Analysis

Statistical analysis was performed using Statistica 13.3 PL (StatSoft, Cracow, Poland). All investigated quantitative variables were checked with the Shapiro–Wilk test to establish the type of distribution. Variables with non-parametric distribution were presented as the median and interquartile range (IQR) 25–75%. The comparison of quantitative variables between the groups was performed using the Kruskal–Wallis test and the U Mann–Whitney test. The Kruskal–Wallis test was used to compare three groups (HIV-infected men before cART, after cART, and controls) in terms of the quantitative variables studied. The statistically significant result of the Kruskal–Wallis test indicated that at least one group differs from the other group. Therefore, a post-hoc test (Dunn test with Bonferroni correction) was then performed to see exactly which groups differed from each other. Within-group comparison between results before and after cART was made using the Wilcoxon test. HIV-infected men were also analyzed in subgroups depending on the number of CD4+ T cells count (below and above 300 cells/μL), CD8+ T cells count (below and above 1000 cells/μL), HIV RNA viral load (below and above 100,000 RNA copies/mL), and the type of therapeutic regimen (INSTIs or PIs). For all analyses, $p < 0.05$ was accepted as a significant value.

3. Results

The study groups consisted of HIV-infected men before cART (A) and one year after the implementation of cART (B), and a control group (C). Demographic and biochemical data (BMI, FBG, TG, LDL-C, HDL-C, and TG) are presented in Table 1.

The median values of age, BMI, FBG, TG, LDL-C, HDL-C, and TG in HIV-infected men before (A) and after treatment (B) were similar in the study groups of patients and the control group and were not statistically significant ($p > 0.05$).

Immunological data concerning the groups of patients before (A) and after cART (B) are provided in Table 2.

The difference in the median values of HIV RNA viral load and CD4+ T cells and CD8+ T cells count in HIV-infected men before (A) and after treatment (B) were statistically significant.

3.1. Panel of Selected Parameters in Plasma of HIV-Infected Men before cART Therapy, after cART Therapy, and in the Control Group with Statistical Analysis

Values of IRS, MSTN, PYY, DPP-4, FETU-A, PTX3, SDF-1, and RANTES before (A) and after (B) cART in the group of HIV-infected men and the control group (C) with statistical analysis are provided in Table 3.

Table 1. Demographic and biochemical data of HIV-infected men before (A) and after cART (B) and the control group (C) with statistical analysis.

Group Characteristics	A	B	C	p *
	Me (IQR)	Me (IQR)	Me (IQR)	
Age (Y)	33 (28–40)	34 (29–41)	36 (30–43)	0.76
BMI [kg/m^2]	24.15 (21.55–24.80)	24.00 (21.56–24.81)	22.30 (18.00–26.80)	0.68
FBG [mg/dL]	96.00 (91.90–98.10)	98.90 (92.40–103.90)	95.00 (87.20–99.20)	0.08
TC [mg/dL]	174.90 (156.00–187.00)	174.00 (155.00–189.00)	180.00 (165.00–195.00)	0.44
LDL-C [mg/dL]	96.00 (85.00–103.00)	99.06 (80.00–110.00)	100.00 (96.00–115.00)	0.11
HDL-C [mg/dL]	76.60 (63.00–84.00)	77.15 (67.00–82.00)	75.00 (59.00–101.00)	0.15
TG [mg/dL]	149.00 (119.00–160.00)	157.20 (100.00–162.00)	163.00 (151.00–175.00)	0.35

Abbreviation: A—HIV-infected men before cART; B—HIV-infected men after cART; C—control group; BMI—body mass index; FBG—fasting blood glucose; TC—total cholesterol; LDL-C—LDL cholesterol; HDL-C—HDL cholesterol; TG—triglycerides; Me—median; IQR—interquartile range; and N—number of participants. * p—statistical significance by Kruskal–Wallis test.

Table 2. Immunological data of HIV-infected men groups before (A) and after cART (B) with statistical analysis.

Group Characteristics	A	B	p *
	Me (IQR)	Me (IQR)	
CD4+ T [cells/μL]	340 (234–386)	570 (398–762)	<0.001
CD8+ T [cells/μL]	999 (717–1190)	855 (706–1062)	0.004
HIV RNA [copies/mL]	148,000 (5190–245,000)	20 (15–34)	<0.001

Abbreviation: A—HIV-infected men before cART; B—HIV-infected men after cART; C—control group; Me—median; IQR—interquartile range; and N—number of participants. * p—statistical significance by Wilcoxon test.

The analysis of examined parameters in HIV-infected men before and after cART and the control group showed statistically significant differences for the following parameters: IRS, MSTN, PYY, GLP-1, PTX 3, and RANTES. No significant differences were found for three parameters: DPP-4, FETU-A, and SDF-1.

For IRS, MSTN, PYY, GLP-1, PTX 3, and RANTES in HIV-infected men before cART (A), the median levels were significantly lower compared to the median levels obtained in the control group (2.4-, 2-, 1.2-, 2.9-, 1.6-, and 2.3-fold, respectively). The median levels of IRS, MSTN, GLP-1, and FETU-A in HIV-infected men before cART (A) were lower than the medians obtained in HIV-infected men after cART (B), but the differences were not statistically significant. The median levels of DPP-4, PTX 3, and RANTES in HIV-infected men before cART (A) were higher than the medians obtained in HIV-infected men after cART (B), but the differences were not statistically significant. The median level for SDF- 1 was the same in HIV-infected men both before and after cART. The only parameter whose median level was statistically significantly higher (two-fold) in HIV-infected men before (A) compared to after cART (B) was PYY (Figure S1A–I).

Table 3. Results for selected parameters in the plasma of HIV-infected men before (A) and after cART (B) and in the control group (C) with statistical analysis.

Groups	A	B	C	*p* *	Post-Hoc
	Me (IQR)	Me (IQR)	Me (IQR)		
IRS [ng/mL]	4.70 (1.60–21.80)	8.90 (2.50–41.30)	11.30 (3.20–38.30)	0.02	A:C = 0.02 B:C = 0.38 A:B = 0.13
MSTN [ng/mL]	162.80 (80.50–542.00)	253.00 (105.20–2060.50)	318.40 (129.40–1575.00)	0.03	A:C = 0.02 B:C = 0.23 A:B = 0.25
PYY [pg/mL]	156.30 (126.90–286.40)	79.20 (31.40–262.60)	181.50 (43.80–446.40)	0.03	A:C = 0.02 B:C = 0.30 A:B = 0.04
GLP-1 [ng/mL]	209.60 (84.80–604.60)	263.40 (132.00–2516.00)	607.10 (184.60–1440.00)	0.01	A:C = 0.004 B:C = 0.24 A:B = 0.19
DPP-4 [ng/mL]	239.80 (181.80–430.40)	109.00 (51.30–1296.50)	174.30 (66.70–1260.00)	0.17	A:C = 1.00 B:C = 0.45 A:B = 0.33
FETU-A [ng/mL]	265.2 (132.30–1387.00)	363.90 (142.00–3568.50)	620.60 (157.50–2302.00)	0.12	A:C = 0.10 B:C = 0.58 A:B = 0.58
PTX 3 [ng/mL]	2.70 (1.10–7.90)	2.60 (1.30–36.10)	4.40 (1.50–17.40)	0.03	A:C = 0.04 B:C = 0.53 A:B = 0.13
SDF-1 [ng/mL]	1.7 (0.70–6.40)	1.70 (0.80–18.80)	3.30 (1.10–8.50)	0.19	A:C = 0.10 B:C = 0.41 A:B = 1.0
RANTES [ng/mL]	330.50 (107.90–782.40)	317.90 (165.90–3129.00)	750.90 (182.60–2625.00)	0.02	A:C = 0.02 B:C = 0.42 A:B = 0.15

Abbreviations: IRS—irisin; MSTN—myostatin; PYY—peptide YY; GLP-1—glucagon-like peptide-1; DPP-4—dipeptidyl peptidase IV; FETU-A—fetuin A; PTX3—pentraxin 3; SDF-1-1—chemokine stromal cell-derived factor 1 regulated on activation; RANTES—normal T cell expressed and secreted; A—HIV-infected men before cART; B—HIV-infected men after cART; C—control group; Me—median; and IQR—interquartile range. * *p*—statistical significance by Kruskal–Wallis test.

3.2. Results of Selected Parameters before and after cART in HIV-Infected Men by CD4+ T and CD8+ T Cells Counts

The values of selected parameters in HIV-infected men both before cART (A) and after treatment (B) depending on the CD4+ T cells count (below and above 300 cells/μL) and the CD8+ T cells count (below and above 1000 cells/μL) at the time of sampling, are presented in Table 4.

Median levels of the studied parameters slightly differed in HIV-infected men with CD4+ T cells ≤300 and CD4+ T cells >300 before (A) and after (B) cART.

In HIV-infected men with CD8+ T cells ≤1000 before cART (A), an increase (almost two-fold) in the median level was observed for all parameters except PYY (3.5-fold decrease) compared to the median level of these parameters after cART. Significant statistical differences were shown only for FETU-A ($p = 0.03$) and PTX-3 ($p = 0.04$).

In HIV-infected men with CD8+ T cells >1000 count, an increase in the median level was observed for all parameters except PYY, DPP-4, and PTX-3 (decrease) compared to the median levels of these parameters after cART.

Table 4. Results of selected parameters before and after cART in HIV-infected men by CD4+ T cells count (below and above 300 cells/μL) and by CD8+ T cells count (below and above 1000 cells/μL) with statistical analysis.

	CD4+ T Cells ≤ 300 [Cells/μL]		p *	**CD8+ T Cells ≤ 1000 [Cells/μL]**		p *
	Me (IQR)			**Me (IQR)**		
	A (*N* = 18)	**B (*N* = 10)**		**A (*N* = 26)**	**B (*N* = 36)**	
IRS [ng/mL]	4.55 (1.70–22.40)	4.70 (1.60–18.10)	0.44	5.20 (1.70–32.80)	10.00 (2.50–75.70)	0.09
MSTN [ng/mL]	149.10 (80.50–903.90)	162.80 (82.40–447.60)	0.07	273.00 (80.50–572.80)	484.70 (127.00- 2960.00)	0.06
PYY [pg/mL]	161.75 (131.40–334.50)	156.30 (125.90–255.60)	0.21	173.20 (126.90–293.20)	158.60 (39.30–611.40)	0.45
GLP-1 [ng/mL]	206.90 (100.10–697.60)	209.60 (82.10–456.20)	0.60	265.65 (83.00–499.30)	559.60 (141.10–3598.00)	0.10
DPP-4 [ng/mL]	245.10 (190.20–488.40)	239.80 (181.95–341.65)	0.68	245.10 (174.50–320.00)	305.20 (54.00–1452.00)	0.45
FETU-A [ng/mL]	269.10 (136.20–1487.00)	265.15 (119.05–820.75)	0.68	344.20 (132.30–1068.00)	1112.10 (173.50–4109.00)	0.03
PTX3 [ng/mL]	2.80 (1.10–8.50)	2.70 (1.00–5.40)	0.69	2.85 (1.10–5.30)	7.50 (1.50–43.70)	0.04
SDF-1 [ng/mL]	1.90 (0.70–9.60)	1.65 (0.70–4.90)	0.59	2.35 (0.70–5.010)	4.20 (1.10–41.10)	0.06
RANTES [ng/mL]	346.40 (127.10–1075.00)	330.50 (106.30–767.20)	0.68	434.70 (127.10–767.20)	816.30 (196.10–4804.00)	0.11
	CD4+ T cells > 300 [cells/μL]		p *	**CD8+ T cells > 1000 [cells/μL]**		p *
	Me (IQR)			**Me (IQR)**		
	A (*N* = 35)	**B (*N* = 43)**		**A (*N* = 27)**	**B (*N* = 17)**	
IRS [ng/mL]	9.50 (2.20–15.30)	9.40 (2.50–49.10)	0.10	2.30 (1.60–18.40)	5.850 (2.25–33.40)	0.15
MSTN [ng/mL]	322.55 (103.40–770.50)	299.50 (105.20–2684.00)	0.30	122.10 (78.50–903.90)	221.10 (107.90–1856.00)	0.20
PYY [pg/mL]	101.70 (32.80–217.20)	83.80 (30.10–452.90)	0.27	155.00 (125.90–286.40)	43.70 (24.70–280.50)	0.86
GLP-1 [ng/mL]	408.40 (124.30–661.80)	340.20 (133.90–3170.00)	0.23	103.20 (84.80–697.60)	171.70 (133.90–2360.00)	0.06
DPP-4 [ng/mL]	176.75 (48.30–512.10)	123.50 (52.40–1356.00)	0.95	229.30 (181.80–430.40)	58.50 (51.90–1356.00)	0.57
FETU-A [ng/mL]	722.95 (160.50–1433.00)	454.30 (133.30–4109.00)	0.24	164.90 (122.50–1586.00)	209.00 (158.00–3290.00)	0.08
PTX3 [ng/mL]	3.25 (1.20–8.90)	2.90 (1.30–39.50)	0.15	2.40 (1.00–9.60)	1.60 (1.20–32.20)	0.11

