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Wpływ terapii antyretrowirusowej na wybrane aspekty ryzyka sercowo-naczyniowego i parametry immunologiczne u zakażonych HIV

Rozprawa na stopień doktora nauk medycznych i nauk o zdrowiu

w dyscyplinie nauki medyczne

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Keywords: human immunodeficiency virus (HIV), antiretroviral treatment, cardiovascular risk, vascular cell adhesion molecule-1 (VCAM-1), lymphocytes T CD4+, immune reconstitution

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1. ***„VCAM-1 as a Biomarker of Endothelial Function among HIV-Infected Patients Receiving and Not Receiving Antiretroviral Therapy”*** (tłum. *„VCAM-1 jako biomarker funkcji śródbłonka u pacjentów zakażonych HIV otrzymujących i nieotrzymujących leczenia antyretrowirusowego”*) Viruses. 2022 Mar 11;14(3):578. doi: 10.3390/v14030578. PMID: 35336985; PMCID: PMC8955345
2. ***„Evaluation of Clinical Biomarkers Related to CD4 Recovery in HIV-Infected Patients—5-Year Observation”*** (tłum. *„Analiza biomarkerów klinicznych związanych z odbudową liczby limfocytów CD4 u pacjentów zakażonych HIV – pięcioletnia obserwacja”*) Viruses. 2022 Oct 18;14(10):2287. doi: 10.3390/v14102287. PMID: 36298842; PMCID: PMC9607521
3. ***„Human Immunodeficiency Virus as a Risk Factor for Cardiovascular Disease”*** (tłum. „*Wirus nabytego niedoboru odporności jako czynnik ryzyka sercowo-naczyniowego”*) Cardiovascular Toxicology. 2024 Jan;24(1):1-14. doi: 10.1007/s12012-023-09815-4. Epub 2023 Nov 20. PMID: 37982976; PMCID: PMC10838226

Spis treści

Wykaz zastosowanych skrótów

AIDS – zespół nabytego niedoboru odporności (ang. *acquired immune deficiency syndrome*)

BMI – wskaźnik masy ciała (ang. *body mass index*)

CDC – Centrum Kontroli Chorób w Atlancie (and. *Centers for Disease Control and Prevention)*

CD14 – antygen różnicowania komórkowego 14 (ang. *cluster of differentiation 14*)

CD4 – antygen różnicowania komórkowego 4 (ang. *cluster of differentiation 4*)

CD8 – antygen różnicowania komórkowego 8 (ang. *cluster of differentiation 8*)

CMV – wirus cytomegalii (ang. *cytomegalovirus*)

DHHS – Departament Zdrowia i Usług Społecznych USA (ang. *U.S. Department of Health and Human Services*)

EBV – wirus Epsteina i Barr (ang. *Epstein-Barr virus*)

HBV – wirus zapalenia wątroby typu B (ang. *hepatitis B virus*)

HIV – ludzki wirus nabytego niedoboru odporności (ang. *human immunodeficiency virus*)

HCV – wirus zapalenia wątroby typu C (ang. *hepatitis C virus*)

HDL – lipoproteina wysokiej gęstości (ang. *high density lipoprotein*)

hs-CRP – wysoko czułe białko C reaktywne (ang. *high-sensitivity C-reactive protein*)

ICAM-1 – międzykomórkowa molekuła adhezyjna-1 (ang. *intercellular adhesion molecule-1*)

InSTI – inhibitory integrazy (ang. *Integrase Strand Transfer Inhibitors*)

IP-10 – proteina 10 indukowana interferonem gamma (ang. *interferon gamma-induced protein 10*)

IL-1β – interleukina-1 beta (ang. *interleukin-1 beta*)

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

LDL – lipoproteina niskiej gęstości (ang. *low density lipoprotein*)

NNRTI – nienukleoz(t)ydowe inhibitory odwrotnej transkryptazy (ang. *non-nucleoside reverse transcriptase inhibitors)*

NRTI – nukleoz(t)ydowe inhibitory odwrotnej transkryptazy (ang. *nucleoside reverse transcriptase inhibitors)*

PI – inhibitory proteazy (ang. *protease inhibitors*)

TAF – alafenamid tenofowiru (ang. *tenofovir alafenamide*)

TDF – dizoproksyl tenofowiru (ang. *tenofovir disoproxil*)

TGF-β – transformujący czynnik wzrostu nowotworów beta (ang. *transforming growth factor beta*)

TNF-α – czynnik martwicy nowotworu alfa (ang. *tumor necrosis factor alpha*)

VCAM-1 - molekuła adhezyjna-1 komórki naczyniowej (ang. *vascular cell adhesion molecule-1*)

Streszczenie w języku polskim

Leczenie antyretrowirusowe znacznie poprawiło jakość i wydłużyło życie pacjentów zakażonych HIV. W głównej mierze jest to wynik zahamowania replikacji HIV, co umożliwia odbudowę immunologiczną. Dzięki temu, przy rosnącej długości życia pacjentów obserwowano zmniejszenie zapadalności na choroby wskaźnikowe AIDS, jednak zauważono częstsze występowanie chorób niezwiązanych z AIDS, zwłaszcza chorób układu sercowo-naczyniowego. Wiele czynników wpływa na zmianę najczęściej występujących schorzeń w tej populacji pacjentów, a jednym z nich jest samo leczenie antyretrowirusowe.