Table 4. *Cont.*

	CD4+ T cells > 300 [cells/μL]		*p* *	CD8+ T cells > 1000 [cells/μL]		*p* *
	Me (IQR)			Me (IQR)		
	A (*N* = 35)	B (*N* = 43)		A (*N* = 27)	B (*N* = 17)	
SDF-1 [ng/mL]	2.40 (0.70–5.20)	1.90 (0.80–23.70)	0.14	0.90 (0.70–7.70)	0.90 (0.80–17.80)	0.12
RANTES [ng/mL]	540.45 (166.60–999.10)	361.40 (165.10–3581.00)	0.12	176.40 (92.30–1075.00)	192.30 (169.30–3436.00)	0.06

Abbreviation: A—HIV-infected man before cART; B—HIV-infected man after cART; Me—median; IQR—interquartile range; *N*—number of participants; IRS—irisin; MSTN—myostatin; PYY—peptide YY; GLP-1—glucagon-like peptide-1; DPP-4—dipeptidyl peptidase IV; FETU-A—fetuin A; PTX3—pentraxin 3; SDF-1—chemokine stromal cell-derived factor 1; and RANTES—regulated on activation, normal T cell expressed and secreted. * *p*—statistical significance by Wilcoxon test.

3.3. Results of Selected Parameters before and after cART in HIV-Infected Men by HIV RNA Viral Load

The concentration and the results of statistical analysis of selected parameters in HIV-infected men before cART (A) depending on the amount of HIV RNA copies (below and above 100,000 copies/mL) at the time of sampling are presented in Table 5.

Table 5. Results of selected parameters before and after cART in HIV-infected men by HIV RNA viral load (below and above 100,000 copies/mL) with statistical analysis.

	HIV RNA ≤ 100,000 [Copies/mL] (*N* = 31)	HIV RNA > 100,000 [Copies/mL] (*N* = 22)	*p* *
	Me (IQR)	Me (IQR)	
	HIV-Infected Men before cART (A)		
IRS [ng/mL]	5.15 (1.60–21.80)	4.10 (1.80–18.10)	0.24
MSTN [ng/mL]	261.60 (83.20–707.60)	128.75 (72.90–445.50)	0.36
PYY [pg/mL]	192.05 (125.85–313.85)	149.35 (126.90–255.60)	0.87
GLP-1 [ng/mL]	243.95 (94.15–684.85)	103.20 (79.40–369.90)	0.89
DPP-4 [ng/mL]	276.45 (183.00–460.05)	229.30 (179.70–281.30)	0.47
FETU-A [ng/mL]	287.95 (133.30–1437.00)	156.00 (121.50–458.30)	0.56
PTX3 [ng/mL]	3.00 (1.10–8.45)	2.40 (0.90–4.20)	0.57
SDF-1 [ng/mL]	2.00 (0.70–8.65)	0.90 (0.70–2.80)	0.50
RANTES [ng/mL]	386.45 (117.50–992.45)	207.80 (88.45–608.55)	0.72

Abbreviation: A—HIV-infected man before cART; B—HIV-infected man after cART; Me—median; IQR—interquartile range; *N*—number of participants; IRS—irisin; MSTN—myostatin; PYY—peptide YY; GLP-1—glucagon-like peptide-1; DPP-4—dipeptidyl peptidase IV; FETU-A—fetuin A; PTX3—pentraxin 3, SDF-1—chemokine stromal cell-derived factor 1; and RANTES—regulated on activation, normal T cell expressed and secreted. * *p*—statistical significance by U Mann–Whitney test.

None of the HIV-infected men with a pre-treatment viral load HIV RNA > 100,000 (copies/mL) maintained such a load after cART (B).

Median levels of all parameters in HIV-infected men with HIV RNA viral load HIV RNA ≤ 100,000 (copies/mL) were higher compared to the results obtained in HIV-infected men with HIV RNA viral load HIV RNA > 100,000 (copies/mL).

3.4. Examined Parameters in HIV-Infected Men Subjected to cART with Protease Inhibitors (PIs) and Integrase Transfer Inhibitors (INSTIs) Treatment

Medians and interquartile ranges for selected parameters in HIV-infected men after cART subgroups with protease inhibitors (PIs) and integrase transfer inhibitors (INSTIs) treatment are presented in Table 6.

Table 6. Results of selected parameters in HIV-infected men after cART in the subgroup with protease inhibitors (PIs) treatment and the subgroup with integrase transfer inhibitors (INSTIs) treatment with statistical analysis.

Parameters	PIs (*N* = 25)	INSTIs (*N* = 28)	*p* *
	Me (IQR)	Me (IQR)	
	HIV-Infected Men after cART (B)		
IRS [ng/mL]	2.90 (2.30–15.90)	10.60 (2.90–77.40)	0.18
MSTN [ng/mL]	160.00 (105.20–917.90)	484.70 (110.60–3589.00)	0.20
PYY [pg/mL]	54.50 (32.60–205.50)	201.20 (30.10–666.60)	0.08
GLP-1 [ng/mL]	159.30 (124.30–709.20)	661.80 (150.00–3484.00)	0.09
DPP-4 [ng/mL]	70.90 (48.30–541.80)	395.80 (52.60–1452.00)	0.14
FETU-A [ng/mL]	224.25 (142.80–1433.00)	1267.00 (173.50–5173.00)	0.33
PTX3 [ng/mL]	1.95 (1.20–9.80)	8.10 (1.50–43.70)	0.31
SDF-1 [ng/mL]	1.15 (0.70–5.20)	5.20 (0.80–41.10)	0.22
RANTES [ng/mL]	240.40 (155.40–999.10)	893.90 (169.30–4804.00)	0.24

Abbreviation: cART—combined antiretroviral therapy; B—HIV-infected man after cART; PIs—protease inhibitors treatment; INSTIs—integrase transfer inhibitors; Me—median; IQR—interquartile range; *N*—number of participants; NS—not statistically significant; IRS—irisin; MSTN—myostatin; PYY—peptide YY; GLP-1—glucagon-like peptide-1; DPP-4—dipeptidyl peptidase IV; FETU-A—fetuin A; PTX 3—pentraxin 3; SDF-1—chemokine stromal cell-derived factor 1; and RANTES—regulated activation normal T cell expressed and secreted. * *p*—statistical significance by Wilcoxon test.

The antiretroviral regimen used, whether cART with protease inhibitors or integrase transfer inhibitors treatment, had no significant effect on the selected parameters' levels.

The median levels of the parameters: IRS, MSTN, PPY, GLP-1, DPP-4, FETU-A, PTX 3, SDF-1, and RANTES were lower in HIV-infected men treated with PIs compared to HIV-infected men treated with INSTIs (3.6-, 3-, 3.7-, 4.2-, 5.6-, 5.7-, 4.2-, 4.7-, 3.7-fold, respectively).

4. Discussion

In the era of effective cART therapy which has significantly extended the life expectancy of HIV-infected patients, the priority is to improve the quality of life of infected people by optimizing cART therapy. Literature data indicate an increased risk of metabolic

disorders in HIV-infected subjects who use cART [22,23]. However, the exact mechanisms of these changes are still not sufficiently understood and there are no precisely defined parameters that can describe these disorders. In the presented study, the level of nine parameters related to carbohydrate and lipid metabolism and inflammation were measured. The abovementioned parameters are some of the main factors contributing to the development of concomitant diseases, especially metabolic diseases, in HIV-infected patients. The levels of selected parameters (IRS, MSTN, PYY, GLP-1, DPP-4, FETU-A, PTX 3, SDF-1, and RANTES) were compared, both before and one year after the cART application, depending on the value of individual parameters characterizing the clinical status of patients (CD4+ T cells and CD8+ T cell count and HIV RNA viral load) and the type of treatment used (PIs or INSTIs). There are no studies similar to our investigation in the scientific literature, which has made the subject of particular interest.

The analysis of HIV-infected men before and after cART and the control group showed statistically significant differences for the following parameters: IRIS, MSTN, PYY, GLP-1, PTX 3, and RANTES, but no significant differences were found for DPP-4, FETU-A, and SDF-1.

Data in the literature indicate a protective function of IRS in the development of diseases such as: obesity, insulin resistance, type two diabetes, cardiovascular diseases, or cancer due to its anti-inflammatory activity and the effect of increasing insulin sensitivity, glycogenesis, and inhibiting gluconeogenesis [24]. IRS levels have been shown to be elevated in obese pre-diabetic subjects and reduced by about 40% in people with T2DM [25]. Sesti et al. [26] showed that in healthy non-diabetic subjects, IRS levels were positively correlated with body fat mass and insulin levels and negatively correlated with insulin-stimulated glucose disposal and insulin clearance, which as the authors concluded, may be associated with compensatory actions in the development of metabolic disorders [26]. Similar correlations between IRS concentration and the parameters of carbohydrate metabolism were noticed by Trombeta et al. [27] and Moreno-Perez et al. [28] in HIV-infected men. However, this study examined plasma IRS levels in both cART-treated and untreated patients, making it impossible to assess the effects of cART [27,28]. Srinivasa et al. [29] measured IRS levels in HIV-infected people with established metabolic syndrome as defined by the National Cholesterol Education Program (NCEP). The authors showed that the level of IRS was statistically significantly lower in the group of HIV-infected patients compared to the control group ($p = 0.003$). In our own study, plasma median levels of IRS were also statistically significantly lower in HIV-infected men before cART compared to the control group ($p = 0.02$). Despite the lack of statistical significance between the groups before and after cART, an upward trend in the IRS median level after cART could be observed, which may indicate a negative impact of HIV infection on IRS levels as well as a beneficial effect of cART. However, compared to our studies, in the studies conducted by Srinivasa et al. [29], patients used cART for over six years on average, so it is possible that the follow-up was too short for us to notice the above dependence or correlations in our own study. It is possible that in the following years of cART use, a further reduction in the level of circulating IRS is observed as a result of the development of metabolic disorders. We have found no other studies showing IRS levels in HIV-infected patients not treated with cART, which would have allowed the effect of HIV infection alone on IRS levels to be assessed.

In addition to preventing muscle hypertrophy, MSTN has also been shown to induce insulin resistance in a mechanism dependent on nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB) and SMAD family member 3 (SMAD3) [10,11]. The concentration of MSTN in the serum depends on nutritional status—reduced levels have been found in people suffering from anorexia nervosa while increased levels have been observed in obese people or people with metabolic syndrome, T2DM, or pre-diabetes [30,31]. Assyov et al. [11] revealed that MSTN serum levels in people with normoglycemia had the lowest levels of MSTN compared to people with T2DM and pre-diabetes. A positive correlation with FBG (fasting blood glucose) and the homeostatic model of assessment for insulin resistance (HOMA-IR) was demonstrated [11]. Lower serum MSTN levels were

also observed in people with metabolic syndrome, central obesity, and higher TG and lower HDL-C levels [32]. In our study, no changes in FBG were found before or after cART in HIV-infected men. These levels were also similar to the results in the control group. The MSTN median level was the lowest in HIV-infected men before cART compared to the control group, and this difference was statistically significant ($p = 0.02$). Other results were obtained by Gonzalez-Cadavid et al. [33], who showed that serum levels of MSTN in HIV-infected men were statistically significantly higher compared to the control group. However, the studied patients were treated with cART and had significant weight loss and AIDS wasting syndrome [33]. The above data may indicate changes in the MSTN level during HIV infection that is dependent on the clinical condition of the patient, and the analysis of the MSTN level alone may not be sufficient. There is also still a need for research on the clinical utility of measuring MSTN in the assessment of comorbidities in HIV-infected individuals.

It has been shown that postprandial secretion of intestinal incretin hormones, mainly GLP-1, is reduced in people with T2DM and weakened in obese people with insulin resistance and normal glucose levels [34]. Andersen et al. [35] controversially showed increased GLP-1 secretion in response to insulinotropic stimuli in HIV-infected patients with impaired glucose tolerance compared to HIV-infected patients with normal glucose tolerance. The authors pointed to the potential existence of a compensatory mechanism as a result of developing insulin resistance [35]. As a result of chronic immune activation and damage to enteroendocrine cells in the course of HIV infection and the development of HIV-associated enteropathy, incretin release is impaired in infected individuals [36]. This was confirmed by our own research in which median GLP-1 was significantly lower in HIV-infected men compared to the control group ($p = 0.004$). Our own research also showed changes in the median level of GLP-1 in the course of HIV infection and an upward trend one year after cART, which confirms earlier data on the influence of infection on the secretion of incretins.

PYY is metabolized through DPP-4 to its active form. Due to the action of PYY on the pancreatic β-cell NPYR1 receptor, it is considered a therapeutic target for antidiabetic therapies [37]. A positive correlation of the PYY level with hs-CRP has also been demonstrated, indicating the inflammatory regulation of the secretion of this neuropeptide. In addition, a correlation with the PYY level has been demonstrated for cardiovascular risk factors (diabetes, hypertension, and hypercholesterolemia) and cardiovascular events [38]. Our own study showed a higher median level of PYY in HIV-infected men before cART compared to after cART ($p = 0.04$) and a lower level of PYY than in the control group ($p = 0.02$). Differences in the median level of PYY before and after cART may be the result of chronic inflammation during infection or the abovementioned HIV-associated enteropathy. These data indicate a multidirectional effect of PYY, and our own studies confirmed the need for further research on the role of changes in GLP-1 or PYY secretion in the course and pathogenesis-associated disorders of HIV.

DPP-4 has been shown to have multidirectional immunomodulatory activity; it regulates the functions of, among others, CD4+ T lymphocytes, natural killer cells, and macrophages [16]. Levels of GLP-1 and PYY are closely related to the activity of DPP-4 peptidase which causes their enzymatic degradation. Hosono et al. [39] showed no statistically significant differences in the plasma levels of DPP-4 in HIV-infected people compared to uninfected controls. However, DPP-4 activity was significantly lower in infected individuals ($p < 0.0001$) and correlated positively with CD4+ T and CD8+ T cell count while correlating inversely with HIV RNA [39]. Songok et al. [40] showed that DPP-4 levels were not significantly different in the HIV-positive group compared to the HIV-negative controls [40]. Similarly in our own study, no statistically significant differences in median DPP-4 levels were found. However, the authors demonstrated that significantly higher plasma levels of DPP-4 occurred in people exposed but resistant to HIV infection. The authors showed that in people resistant to infection, the expression of DPP-4 in CD4+ T cells was significantly increased compared to HIV-negative and unexposed people ($p = 0.0003$) [40]. Opposite

results were shown in our own studies, where the median plasma level of DPP-4 was the highest in HIV-infected patients and decreased after the administration of the therapy. These data may indicate a lack of correlation between enzymatic activity, expression in CD4+ T cells, and the plasma level of DPP-4 in the fasting state.

It has been shown that high levels of FETU-A can be considered a risk factor for insulin resistance, T2DM, CVD by inhibition of adiponectin expression, peroxisome proliferator-activated receptor-γ (PPARγ) or activating the toll-like receptor 4 (TLR-4), and as a factor reducing the risk of coronary artery disease [41]. The level of FETU-A, as a negative acute phase protein, decreases in the course of inflammation and increases again after recovery [41]. FETU-A may therefore be useful in monitoring inflammation in HIV patients. In our study, median plasma levels of FETU-A did not differ significantly in HIV-infected men before and after cART and in the control group. However, the median level of FETU-A was the lowest in the group before cART and the highest in the control group, which confirms that the changes in the FETU-A level were a result of the development of inflammation that is characteristic of HIV infection [7]. Statistically significant lower plasma FETU-A levels in HIV-infected men with CD8+ T-cell count ≤1000 cells/μL before cART compared to after cART with CD8+ T-cell count ≤1000 cells/μL (p = 0.03) were also observed. The obtained results indicate that the level of FETU-A in HIV-infected men increased as a result of the applied treatment. However, more research is needed to determine the usefulness of FETU-A in monitoring the course of HIV infection and the effectiveness of therapy.

PTX 3 is considered to be an independent marker in the diagnosis of coronary artery disease, and its concentration is negatively correlated with the course of the disease. The concentration of PTX 3 increases in asymptomatic atherosclerosis or coronary diseases and acute coronary syndrome [18]. It has also been shown to have a prognostic value in the course of some viral and bacterial infections [42,43]. To the best of our knowledge, there are no other studies examining the PTX 3 level in HIV-positive patients. Our research yielded unexpected results. The PTX 3 median level was statistically significantly higher in the control group compared to HIV-infected men before cART (p = 0.03). These data may suggest the existence of separate mechanisms of PTX 3 release impairment due to HIV infection, indicating the need for further research in this aspect. Interesting results were also obtained by analyzing the median PTX 3 level in subgroups depending on CD8+ T-cell count. Statistically significant lower median plasma levels of PTX 3 were found in the group of HIV-infected men before cART with CD8+ T cell count ≤1000 cells/μL compared to after cART with CD8+ T cell count ≤1000 cells/μL. As a result of chronic inflammation in the course of HIV, there was excessive activation of CD8+ T cells as a result of the action of pro-inflammatory cytokines. These data may confirm previous reports, which showed that there is insufficient effectiveness of cART in normalizing CD8+ T cell count and intensified inflammatory processes, even in patients treated with cART [44].

In our own study, the median SDF-1 level did not differ significantly between HIV-infected men before cART, after cART, and in the control group. Similarly Yeregui et al. [23] did not find statistically significant differences in SDF-1 level of HIV-infected individuals starting cART with CD4+ T cell count ≤200 cells/μL compared to patients starting cART with CD4+ T cell count >200 cells/μL. However, the authors achieved statistically significant higher levels of SDF-1 in patients starting cART with CD4+ T cell count ≤200 cells/μL, whom after one year of cART achieved no more than CD4+ T cell count <250 cells/μL (immunological nonrecovery) compared to those who attained immunological recovery, indicating a prognostic value in poor immunological recovery in response to cART. However, the authors did not compare SDF-1 levels to the control group or SDF-1 levels before and after cART. In our own study, no significant differences were found between the level of SDF-1 in subgroups depending on CD4+ T cell count (≤300 cells/μL and >300 cells/μL) in HIV-infected men before and after cART. However, further studies are needed to confirm the prognostic role in poor immunological recovery for SDF-1 in patients with lower CD4+ T cell count.

Elevated plasma levels of RANTES have been shown to correlate with the progression of coronary artery disease and acute coronary syndrome. In healthy people, they were prognostic indicators of the metabolic syndrome [23]. Increased levels of RANTES have also been demonstrated in diseases with chronic immune activation [24]. RANTES controls HIV infection through its chemotactic properties for immune cells and by limiting the interaction of the virus with the CCR5 coreceptor [45,46]. In our study, the median plasma level of RANTES in HIV-infected men before cART was statistically significantly lower compared to the control group ($p = 0.02$), which may indicate a weakened immune response in the course of infection or impaired CD8+ cell function. Further research is necessary to provide information on changes in RANTES levels in subsequent years of cART use and to determine the usefulness of this parameter in monitoring the course of HIV infection or treatment effectiveness.

Limitations

Our study had some limitations. Due to the too short (one year) follow-up, no significant changes in the basic biochemical parameters such as FBG, TG, LDL-C, HDL-C, and BMI were observed. It is possible that the exposure time to the drug was too short to cause the changes due to the use of cART that are widely described in the literature [3–5]. Therefore, in the above study, changes in the median levels of parameters before cART were mainly observed compared to the control group. It was also not possible to test some parameters after nutritional stimuli (GLP-1 or PYY) or after physical activity (IRS), or measure the activity enzyme (DPP-4). Too short of a follow-up period likely prevented significant changes in the levels of the studied parameters as a result of cART from showing, depending on the therapeutic regimen used (PIs or INSTIs). However, it was noted that the median levels of all parameters tested in patients treated with INSTIs were higher than in patients treated with PIs, providing an interesting area for future research to be continued in a long-term follow-up.

5. Conclusions

Statistically significant reduced median levels of IRS, MSTN, PYY, GLP-1, PTX 3, and RANTES were demonstrated in HIV-infected men before cART compared to the control group. Statistically significant differences were also demonstrated in the median levels of FETU-A and PTX 3 between HIV-infected men before cART with CD8+ T cells count $\leq$1000 cells/μL and after cART with CD8+ T cells count $\leq$1000 cells/μL. Higher median levels of the tested parameters were also noted in men treated with INSTIs compared to PIs. The obtained results indicated significant changes in the levels of selected parameters as a result of HIV infection and changes along with the implementation of cART even in a relatively short observation period of one year, which indicated the need for further research on the diagnostic and prognostic value of the parameters. The obtained data may be useful in monitoring the course of HIV infection, treatment efficacy, or optimization of therapy. This may be helpful in the prevention or development of comorbidities associated with HIV infection and chronic cART use.

The obtained results may also indicate the need for further research in terms of the role of the studied parameters in the pathogenesis of disorders comorbid with HIV infection.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/jcm11061713/s1, Figure S1. The results of selected parameters in plasma obtained from HIV-infected men before and after cART and depending on the HIV RNA viral load, CD4+ and CD8+ T cells count, and the type of therapeutic regimen and control group are presented in (A–I).

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Załącznik 4

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Article

Differences in Expression of Selected Interleukins in HIV-Infected Subjects Undergoing Antiretroviral Therapy

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Abstract: The use of combined antiretroviral therapy (cART) inhibits the replication of the Human Immunodeficiency Virus (HIV) and thus may affect the functioning of the immune system, e.g., induce changes in the expression of certain cytokines. The aim was to examine the effect of cART on the expression of selected cytokines: interleukin -4, -7 and -15 in HIV-infected subjects. The test material was the plasma of HIV-infected men and healthy men (C, control group). The levels of interleukin were measured by immunoenzymatic method before cART and one year after treatment in relation to the C group. HIV-infected men were analyzed in subgroups depending on the HIV-RNA viral load, $CD4^+$ and $CD8^+$T-cell counts, and the type of therapeutic regimen. A significantly higher level of IL-4 was demonstrated in HIV-infected men before cART compared to those after treatment and in the control group. The use of cART resulted in a significant decrease in the level of IL-7 in HIV-infected men; however, high levels of IL-7 were associated with a low number of $CD4^+$ T cells and $CD8^+$ T cells. An increase in the level of IL-15 in HIV-infected men was noted after the use of cART. There was no difference in the expression of interleukins depending on the treatment regimen used. The study showed the effect of cART on the expression of interleukins, especially IL-4 and IL-7. Further research in this direction seems promising, confirming the role of these interleukins in the course of the disease.

Keywords: interleukin-4; interleukin-7; interleukin-15; HIV; combined antiretroviral therapy

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1. Introduction

The pathogenesis of HIV infection and the development of AIDS are a consequence of the infectious properties of the virus and the host's immune response to the virus. The balance between the effectiveness of these two components determines the outcomes of infection, from the development of AIDS to long-term survival [1,2].

The discovery of the multistage HIV replication cycle in human $CD4^+$ T cells has identified potential drug targets for arresting or slowing down the process of viral replication. The first approved drug was zidovudine, a nucleoside reverse transcriptase inhibitor (NRTI). Although NRTI monotherapy was shown to reduce viral load, delay disease progression, and prolong survival, the use of a single agent did not result in sustained viral suppression. The use of three HIV protease inhibitors (PI) radically changed the course of the HIV epidemic. The administration of a PI and two NRTI therapies resulted in the rapid reduction of HIV, improvement in immune function, regression of difficult-to-treat opportunistic infections such as Kaposi's sarcoma and progressive multifocal leukoencephalopathy, and reduced mortality. From that moment forward, combined antiretroviral therapy (cART) became the basis of the treatment of HIV infection. The strategic use of three drugs from each of the two classes enables the achievement and maintenance of an undetectable viral load, effectively bringing the disease into remission. The most common combinations are

two NRTIs and one non-nucleoside reverse transcriptase inhibitor (NNRTI) or two NRTIs and one PI [3,4].

The immune response is tightly regulated by various subsets of $CD4^+$ Th cells (lymphocytes T $CD4^+$ helper). T-cell clones show at least one of two distinct cytokine production profiles, including $CD4^+$ Th1 cells and $CD4^+$ Th2 cells. The cytokines produced by Th1 stimulate macrophages and activate $CD8^+$ cells, thereby promoting a response based on traditional cellular immunity. The cytokines produced by Th2 cells activate eosinophil function and Th cells to produce immunoglobulin G1 and immunoglobulin E antibodies, thereby promoting response to traditional humoral immunity and suppressing cellular immunity [4,5].

While the mechanisms underlying Th1 cell dominance over Th2 are not fully understood, it is clear that Th1 cells are prevalent in immunologically healthy individuals. During HIV infection and the resulting disturbance of immune regulation, the Th2 cells' response is superior to the Th1 cells' response. Thus, cellular immunity is greatly reduced and ultimately destroyed during HIV-induced immunosuppression [6].

The use of cART has an inhibitory effect on the replication of the virus and thus may affect changes in the functioning of the immune system, e.g., changes in the expression of certain cytokines (interleukins) in HIV-infected subjects [7].

Interleukin 4 (IL-4) is a pleiotropic cytokine produced by Th2 cells, mast cells and basophils. Its activity is broad, affecting many populations of cells of the immune system and showing antagonistic effects (in most cases) to interferon-γ. It also affects T cells, directing their development towards Th2 cells. IL-4 may inhibit cellular and humoral immunity [8]. IL-4 regulates the differentiation of precursor T helper cells into those of the Th2 subset that mediate humoral immunity and modulate antibody production. With respect to HIV-1 infection, IL-4 was reported to differentially regulate two major HIV-1 coreceptors, CXCR4 for SI (syncytium-inducing) variants and CCR5 for NSI (no syncytium-inducing) viruses. IL-4 down-regulates CCR5 expression and thus inhibits replication of HIV-1 NSI isolates in human T-cells and macrophages.

On the other hand, IL-4 upregulates the expression of CXCR4. In addition, IL-4 stimulates the expression of HIV-1 through the activation of viral transcription. The combination of these effects of IL-4 on HIV-1 replication may be involved in the phenotypic switch from NSI to SI as well as disease progression in HIV-1 infection. Thus, IL-4 could be an important factor in viral evolution and AIDS pathogenesis [9].

Interleukin 7 (IL-7) is a hematopoietic growth factor secreted by stromal cells in the bone marrow and thymus. It is also produced by keratinocytes, dendritic cells, hepatocytes, neurons, and epithelial cells but is not produced by lymphocytes. IL-7 is needed for the development and maintenance of T cells and the restoration of mature T-cell homeostasis. Under normal conditions, its resource is relatively limited [10,11]. IL-7 plays an important role in immune system function, especially in the development of T cells, including $CD4^+$ cells. IL-7 may improve HIV-specific immune responses by increasing the number of $CD4^+$ cells and boosting immune response. Recently, IL-7 therapy has been demonstrated to favorably impact T-cell functions by promoting their proliferation and expansion in subjects receiving cART. However, the effects of IL-7 administration on the mechanisms of HIV persistence and the size of the latent reservoir are still unclear [12,13].

Interleukin 15 (IL-15) is a pleiotropic and multifunctional cytokine that has a diverse range of biological effects in the body produced by monocytes, macrophages, and dendritic cells. It plays a key role in the host's defense against viral and non-viral intracellular pathogens. IL-15 induces the proliferation of natural killer cells and their antibody-dependent cytotoxicity and stimulates their production of cytokines and chemokines. It activates neutrophils, acts on activated T cells, and is involved in the maintenance of memory T cells. It is involved in the differentiation of dendritic cells and induces angiogenesis and muscle differentiation [14,15]. IL-15 plays an essential role in the development and maturation of NK. This interleukin enhances the cytolytic potential of NK cells. NK cells play an important role in host defense by directly killing virus-infected cells and activating

macrophages through INF-γ synthesis. Thus, the production of an optimum level of IL-15 may represent a prerequisite for normal maintenance of NK activity, the antimicrobial potential of macrophages, and the induction of pathogen-specific immune responses. Virus-induced IL-15 gene activation is linked to the massive proliferation of $CD8^+$ T cells and the maintenance of virus-specific memory T cells (15). IL-15 has also been shown to substitute for $CD4^+$ T-cell help in the induction and maintenance of antiviral CTL (cytotoxic T lymphocytes) responses (16). This IL-15 function may be crucial in HIV infection, particularly in later stages, when $CD4^+$ T-cell numbers decline precipitously [14,16].

In the authors' research, an attempt was made to assess the expression of three selected interleukins, IL-4, -7 and -15, in HIV-infected men and to examine the effect of one year of antiretroviral therapy on their change. The analyses took into account the influence of parameters characterizing the state of the immune system, such as HIV-RNA viral load, $CD4^+$ and $CD8^+$ T-cell counts, as well as the type of antiretroviral treatment regimen used on the blood expression of the IL-4, IL-7 and IL-15. The interdependencies between interleukins were also analyzed to investigate the contribution of these cytokines to HIV infection.

2. Individuals and Methods

2.1. Participants' Selection and Collection of Data

The research material was plasma obtained from whole blood samples taken from 30 HIV-infected subjects (men) treated at the Center for Prevention and Treatment of Infectious Diseases and Addictions and the Department of Infectious Diseases, Liver and Acquired Immunity Defects of the Wroclaw Medical University.

In the group of HIV-infected men, inclusion criteria were the patient's consent for tests and confirmation of the presence of HIV infection. Exclusion criteria were diseases such as diabetes, cancer, hypertension, hepatitis B or C virus infection, especially urinary tract diseases, and concomitant use of drugs other than cART. The control group consisted of 28 HIV-negative healthy volunteers (men).

HIV-infected men were treated with two therapeutic regimens, which included two NRTIs (emtricitabine and tenofovir alafenamide) in combination with PIs (ritonavir-boosted lopinavir or cobicistat-boosted darunavir) or INSTIs (dolutegravir).

Patients were also analyzed in subgroups depending on HIV-RNA viral load (below and above 100,000 RNA copies/mL), $CD4^+$ T-cell count (below and above 300 cells/μL), $CD8^+$ T-cell count (below and above 1000 cells/μL), and the type of therapeutic regimen (INSTIs or PIs) both before and after one year of anti-HIV therapy.

Data on HIV-RNA viral load levels, $CD4^+$ and $CD8^+$ T-cell counts were obtained from medical records. HIV-RNA isolation was performed using a system viral nucleic acid kit (Roche Diagnostics, Mannheim, Germany). HIV-1 viral load was measured by real-time PCR assay (COBAS TaqMan HIV-1 test v. 2.0, Roche Diagnostics, Basel, Switzerland). The detection limit was 40 copies/mL. The $CD4^+$ and $CD8^+$ T-cell counts were tested by flow cytometry using the FACSCount Becton Dickinson system (BD Biosciences, San Jose, CA, USA).

2.2. Blood Collection and Enzyme-Linked Immunosorbent Assay (ELISA)

Blood was drawn from HIV-infected men twice—the first time prior to the initiation of antiretroviral therapy and again one year after the initiation of treatment.

Fasting blood samples were collected from HIV-infected men and from healthy (HIV-uninfected) men in anticoagulant tubes (5 mL blood, containing 1.6 mg/mL EDTA, Sarstedt, Poland). The samples were centrifuged at 1500× *g* for 10 min to separate plasma. Plasma was aliquoted and frozen at −80 °C until assayed.

The concentration of the studied interleukins was measured using the enzyme-linked immunosorbent assay method (ELISA). The following enzyme immunoassays were used in the studies: Human IL-4 Immunoassay (R&D Systems, Inc., Minneapolis, MN, USA); Human IL-7 ELISA Kit (Thermo Fisher Scientific Inc., Waltham, MA, USA); Human IL-15 Immunoassay (R&D Systems, Inc., Minneapolis, MN, USA), according to the manufac-

turer's instructions. Absorbance was read at 450 nm with a microplate reader (STAT FAX 2100, Awareness Technology, Inc., Palm City, FL, USA).

All participants were informed about the purpose of the study and gave their written consent. The study was approved by the Ethics Committee of the Medical University in Wroclaw (KB-597/2019) and with the Helsinki Declaration.

2.3. Statistical Analysis

The statistical analysis of the obtained results was performed using Statistica 13.3 PL by StatSoft. The Kolmogorov–Smirnov and Lilliefors test was used to check the data distribution. Data distributions of tested interleukins (IL-4, IL-7, IL-15) were non-parametric; therefore, Mann–Whitney *U*-tests were selected for the analysis. Correlations between parameters were assessed using the Spearman test. The comparison of results within subgroups was performed using the Wilcoxon test. In all analyzes, $p < 0.05$ was considered a significant value.

3. Results

3.1. Study Population

The number of HIV-infected men ($n = 30$) was similar to that of men not infected with HIV ($n = 28$). The mean age was 33 years (25–54) among HIV—infected men and 36 (22–58) in the control group (C, HIV(-) men), and did not differ statistically ($p > 0.05$).

3.2. Assessment of Immunological Parameters in HIV-Infected Men

The assessment parameters such as HIV viral load, $CD4^+$ and $CD8^+$ T-cell counts, characterizing HIV- infected men before cART (A) treatment and after one year of cART (B) are presented in Table 1.

Table 1. Analysis of immunological parameters characterizing HIV-infected men before (A) and after cART (B) depending on the HIV viral load, $CD4^+$ and $CD8^+$ T-cell counts.

Groups Characteristic	HIV-Infected Men before cART (A)	HIV-Infected Men after cART (B)	* *p*
	Mean (Min–Max)	Mean (Min–Max)	
HIV-RNA (copies/mL)	222 157.00 (39.00–2,030,000.00)	14.50 (0.00–85,000.00)	<0.001
$CD4^+$ T cells (cells/μL)	351.00 (20.00–960.00)	600.00 (122.00–1407.00)	<0.001
$CD8^+$ T cells (cells/μL)	1 094.00 (386.00–3388.00)	918.00 (257.00–2000.00)	0.004
$CD4^+/CD8^+$ ratio	0.34 (0.03–0.89)	0.75 (0.22–1.74)	<0.001

Abbreviation: N—number of men; NS—statistically insignificant value; * $p < 0.05$ statistical significance of the Wilcoxon test.

The number of HIV-RNA copies and the amount of $CD4^+$ and $CD8^+$ T cells were statistically significantly different between the groups of patients before cART (A) compared to the values of these parameters obtained in the group of patients after therapy (B) (Table 1).

In HIV-infected men before cART, the mean HIV-RNA viral load was more than 15,000-fold higher than the values obtained after one year of cART.

The mean amount of $CD4^+$ T cells after cART increased 1.7-fold relative to $CD4^+$ T-cell count prior to administration of antiretroviral therapy.

Treatment with cART decreased (2.2-fold) the mean $CD8^+$ T-cell count compared to untreated HIV-infected men.

The $CD4^+/CD8^+$ ratio in HIV-infected men after cART was 2.2-fold higher compared to the $CD4^+/CD8^+$ ratio in HIV-infected men before cART.

3.3. Interleukins (IL-4, -7, -15) in Plasma of HIV-Infected Men before cART Therapy, after cART Therapy and in the Control Group

The obtained values of interleukins (IL-4, IL-7, and IL-15) in HIV-infected men before cART, after cART, and in the control group are presented in Table 2.

Table 2. Results for IL-4, -7, -15 in HIV-infected men before cART(A), after cART(B) and in the control group (C) with statistical analysis.

HIV-Infected Men before cART (A) **Mean ± SD** **Me** **(IQR)**	**HIV-Infected Men after cART (B)** **Mean ± SD** **Me** **(IQR)**	**HIV-Uninfected Men (C)** **Mean ± SD** **Me** **(IQR)**	***p***
	IL-4 (pg/mL)		
5.39 ± 5.02 2.90 (2.00–7.00)	3.39 ± 2.68 3.20 (1.40–4.70)	2.68 ± 1.02 2.80 (2.00–3.45)	A:C = 0.03 * B:C = NS A:B = 0.04 **
	IL-7 (pg/mL)		
575.00 ± 700.20 208.35 (75.00–887.40)	388.31 ± 464.27 162.40 (61.40–720.60)	383.24 ± 270.42 348.00 (133.00–508.35)	A:C = NS B:C = NS A:B = 0.02 **
	IL-15 (pg/mL)		
2.56 ± 1.78 1.95 (1.20–4.20)	3.69 ± 3.58 2.55 (1.70–3.40)	2.25 ± 1.34 2.05 (1.30–3.15)	A:C = NS B:C = NS A:B = NS

Abbreviation: SD—standard deviation; IQR—(25–75%); IL—interleukin; NS—not statistically significant; The *p*-values were calculated using the Mann–Whitney *U*-test * and Wilcoxon test **; $p < 0.05$—statistically significant.

The mean plasma IL-4 concentration value of HIV-infected men before (A) cART was twice as high as in healthy uninfected control men (C). There was a statistically significant difference between IL-4 concentrations in these patients relative to the control ($p = 0.03$). The mean concentration of IL-4 in the group of HIV-infected men after cART (B) therapy was 1.3-fold higher than the value obtained in the control group, but the difference was not statistically significant. A statistically significant difference was found between the values of IL-4 concentrations in HIV-infected men before cART (A) compared to HIV-infected men after antiretroviral treatment (B), ($p = 0.04$).

The mean value of IL-7 plasma concentration in HIV-infected men before (A) cART was almost 1.5-fold higher compared to cART (B). A statistically significant difference was demonstrated between the values of IL-7 concentrations in the HIV-infected before cART (A) compared to men after cART (B). A statistically significant difference was demonstrated ($p = 0.02$). The mean concentration of IL-7 in the group of HIV-infected men before cART (A) was also 1.5-fold higher compared to the value obtained in the control group, but the difference was not statistically significant. Mean values of IL-7 concentration among men after cART (B) and in the control group were at a similar level, and no statistically significant difference was found.

The mean value of IL-15 concentration in the plasma of HIV-infected men before treatment (A) was similar to the mean value of IL-15 concentration obtained in the control group. There was no statistically significant difference between these groups. The mean value of IL-15 concentration in men after treatment (B) in comparison to the mean value of IL-15 concentration in the control group was 1.6-fold higher. There was no statistically significant difference between IL-15 levels in men after cART (B) compared to men in the control group. The pre-treatment IL-15 concentration value in HIV-infected men (A), compared to HIV-infected men after treatment (B), was 1.4 times lower, but no statistically significant difference was found.

3.4. Results of IL-4, -7 and -15 before and after cART in HIV-Infected Men by HIV-RNA Viral Load

The analysis of IL-4, IL-7 and IL-15 was performed in subgroups of HIV-infected men both before cART (A) and after treatment (B), depending on the amount of HIV-RNA copies (below and above 100,000 copies/mL). The results are presented in Table 3.

Table 3. Results of IL-4, -7 and -15 before (group A) and after (group B) cART in HIV-infected men by HIV-RNA viral load (below and above 100,000 copies/mL) with statistical analysis.

HIV-Infected Men before cART (A)	**HIV-RNA ≤ 100,000 (Copies/mL) (N = 16)**	**HIV-RNA > 100,000 (Copies/mL) (N = 14)**	***p***
	Me (IQR)	**Me (IQR)**	
IL-4 (pg/mL)	3.00 (1.85–5.10)	4.90 (2.60–7.00)	0.001 *
IL-7 (pg/mL)	224.05 (82.25–1028.20)	193.00 (74.40–1215.00)	NS
IL-15 (pg/mL)	2.15 (1.15–4.75)	1.40 (1.20–2.80)	NS
HIV-Infected Men after cART (B)	**HIV-RNA ≤ 100,000 [Copies/mL] (N = 30)**	**HIV-RNA > 100,000 (Copies/mL) (N = 0)**	***p***
	Me (IQR)	**Me (IQR)**	
IL-4 (pg/mL)	2.90 (2.00–7.00)	there were no HIV-infected men with HIV-RNA > 100,000 copies/mL in this subgroup	-
IL-7 (pg/mL)	162.40 (61.40–720.60)		
IL-15 (pg/mL)	2.55 (1.70–3.40)		

Abbreviation: Me—median; IQR—Interquartile range; N- number of participants; NS—not statistically significant; the *p*-values were calculated using the Wilcoxon test *; $p < 0.05$—statistically significant.

Analyzing the values of IL-4 levels in HIV-infected men before cART (A) depending on the number of HIV-RNA copies ≤ 100,000/mL and >100,000/mL, a statistically significant difference was demonstrated ($p = 0.001$). The median IL-4 was 1.8-fold higher in the subgroup of HIV -infected men with a HIV-RNA > 100,000/mL copy count.

In HIV-infected men with HIV-RNA ≤ 100,000 copies/mL] (A) the median levels of IL-7 and IL-15 were 1.2-fold and 1.6-fold higher compared to the median levels of these interleukins in HIV-infected men with HIV-RNA > 100,000/mL, respectively, but no statistically significant differences were found.

There was no difference in IL-4, -7 and -15 levels in HIV-infected men before cART (A) with HIV-RNA ≤ 100,000 [copies/mL] compared to these interleukins in HIV-infected men with HIV-RNA ≤ 100,000 copies/mL after cART (B).

HIV-infected men with a viral load of HIV-RNA > 100,000 [copies/mL] were not found to be present after cART (B).

Additionally, interleukin levels were compared in HIV-infected men after cART (B) with no HIV detected (N = 19) to HIV-infected men with low HIV levels detected (N = 11; a viral load of HIV-RNA mean 40 copies/mL).

The medians (IQR) for IL-4, -7, and -15 in plasma of HIV-infected men after cART (B) who had a mean HIV-RNA value of 40 copies/mL were 1.80 (0.50–3.70), 260.20 (61.40–720.60) and 1.90 (1.40–3.40), respectively. The medians (IQR) for IL-4, -7 and -15 in the plasma of HIV-infected men after cART (B) with no HIV-RNA detected were 3.50 (1.80–5.90), 128.10 (49.20–770.00) and 2.7 (1.80–3.70), respectively. The differences between interleukin levels in HIV-infected men with a low viral load of HIV-RNA and interleukin levels in men with undetectable HIV were not statistically significant.

3.5. Results of IL-4, -7 and -15 before and after cART in HIV-Infected Men by $CD4^+$ T Cell Count

The analysis of IL-4, IL-7 and IL-15 was performed in subgroups of HIV-infected men both before cART (A) and after treatment (B), depending on the $CD4^+$ T-cell count (below and above 300 cells/μL). The results are presented in Table 4.

Table 4. Results of IL-4, -7 and -15 before (group A) and after (group B) cART in HIV-infected men by $CD4^+$ T-cell count (below and above 300 cells/μL) with statistical analysis.

HIV-Infected Men before cART (A)	**$CD4^+$ T Cells ≤ 300 (Cells/μL) (N = 11)**	**$CD4^+$ T Cells > 300 (Cells/μL) (N = 19)**	***p***
	Me (IQR)	**Me (IQR)**	
IL-4 (pg/mL)	2.90 (1.70–7.00)	2.90 (2.60–9.10)	NS
IL-7 (pg/mL)	482.50 (89.50–1627.00)	148.30 (74.40–789.50)	0.026 *
IL-15 (pg/mL)	2.00 (1.20–4.20)	1.90 (1.10–4.30)	NS
HIV-Infected Men after cART (B)	**$CD4^+$ T Cells ≤ 300 (Cells/μL) (N = 4)**	**$CD4^+$ T Cells > 300 (Cells/μL) (N = 26)**	***p***
	Me (IQR)	**Me (IQR)**	
IL-4 (pg/mL)	4.90 (4.10–5.70)	2.60 (1.10–4.10)	NS
IL-7 (pg/mL)	118.05 (26.80–896.05)	162.40 (81.50–720.60)	NS
IL-15 (pg/mL)	3.10 (2.45–9.60)	2.25 (1.60–3.40)	NS

Abbreviation: Me—median; IQR—Interquartile range; N—number of participants; NS—not statistically significant; the *p*-values were calculated using the Wilcoxon test *; $p < 0.05$—statistically significant.

In HIV-infected men with $CD4^+$ T cell ≤ 300 count [cells/μL] before cART (A), the median levels of IL-4 and IL-15 were similar to the median levels of these interleukins in HIV-infected men with a $CD4^+$ T cell > 300 count [cells/μL] (A) and were not statistically significantly different.

The median level of IL-7 in HIV-infected men with a $CD4^+$ T cell ≤ 300 count [cells/μL] (A) was 2.3-fold higher compared to HIV -infected men with a $CD4^+$ T cell > 300 count [cells/μL] (A), and the difference was shown to be statistically significant ($p = 0.026$).

In HIV-infected men with a $CD4^+$ T cell ≤ 300 count [cells/μL] after cART (B), the median levels of IL-4 and IL-15 were 1.9-fold and 1.4-fold higher. IL-7 was 0.7-fold lower compared to the median levels of these interleukins in HIV-infected men with a $CD4^+$ T cell > 300 count [cells/μL] (B), but no statistically significant differences were found.

There was no difference in IL-4, IL-7 and IL-15 levels in HIV-infected men before cART with $CD4^+$ T cell ≤ 300 count [cells/μL] (A) compared to these interleukins in HIV-infected men with $CD4^+$ T cell ≤ 300 count [cells/μL] after cART (B). Similarly, no difference was demonstrated in IL-4, -7 and -15 levels in HIV-infected men before cART with $CD4^+$ T cell > 300 count [cells/μL] (A) compared to these interleukins in HIV- infected men with $CD4^+$ T cell > 300 count [cells/μL] after cART (B).

3.6. Results of IL-4, -7 and -15 before and after cART in HIV-Infected Men by $CD8^+$ T Cell Count

The analysis of IL-4, IL-7 and IL-15 was performed in subgroups of HIV-infected men both before cART (A), and after treatment (B), depending on the $CD8^+$ T-cell count (below and above 1000 cells/μL). The results are presented in Table 5.

In HIV-infected men with a lymphocyte T $CD8^+$ count ≤ 1000 [cells/μL] (A), the median levels of IL-4 and IL-15 were 1.1-fold and 2.3-fold higher, respectively, than the median levels of these interleukins in men with a $CD8^+$ T-cell count > 1000 [cells/μL] (A) and were not statistically significantly different.

The median level of IL-7 in HIV-infected men with a $CD8^+$ T-cell count ≤ 1000 [cells/μL] (A) was 6-fold higher compared to HIV-infected men with a $CD8^+$T-cell count > 1000 [cells/μL] (A), and the difference was shown to be statistically significant ($p = 0.027$).

In HIV-infected men with a $CD8^+$ T-cell count ≤ 1000 [cells/μL] after cART (B), the median levels of IL-4 and IL-15 were higher by 1.8-fold and 1.4-fold, but IL-7 was 1.5-fold lower compared to the median levels of these interleukins in HIV-infected men with $CD8^+$ T-cell count > 1000 [cells/μL] (B) respectively, but no statistically significant differences were found.

Table 5. Results of IL-4, -7 and -15 before (group A) and after (group B) cART in HIV-infected men by CD8+ T-cell count (below and above 1000 cells/μL) with statistical analysis.

HIV-Infected Men before cART (A)	**$CD8^+$ T Cells $\leq$ 1000 (Cells/μL) (N = 13)**	**$CD8^+$ T Cells > 1000 (Cells/μL) (N = 17)**	*p*
	Me (IQR)	**Me (IQR)**	
IL-4 (pg/mL)	3.10 (2.00–7.00)	2.90 (2.60–6.50)	NS
IL-7 (pg/mL)	789.50 (97.10–1554.00)	133.90 (74.40–269.10)	0.027 *
IL-15 (pg/mL)	3.20 (1.20–4.40)	1.40 (1.20–2.30)	NS
HIV-Infected Men after cART (B)	**$CD8^+$ T Cells $\leq$ 1000 (Cells/μL) (N = 9)**	**$CD8^+$ T Cells > 1000 (Cells/μL) (N = 21)**	*p*
	Me (IQR)	**Me (IQR)**	
IL-4 (pg/mL)	1.80 (0.50–4.10)	3.40 (1.80–4.80)	NS
IL-7 (pg/mL)	199.10 (51.20–552.80)	132.20 (87.20–720.60)	NS
IL-15 (pg/mL)	1.90 (1.50–3.30)	2.70 (1.80–3.40)	NS

Abbreviation: Me—median; IQR—Interquartile range; N—number of participants; NS—not statistically significant; the *p*-values were calculated using the Wilcoxon test *; $p < 0.05$—statistically significant.

There was no difference in IL-4, IL-7 and IL-15 levels in HIV-infected men before cART with a $CD8^+$ T-cell count $\leq$ 1000 [cells/μL] (A) compared to these interleukins in men with a $CD8^+$ T-cell count $\leq$ 1000 [cells/μL] after cART (B). Similarly, no difference was demonstrated in IL-4, IL-7 and IL-15 levels in HIV-infected men before cART with $CD8^+$ T-cell count > 1000 [cells/μL] (A) compared to these interleukins in men with a $CD8^+$ T-cell count > 1000 [cells/μL] after cART (B).

3.7. Results of IL-4, -7 and -15 before and after cART in HIV-Infected Men in Terms of the $CD4^+/CD8^+$ Ratio

In group A, before cART, the $CD4^+/CD8^+$ ratio $\leq$ 1.00 had all HIV-infected men. In group B, after cART, the $CD4^+/CD8^+$ ratio > 1.00 had 6 (20%) HIV- infected men. The analysis was performed only in group B after cART.

Median (IQR) for IL-4, -7 and -15 in plasma of HIV-infected men after cART (B) in the subgroup with $CD4^+/CD8^+$ ratio $\leq$ 1.00 were 3.45 (1.7–4.9) pg/mL, 163.60 (66.35–751.35) pg/mL and 2.65 (1.85–3.75) pg/mL, respectively.

Median (IQR) for IL-4, -7 and -15 in plasma of HIV-infected men after cART (B) in the subgroup with $CD4^+/CD8^+$ ratio > 1.00 were 1.60 (1.10–1.90) pg/mL, 162.40 (61.40–339.7) pg/mL and 1.75 (1.60–2.90) pg/mL, respectively.

The level of IL-4 in HIV-infected men (B) having a $CD4^+/CD8^+$ ratio $\leq$ 1, was higher at 2.2-fold compared to HIV-infected men with $CD4^+/CD8^+ > 1$ and was statistically significant ($p = 0.04$).

The level of IL-7 in HIV-infected men (B) having a $CD4^+/CD8^+$ ratio $\leq$ 1 was similar to that of HIV-infected men with $CD4^+/CD8^+ > 1$.

The level of IL-15 in HIV-infected men (B) having a $CD4^+/CD8^+$ ratio $\leq$ 1, was higher at 1.5-fold compared to HIV-infected men with $CD4^+/CD8^+ > 1$, but not was statistically significant.

3.8. IL-4, -7 and -15 Results in HIV-Infected Men Subjected to cART with Protease Inhibitors (PIs) and Integrase Transfer Inhibitors (INSTIs) Treatment

Medians and interquartile ranges for IL-4, IL-7 and IL-15 in the plasma of HIV- infected men after cART subgroups with Protease inhibitors (PIs) and Integrase transfer inhibitors (INSTIs) treatment are presented in Table 6.

Table 6. Results of Il-4, -7 and -15 in HIV-infected men after cART in the subgroup with Protease inhibitors (PIs) treatment and the subgroup with Integrase transfer inhibitors (INSTIs) treatment with statistical analysis.

	HIV-Infected Men with cART		
ILs	**INSTIs (N = 16) Me (IQR)**	**PIs (N = 14) Me (IQR)**	***p***
IL-4 (pg/mL)	3.65 (1.00–5.35)	2.85 (1.80–3.50)	NS
IL-7 (pg/mL)	196.20 (66.35–636.70)	160.35 (61.40–770.00)	NS
IL-15 (pg/mL)	2.25 (1.70–5.05)	2.75 (1.70–3.30)	NS

Abbreviation: INSTIs—Integrase transfer inhibitors; PIs—Protease inhibitors; Me—median; IQR—Interquartile range.

The antiretroviral regimen used, whether cART with Protease inhibitors or Integrase transfer inhibitors treatment, had no significant effect on IL-4, IL-7 and IL-15 levels.

3.9. Correlations between Interleukins

The analysis of mutual linear relationships between the studied interleukins showed the presence of three significant correlations. These correlations are presented in Figure 1A,B.

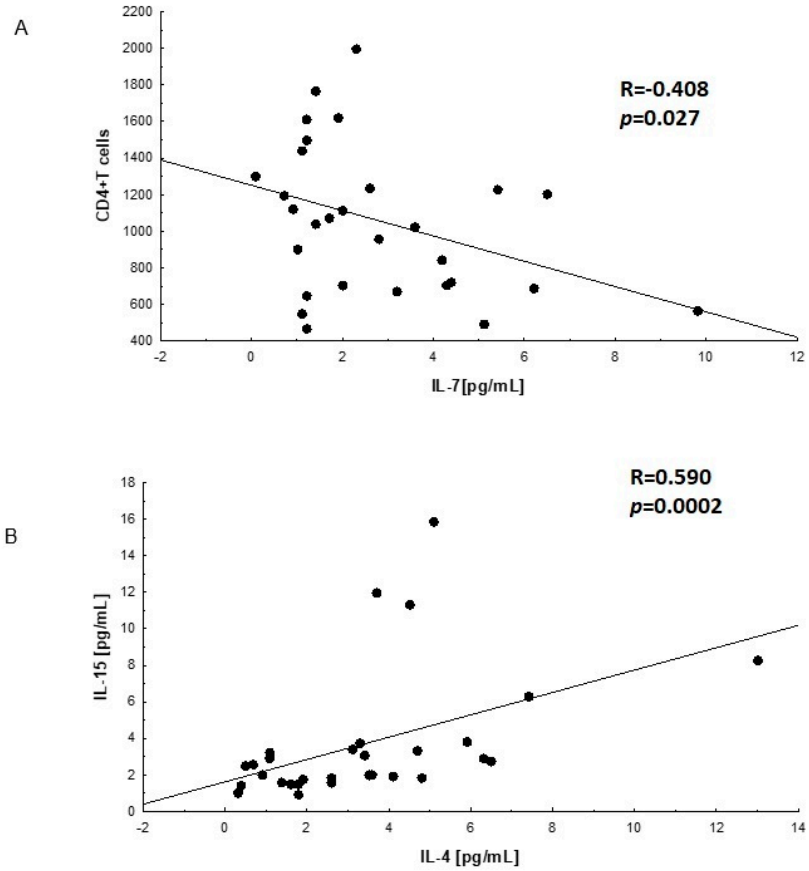

Figure 1. Correlations between interleukins. (**A**)—linear relationship between IL-7 concentration and the number of lymphocytes T CD4$^+$ in plasma of HIV-infected men before cART; (**B**)—linear relationship between IL-4 and IL-15 in plasma of HIV-infected men after cART; the correlations were evaluated with Spearman's non-parametric test.

A negative correlation was found between the number of CD4$^+$ T cells in the group of HIV-infected men before cART (A) and the concentration of IL-7 in the plasma of these HIV-infected men. The Spearman R coefficient was R = −0.408 (p = 0.027).

In the subgroup of HIV-infected men after cART (B) therapy, a mutually positive relationship between IL-4 and IL-15 was demonstrated. The value of the Spearman R coefficient = 0.590 (p = 0.0002).

Visualizations of the IL-4, -7 and -15 results obtained are shown in Figure S1 (Supplementary Materials).

4. Discussion

In the subgroup of HIV-infected men after cART (B) therapy, a mutually positive relationship between IL-4 and IL-15 was demonstrated. The value of the Spearman R coefficient = 0.590 (p = 0.0002).

Visualizations of the IL-4, -7 and -15 results obtained are shown in Figure S1 (Supplementary Materials).

4. Discussion

In the authors' research, an attempt was made to evaluate the expression of three selected interleukins important to the course of the disease—IL-4, -7 and -15—in HIV-infected men to investigate the effect of the applied therapy in a one-year follow-up.

IL-4 synthesis is stimulated during the development of AIDS as a result of HIV-induced domination of Th2 cell stimulation [17]. This thesis is reflected in the results obtained in our study, where a significantly higher level of IL-4 was observed in untreated HIV-infected patients compared to patients treated with cART (p = 0.04) and in the control group (p = 0.03). The concentration of IL-4 in HIV-infected men after one year of cART was comparable to the concentration of IL-4 in uninfected men.

Osuji et al. [7] investigated the levels of pro-inflammatory and anti-inflammatory cytokines in serum in people infected with HIV. The study results showed significant differences in the levels of anti-inflammatory cytokines IL-4, IL-10 and TGF-β before starting cART therapy, 6 months after cART and 1 year after cART treatment. Anti-inflammatory cytokine levels, including IL-4, were increased in pre-treatment HIV-infected patients compared with 6 and 12 months of treatment and compared to the control group. IL-4 and IL-10 showed no significant difference after 1 year of cART treatment compared to the control group.

IL-4 promotes a number of immune functions that influence macrophage differentiation, the differentiation of $CD4^+$ T cells into Th2 cells, and the inhibition of the secretion of various pro-inflammatory cytokines [18]. The results suggest a link between an increase in IL-4 production during retrovirus-induced immunosuppression and the suppression of cellular immune responses [19].

Our own studies showed a significantly higher level of IL-7 in HIV-infected men before cART compared to the level of this interleukin in patients after therapy and in the control group. It was noted that a higher level of IL-7 was associated with a lower count of $CD4^+$ and $CD8^+$ T cells in HIV-infected patients prior to cART, which was further confirmed by a negative correlation between IL-7 and $CD4^+$ T cells.

IL-7 is implicated in thymopoiesis and regulates peripheral naive T-cell survival by modulating the expression of the anti-apoptotic molecule Bcl-2 and sustains peripheral T-cell expansion in response to antigenic stimulation. Infection with HIV leads to T lymphopenia and immune dysfunction. Increased IL-7 plasma levels are observed in HIV-infected patients. The existence of an inverse correlation between IL-7 plasma levels and the $CD4^+$ T-cell count suggests that a direct feedback mechanism is working to restore peripheral T-cell counts in lymphopenic patients. Here, IL-7 plasma levels are a good predictive marker of $CD4^+$ T-cell restoration during therapy. The cART considerably slows disease progression, decreases the viral load, and significantly increases peripheral $CD4^+$ T-cell counts [20].

Progressive HIV infection is associated with a complex deregulation of the IL-7/IL-7R receptor pathways, including increased plasma levels of IL-7 with a decrease in the percentage of $CD4^+$ and $CD8^+$ T cells expressed by the CD127 receptor [21].

Hodge et al. [22] explored how immune reconstitution through antiretroviral therapy in HIV-infected patients affects IL-7 serum levels, expression of the IL-7 receptor (CD127), and T-cell cycling. Immunophenotypic analysis of T cells from 29 HIV-uninfected controls and 43 untreated HIV-infected patients (30 of whom were followed longitudinally for $\leq$24 months on cART) was performed. Restoration of both $CD4^+$ and $CD8^+$ T cells was driven by increases in $CD127^+$ naive and central memory T cells. $CD4^+$ T-cell subsets were not fully restored after 2 years of cART, whereas serum IL-7 levels normalized by

1 year of ART. Mathematical modeling indicated that changes in serum IL-7 levels could be accounted for by changes in the receptor concentration. These data suggest that T-cell restoration after cART in HIV infection is driven predominantly by $CD127^+$ cells and that decreases in serum IL-7 can be largely explained by improved CD127-mediated clearance.

Benito et al. [23] found that pre-cART IL-7 levels were high and interleukin-7 receptor-α (IL7Rα) expression was reduced in HIV-infected patients. This downregulation mechanism of the receptor IL7Rα is mainly related to the activation of T cells. Observations suggest that the establishment of an IL-7/IL-7R balance during HIV cART treatment can remedy the viral damage.

Ikomey et al. [24] observed higher levels of pro-inflammatory IL-2 and IL-7 in a failed cART regimen compared to lower levels of pro-inflammatory cytokines IL-2 and IL-7 in a successful cART regimen. This also suggests a possible correlation between low viral load and low pro-inflammatory cytokines. Thus, the effective decrease in viral load may have an association with decreased levels of pro-inflammatory cytokines.

Administration of IL-7 to HIV-infected patients treated with antiretroviral therapy causes a selective increase in the fraction of virgin T cells and $CD4^+$ T cells, suggesting a beneficial effect on the overall function of $CD4^+$ T cells [25].

In our own studies, although no statistical significance was found between IL-15 levels in HIV-infected men before and after cART and in relation to the control group, some changes in its expression were noticed. The mean concentration of IL-15 in HIV-infected patients not treated with cART was lower compared to its concentration in HIV-infected men after one year of treatment and was similar to that obtained in healthy men.

Such a tendency was noticed by other authors studying this interleukin in the course of HIV infection Ahmad et al. [26] showed that the level of IL-15 is significantly reduced in the plasma of HIV-infected patients compared to the control group.

Studies have shown that IL-15 is produced during acute HIV and SIV (Simian Immunodeficiency Virus) infection and can affect viremia and viral threshold. Although the role of intrinsic IL-15 during chronic infection is less defined, scientists have demonstrated in vivo that administration of IL-15 does not increase viral replication in SIV-infected animals [27].

D'Ettorre et al. [28], investigating immunological and virological interactions between IL-15 and HIV in untreated and cART-treated patients, found that the production of IL-15 by peripheral blood mononuclear cells was significantly reduced in untreated patients and patients after failed antiretroviral therapy. On the other hand, in patients who responded to cART, the production of IL-15 was comparable to that of healthy subjects. In addition, they showed that IL-15 was able to stimulate HIV-positive monocytes to produce chemokines such as IL-8 and MCP-1 (Monocyte Chemoattractant Protein-1), which specifically attract neutrophils and monocytes to the site of inflammation. This possibly improves the immune response to pathogens during HIV infection.

Albino et al. [29] unveiled that IL-15 (also IL-6 and -12) levels corresponded with immune activation after prolonged cART, thus implying that prolonged cART results in immune activation.

Structured treatment interruption (STI) may help to alleviate the problems associated with long-term cART in HIV-infected patients. A study by Amicosante et al. analyzed the role that baseline levels of cytokines in plasma play as markers of a favorable outcome of STI. Two groups of patients were defined: STI responders and STI nonresponders. STI responders showed a higher baseline concentration of IL-15 in plasma than did STI nonresponders and showed lower levels of tumor necrosis factor (TNF)-alpha during STI. No differences were observed in levels of IL-2, IL-7, or interferon-alpha in plasma. Authors showed that levels of TNF-alpha in plasma correlate with HIV viremia and monitoring baseline levels of IL-15 in plasma allows for the identification of a favorable outcome of STI [30].

By examining the interdependencies between selected interleukins, a positive correlation between IL-4 and IL-15 was demonstrated. With an increase in IL-15, an increase in IL-4 is noticeable, which may indicate that IL-15 can stimulate the production of anti-

inflammatory IL-4. The obtained results may confirm the data on the role of IL-4 and IL-15 in mediating the development of specific $CD8^+$ T cell memory during viral infections, which was confirmed by in vivo studies [31]. However, the exact role of this interaction in HIV infection is unknown. In our study, we showed the need for further research in this area.

The authors' studies showed that the level of $CD8^+$ T cells was higher in HIV-infected men before treatment than in patients after cART ($p = 0.004$). HIV-specific $CD8^+$ T cells are prone to apoptosis, affecting their ability to control HIV infection. Since immune responses mediated by $CD8^+$ T cells play a key role in controlling HIV infection, increasing the survival and effector function of HIV-specific $CD8^+$ T cells may increase their ability to control HIV [14].

Our own studies did not show significant changes in the level of IL-4, -7, -15 depending on the applied cART treatment regimen (INSTIs vs. PIs). There is no information in the available literature on the study of differences in the expression of these interleukins depending on the type of antiretroviral treatment regimen.

Each cytokine plays a specific role in the regulation of a target cell, but when combined with another cytokine of the same family, this role becomes more enhanced and more effective. The ability of IL-7 and IL-15 to expand and/or enhance effector cell function may be of therapeutic benefit to HIV-infected patients. By examining the functional effects of these cytokines on HIV-specific immunity and HIV-carrier cells, researchers showed that both IL-7 and IL-15 enhance natural killer (NK) cell function more, while IL-7 enhances NK cell function by upregulating an apoptosis-inducing ligand associated with tumor necrosis factor (TNF). The close association and synergism provided by cytokines suggest the necessity to treat them as a whole during HIV treatment [32,33].

We are aware of the limitations of our work and believe that further studies should be conducted on a larger group of patients. The presented research was conducted on a group of men, and the expression of selected interleukins in women should be checked— the authors intend to analyze this matter in further research. The cART period was limited to one year; after a longer follow-up period, research should be continued to see if interleukin expression changes with long-term antiretroviral therapy.

5. Conclusions

The research results suggest the involvement of selected interleukins in HIV infection. The use of cART modulates the expression of the interleukins tested, especially in the case of IL-4 and IL-7. The type of treatment regimen did not significantly affect the level of interleukins in the plasma of HIV-infected patients, which may be important for the course of the disease.

Due to the existing global problem related to HIV infection, the development of AIDS, and the effectiveness of antiretroviral therapy, it seems fitting for research to explore this topic. It is for these reasons that this work was created. The obtained results, which deepen existing knowledge about changes in interleukins during the course of HIV infection, are encouraging; however, further research in this direction is needed to explain these mechanisms of action in detail.

Supplementary Materials: The following are available online at https://www.mdpi.com/article/10.3390/v14050997/s1, Figure S1. The level of IL-4 (2A), IL-7 (2B) and IL-15 (2C) in blood plasma obtained from HIV-infected men before and after cART depending on the HIV-RNA viral load, $CD4^+$ and $CD8^+$ T-cell counts and on the therapy regimen (PI or INSTI) and control groups. The *p*-values were calculated using the Mann-Whitney and Wilcoxon tests; * $p < 0.05$; ** $p \leq 0.001$.

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Załącznik 5

Wrocław, 06.06.2022 r.

KAROLINA JURKOWSKA

Wykaz publikacji

1. Publikacje w czasopismach naukowych

1.1 Publikacje w czasopiśmie naukowym posiadającym Impact Factor

Lp	Opis bibliograficzny	IF	PK
1.	**Badania nad nowymi lekami w terapii raka pęcherza moczowego (Research on new drugs in the therapy of bladder cancer (BC)).** [AUT.] KAROLINA JURKOWSKA, ANNA DŁUGOSZ. *Post.Hig.Med.Dośw.* 2018 Vol.72 s.442-448, ryc., bibliogr. 33 poz., streszcz., summ. DOI: 10.5604/01.3001.0012.0539	1,106	15,00
2.	**The impact of metalloestrogens on the physiology of male reproductive health as a current problem of the XXI century.** [AUT.] K[AROLINA] JURKOWSKA, E[WA] M[ARIA] KRATZ, E[WA] SAWICKA, A[GNIESZKA] PIWOWAR. *J.Physiol.Pharmacol.* 2019 Vol.70 no.3 s.337-355, ryc., tab., bibliogr. 196 poz., summ. DOI: 10.26402/jpp.2019.3.02	2,644	100,00
3.	**Chromium (III) and chromium (VI) as important players in the induction of genotoxicity - current view.** [AUT.] EWA SAWICKA, [AUT. KORESP.] KAROLINA JURKOWSKA, [AUT.] AGNIESZKA PIWOWAR. *Ann.Agric.Environ.Med.* 2021 Vol.28 no.1 s.1-10, tab., bibliogr. 89 poz., summ. DOI: 10.26444/aaem/118228	1,447*	100,00
4.	**Sirtuins as interesting players in the course of HIV infection and comorbidities.** [AUT. KORESP.] KAROLINA JURKOWSKA, [AUT.] BEATA SZYMAŃSKA, BRYGIDA KNYSZ, AMADEUSZ KUŹNIARSKI, AGNIESZKA PIWOWAR. *Cells* 2021 Vol.10 no.10 art.2739 [35 s.], ryc., bibliogr. 175 poz., summ. DOI: 10.3390/cells10102739	6,600*	140,00
5.	**The relationship between interleukin-13 and angiogenin in patients with bladder cancer.** [AUT.] B[EATA] SZYMAŃSKA, E[WA] SAWICKA, K[AROLINA] JURKOWSKA, M[ICHAŁ] MATUSZEWSKI, J[ANUSZ] DEMBOWSKI, A[GNIESZKA] PIWOWAR. *J.Physiol.Pharmacol.* 2021 Vol.72 no.4 s.615-622, ryc., tab., bibliogr. 41 poz., summ. DOI: 10.26402/jpp.2021.4.13	3,011*	100,00
6.	**Differences in expression of selected interleukins in HIV-infected subjects undergoing antiretroviral therapy.** [AUT.] BEATA SZYMAŃSKA, [AUT. KORESP.] KAROLINA JURKOWSKA, [AUT.] BRYGIDA KNYSZ, AGNIESZKA PIWOWAR. *Viruses-Basel* 2022 Vol.14 no.5 art.997 [14 s.], ryc., tab., bibliogr. 33 poz., summ. DOI: 10.3390/v14050997	5,048*	100,00
7.	**Effect of combined antiretroviral therapy on the levels of selected parameters reflecting metabolic and inflammatory disturbances in HIV-infected patients.** [AUT.] KAROLINA JURKOWSKA, [AUT. KORESP.] BEATA SZYMAŃSKA, [AUT.] BRYGIDA KNYSZ, AGNIESZKA PIWOWAR. *J.Clin.Med.* 2022 Vol.11 no.6 art.1713 [14 s.], tab., bibliogr. 46 poz., summ. DOI: 10.3390/jcm11061713	4,242*	140,00

Lp	Opis bibliograficzny	IF	PK
8.	**The effect of antiretroviral therapy on SIRT1, SIRT3 and SIRT6 expression in HIV-infected patients.** [AUT.] KAROLINA JURKOWSKA, [AUT. KORESP.] BEATA SZYMAŃSKA, [AUT.] BRYGIDA KNYSZ, AGNIESZKA PIWOWAR. *Molecules* 2022 Vol.27 no.4 art.1358 [13 s.], tab., bibliogr. 40 poz., summ. DOI: 10.3390/molecules27041358	4,412*	140,00
SUMA:		**28,510**	**835,00**

*IF za rok 2020

1.2 Publikacja w czasopiśmie naukowym nieposiadającym IF

Lp	Opis bibliograficzny	PK
1.	**Powszechność oraz sposoby stosowania popularnych leków przeciwbólowych: kwasu acetylosalicylowego, ibuprofenu i paracetamolu (badanie ankietowe) (Commonness and methods of using popular analgesic drugs: acetylsalicylic acid, ibuprofen and paracetamol (questionnaire survey)).** [AUT.] KAROLINA JURKOWSKA, MAŁGORZATA KASZCZYSZYN, KATARZYNA KOWALCZYK, PIOTR OKOŃ, EWA SAWICKA. *Farm.Pol.* 2016 T.72 nr 10 s.638-643, ryc., bibliogr. 8 poz., summ.	8,00
2.	**Niedokrwistości indukowane lekami jako ważny aspekt w opiece nad pacjentem (Drug-induced anemia as an important aspect in patient care).** [AUT.] MAŁGORZATA TERPIŃSKA, SYLWIA PŁACZKOWSKA, KAROLINA JURKOWSKA, AGNIESZKA PIWOWAR. *Farm.Pol.* 2018 T.74 nr 1 s.52-63, ryc., tab., bibliogr. 39 poz., summ.	8,00
3.	**Chrom - pierwiastek już dobrze znany czy wciąż nieznany - dwa oblicza działania (Chrome - a just well-known or still unknown element - two faces of action).** [AUT.] KAROLINA JURKOWSKA, EWA SAWICKA, AGNIESZKA PIWOWAR. *Farm.Pol.* 2019 T.75 nr 4 s.208-218, ryc., tab., bibliogr. 60 poz., summ.	70,00
4.	**Estrogenowe działanie chromu - ważny metaloestrogen w modulowaniu szlaków endokrynnych (Estrogenic effect of chromium - an important metalloestrogen in the modulation of endocrine pathways).** [AUT.] EWA SAWICKA, KAROLINA JURKOWSKA, AGNIESZKA PIWOWAR. *Farm.Pol.* 2019 T.75 nr 7 s.357-364, ryc., bibliogr. 58 poz., summ. DOI: 10.32383/farmpol/116118	70,00
SUMA:		**156,00**

4. Streszczenia zjazdowe

Lp	Opis bibliograficzny
1.	**Rola witaminy K_2 w suplementacji witaminą D_3 i preparatami wapnia.** [AUT.] KATARZYNA KOWALCZYK, KAROLINA JURKOWSKA, MAŁGORZATA KASZCZYSZYN, PIOTR OKOŃ, EWA SAWICKA. W: II Ogólnopolska Konferencja Naukowa "Współczesne zastosowanie substancji pochodzenia naturalnego w farmacji i medycynie". Wrocław, 20 maja 2016 r. Książka abstraktów, s.26.
2.	**Metalloestrogens as a new but important player in male infertility problem.** [AUT.] K[AROLINA] JURKOWSKA, E[WA] M[ARIA] KRATZ, E[WA] SAWICKA, A[GNIESZKA] PIWOWAR. W: 3rd Wroclaw Scientific Meetings. Wrocław, 1st-2nd March 2019 Wrocław 2019, Wydawnictwo Naukowe TYGIEL sp. z o.o, s.83 poz.P29, bibliogr. 5 poz, 978-83-65932-64-8.

Lp	Opis bibliograficzny
3.	**Analysis of interleukin 13 and angiogenin in patients with bladder cancer.** [AUT.] B[EATA] SZYMAŃSKA, K[AROLINA] JURKOWSKA, E[WA] SAWICKA, M[ICHAŁ] MATUSZEWSKI, J[ANUSZ] DEMBOWSKI, A[GNIESZKA] PIWOWAR. W: 4th International Wroclaw Scientific Meetings. Wrocław, 09-10 October 2020 Wrocław 2020, Wydawnictwo Naukowe TYGIEL sp. z o.o, s.237-238, bibliogr. 6 poz, 978-83-66489-37-0.

Sumaryczny Impact Factor: 28,510 *(liczba prac: 8)*

	Punktacja MNiSW
do roku 2018	31,00
od roku 2019	960,00
Razem:	**991,00**

Załącznik 6

KOMISJA BIOETYCZNA
przy
Uniwersytecie Medycznym
we Wrocławiu
ul. Pasteura 1; 50-367 WROCŁAW

OPINIA KOMISJI BIOETYCZNEJ Nr KB – 597/2019

Komisja Bioetyczna przy Uniwersytecie Medycznym we Wrocławiu, powołana zarządzeniem Rektora Uniwersytetu Medycznego we Wrocławiu nr 133/XV R/2017 z dnia 21 grudnia 2017 r. oraz działająca w trybie przewidzianym rozporządzeniem Ministra Zdrowia i Opieki Społecznej z dnia 11 maja 1999 r. (Dz.U. nr 47, poz. 480) na podstawie ustawy o zawodzie lekarza z dnia 5 grudnia 1996 r. (Dz.U. nr 28 z 1997 r. poz. 152 z późniejszymi zmianami) w składzie:

prof. dr hab. Jacek Daroszewski (choroby wewnętrzne, endokrynologia, diabetologia)
prof. dr hab. Krzysztof Grabowski (chirurgia)
dr Henryk Kaczkowski (chirurgia szczękowa, chirurgia stomatologiczna)
mgr Irena Knabel-Krzyszowska (farmacja)
prof. dr hab. Jerzy Liebhart (choroby wewnętrzne, alergologia)
ks. dr hab. Piotr Mrzygłód, prof. nadzw. (duchowny)
mgr Luiza Műller (prawo)
dr hab. Sławomir Sidorowicz (psychiatria)
prof. dr hab. Leszek Szenborn (pediatria, choroby zakaźne)
Danuta Tarkowska (pielęgniarstwo)
prof. dr hab. Anna Wiela-Hojeńska (farmakologia kliniczna)
dr hab. Andrzej Wojnar, prof. nadzw. (histopatologia, dermatologia) przedstawiciel Dolnośląskiej Izby Lekarskiej)
dr hab. Jacek Zieliński (filozofia)

pod przewodnictwem
prof. dr hab. Jana Kornafela (ginekologia i położnictwo, onkologia)

Przestrzegając w działalności zasad Good Clinical Practice oraz zasad Deklaracji Helsińskiej, po zapoznaniu się z projektem badawczym pt.:

„Przydatność wybranych wskaźników biochemicznych w ocenie ryzyka wystąpienia chorób współistniejących i zaburzeń metabolicznych u osób zakażonych HIV"

zgłoszonym przez **mgr farmacji Karolinę Jurkowską** uczestnika studiów doktoranckich w Katedrze i Zakładzie Toksykologii Uniwersytetu Medycznego we Wrocławiu oraz złożonymi wraz z wnioskiem dokumentami, w tajnym głosowaniu postanowiła **wyrazić zgodę** na przeprowadzenie badania w Katedrze i Zakładzie Toksykologii pod nadzorem prof. dr hab. Agnieszki Piwowar **pod warunkiem zachowania anonimowości uzyskanych danych**.

Uwaga: Badanie to zostało objęte ubezpieczeniem odpowiedzialności cywilnej Uniwersytetu Medycznego we Wrocławiu z tytułu prowadzonej działalności.

Pouczenie: W ciągu 14 dni od otrzymania decyzji wnioskodawcy przysługuje prawo odwołania do Komisji Odwoławczej za pośrednictwem Komisji Bioetycznej UM we Wrocławiu.

Opinia powyższa dotyczy projektu badawczego będącego podstawą rozprawy doktorskiej.

Wrocław, dnia 22.07.2019 r.

Załącznik 7

Wrocław, 13.06.2022
miejscowość, data

prof. dr hab. Agnieszka Piwowar
imię i nazwisko (stopień, tytuł)

Katedra i Zakład Toksykologii
Wydział Farmaceutyczny
miejsce zatrudnienia

OŚWIADCZENIE

współautora określające indywidualny wkład w postawnie rozprawy doktorskiej

Oświadczam, że:

w pracy: **Jurkowska K, Szymańska B, Knysz B, Kuźniarski A, Piwowar A. Sirtuins as Interesting Players in the Course of HIV Infection and Comorbidities. Cells. 2021; 10(10):2739.**

mój udział polegał na współuczestniczeniu w opracowywaniu koncepcji pracy, przygotowywaniu przeglądu literatury, współtworzeniu tekstu manuskryptu, ocenie i korekcie merytorycznej tekstu manuskryptu oraz pozyskaniu źródeł finansowania.

w pracach:

Jurkowska K, Szymańska B, Knysz B, Piwowar A. The Effect of Antiretroviral Therapy on SIRT1, SIRT3 and SIRT6 Expression in HIV-Infected Patients. Molecules. 2022; 27(4):1358

Jurkowska K, Szymańska B, Knysz B, Piwowar A. Effect of Combined Antiretroviral Therapy on the Levels of Selected Parameters Reflecting Metabolic and Inflammatory Disturbances in HIV-Infected Patients. Journal of Clinical Medicine. 2022; 11(6):1713.

Szymańska B, Jurkowska K, Knysz B, Piwowar A. Differences in Expression of Selected Interleukins in HIV-Infected Subjects Undergoing Antiretroviral Therapy. Vi-ruses. 2022; 14(5):997

mój udział polegał na współuczestniczeniu w opracowywaniu koncepcji pracy, interpretacji wyników oraz ocenie merytorycznej i korekcie tekstu manuskryptu oraz pozyskaniu źródeł finansowania.

Jednocześnie wyrażam zgodę na przedłożenie w/w prac przez mgr Karoliny Jurkowskiej jako część rozprawy doktorskiej w formie spójnego tematycznie zbioru artykułów naukowych opublikowanych w czasopismach naukowych.

Uniwersytet Medyczny we Wrocławiu
KATEDRA i ZAKŁAD TOKSYKOLOGII
kierownik
prof. dr hab. Agnieszka Piwowar

podpis współautora

Wrocław, 13.06.2022
miejscowość, data

prof. dr hab. Brygida Knysz
imię i nazwisko (stopień, tytuł)

Katedra i Klinika Chorób Zakaźnych, Chorób Wątroby
i Nabytych Niedoborów Odpornościowych
Wydział Lekarski
miejsce zatrudnienia

OŚWIADCZENIE

współautora określające indywidualny wkład w postawnie rozprawy doktorskiej

Oświadczam, że:

w pracy: **Jurkowska K, Szymańska B, Knysz B, Kuźniarski A, Piwowar A. Sirtuins as Interesting Players in the Course of HIV Infection and Comorbidities. Cells. 2021; 10(10):2739.**

mój udział polegał na ocenie merytorycznej tekstu manuskryptu oraz pomocy w opracowywaniu koncepcji pracy.

w pracach:

Jurkowska K, Szymańska B, Knysz B, Piwowar A. The Effect of Antiretroviral Therapy on SIRT1, SIRT3 and SIRT6 Expression in HIV-Infected Patients. Molecules. 2022; 27(4):1358

Jurkowska K, Szymańska B, Knysz B, Piwowar A. Effect of Combined Antiretroviral Therapy on the Levels of Selected Parameters Reflecting Metabolic and Inflammatory Disturbances in HIV-Infected Patients. Journal of Clinical Medicine. 2022; 11(6):1713.

Szymańska B, Jurkowska K, Knysz B, Piwowar A. Differences in Expression of Selected Interleukins in HIV-Infected Subjects Undergoing Antiretroviral Therapy. Viruses. 2022; 14(5):997

mój udział polegał na współuczestnictwie w pozyskiwaniu danych, interpretacji wyników, pomocy w opracowywaniu koncepcji pracy oraz ocenie merytorycznej tekstu manuskryptu.

Jednocześnie wyrażam zgodę na przedłożenie w/w prac przez mgr Karoliny Jurkowskiej jako część rozprawy doktorskiej w formie spójnego tematycznie zbioru artykułów naukowych opublikowanych w czasopismach naukowych.

Brygida Knysz
podpis współautora

Wrocław, 13.06.2022
miejscowość, data

dr n. farm Beata Szymańska
imię i nazwisko (stopień, tytuł)

Katedra i Zakład Toksykologii
Wydział Farmaceutyczny
miejsce zatrudnienia

OŚWIADCZENIE

współautora określające indywidualny wkład w postawnie rozprawy doktorskiej

Oświadczam, że:

w pracy: **Jurkowska K, Szymańska B, Knysz B, Kuźniarski A, Piwowar A. Sirtuins as Interesting Players in the Course of HIV Infection and Comorbidities. Cells. 2021; 10(10):2739.**

mój udział polegał na uczestnictwie w opracowywaniu koncepcji pracy oraz w opracowywaniu tekstu artykułu.

w pracach:

Jurkowska K, Szymańska B, Knysz B, Piwowar A. The Effect of Antiretroviral Therapy on SIRT1, SIRT3 and SIRT6 Expression in HIV-Infected Patients. Molecules. 2022; 27(4):1358

Jurkowska K, Szymańska B, Knysz B, Piwowar A. Effect of Combined Antiretroviral Therapy on the Levels of Selected Parameters Reflecting Metabolic and Inflammatory Disturbances in HIV-Infected Patients. Journal of Clinical Medicine. 2022; 11(6):1713.

Szymańska B, Jurkowska K, Knysz B, Piwowar A. Differences in Expression of Selected Interleukins in HIV-Infected Subjects Undergoing Antiretroviral Therapy. Viruses. 2022; 14(5):997

mój udział polegał na współuczestnictwie w planowaniu i wykonywaniu badań, analizie statystycznej oraz interpretacji wyników oraz pomocy w stworzeniu manuskryptu prac. W dwóch pracach jestem autorem korespondencyjnym.

Jednocześnie wyrażam zgodę na przedłożenie w/w prac przez mgr Karoliny Jurkowskiej jako część rozprawy doktorskiej w formie spójnego tematycznie zbioru artykułów naukowych opublikowanych w czasopismach naukowych.

Beata Szymańska
---
podpis współautora

Wrocław, 13.06.2022
miejscowość, data

lek. dent. Amadeusz Kuźniarski
imię i nazwisko (stopień, tytuł)

Katedra i Zakład Protetyki Stomatologicznej
Wydział Lekarsko-Stomatologiczny
miejsce zatrudnienia

OŚWIADCZENIE

współautora określające indywidualny wkład w postawnie rozprawy doktorskiej

Oświadczam, że:

w pracy: **Jurkowska K, Szymańska B, Knysz B, Kuźniarski A, Piwowar A. Sirtuins as Interesting Players in the Course of HIV Infection and Comorbidities. Cells. 2021; 10(10):2739.**

mój udział polegał na współuczestnictwie w przygotowywaniu rysunków oraz podrozdziału 10 pracy.

Jednocześnie wyrażam zgodę na przedłożenie w/w prac przez mgr Karoliny Jurkowskiej jako część rozprawy doktorskiej w formie spójnego tematycznie zbioru artykułów naukowych opublikowanych w czasopismach naukowych.

podpis współautora