W skład rozprawy wchodzą trzy prace: dwie prace oryginalne oraz jedna praca poglądowa. Celem prac była ocena wpływu leczenia antyretrowirusowego na wybrane aspekty ryzyka sercowo-naczyniowego i odbudowę immunologiczną u pacjentów zakażonych HIV. Dokonano tego poprzez ocenę stężenia VCAM-1 i profilu lipidowego w zależności od stosowania leczenia antyretrowirusowego i jego długości. Przeprowadzono również pięcioletnią obserwację zmian liczby limfocytów T CD4+ i stosunku limfocytów T CD4+:CD8+ po włączeniu leczenia antyretrowirusowego. Dodatkowym celem była analiza czynników wpływających na stężenia VCAM-1 oraz analiza czynników wpływających na normalizację parametrów immunologicznych. Celem pracy poglądowej było zgromadzenie aktualnej wiedzy dotyczącej czynników wpływających na ryzyko sercowo-naczyniowe u osób zakażonych HIV, z uwzględnieniem leczenia antyretrowirusowego i parametrów immunologicznych.

Wyniki badań wykazały, że u pacjentów leczonych antyretrowirusowo przez ponad rok obserwowano niższe stężenia biomarkera związanego ze stanem zapalnym i miażdżycą śródbłonka naczyń VCAM-1 niż u pacjentów z nieleczonym zakażeniem HIV. Jednocześnie obserwowano wyższe stężenia cholesterolu całkowitego i LDL niż u pacjentów nieleczonych, co utrudnia jednoznaczną ocenę ryzyka sercowo-naczyniowego. Przeprowadzone badania wykazały również, że pomimo niewykrywalnej wiremii HIV, u większości pacjentów nie doszło do pełnej odbudowy układu immunologicznego. Do czynników wpływających korzystnie na szansę normalizacji parametrów immunologicznych należał wiek poniżej 35 lat, wysoka liczba limfocytów T CD4+ i wysoka wiremia HIV w momencie włączenia leczenia antyretrowirusowego oraz rozpoczęcie leczenia w fazie ostrej choroby retrowirusowej.

Uzyskane wyniki wskazują, że leczenie antyretrowirusowe może mieć zarówno pozytywny, jak i negatywny wpływ na ryzyko sercowo-naczyniowe, a także nie gwarantuje pełnej odbudowy immunologicznej, jednak jego brak prowadzi do postępującej dysfunkcji immunologicznej i śmierci. Wcześnie rozpoznanie zakażenia HIV i optymalnie szybkie włączenie leczenia antyretrowirusowego zwiększa szansę na pełną odbudowę układu immunologicznego. Dodatkowo, wszyscy pacjenci poddawani leczeniu antyretrowirusowemu powinni być regularnie oceniani pod kątem wystąpienia czynników ryzyka chorób układu sercowo-naczyniowego.

Streszczenie w języku angielskim

The impact of antiretroviral therapy on selected aspects of cardiovascular risk and immune recovery among people with HIV

Antiretroviral therapy significantly contributed to the improvement in quality of life and life expectancy of people living with HIV. It was possible due to immune reconstitution following the inhibition of HIV replication. Antiretroviral therapy has decreased the number of AIDS-related diseases, while non-AIDS conditions, especially cardiovascular disease, started to occur more often. There are many factors impacting the change in most prevalent diseases in this population and one of them is antiretroviral therapy itself.

The dissertation consists of three publications: two original papers and one review article. The aim of the original manuscripts was the assessment of the impact of antiretroviral therapy on selected aspects of cardiovascular risk and immune reconstitution in people living with HIV. It was performed by the analysis of VCAM-1 concentration and lipid profile depending on applying antiretroviral treatment and its length. 5-year observation of changes in lymphocyte T CD4+ and lymphocyte T CD4+:CD8+ after antiretroviral treatment implementation was also performed. The additional purpose was the analysis of factors impacting VCAM-1 concentration and the chance of normalizing immune parameters. The aim of the review article was to summarize most recent knowledge concerning factors impacting cardiovascular risk in people living with HIV, including antiretroviral treatment and immune parameters.

The studies have shown that people living with HIV undergoing antiretroviral therapy longer than a year had significantly lower VCAM-1 concentration than patients not receiving therapy. Simultaneously, higher concentrations of total and LDL cholesterol were observed in patients undergoing treatment comparison to the treatment-naïve patients, which makes it difficult to assess cardiovascular risk explicitly. Studies have also shown that despite undetectable HIV viral load, majority of patients did not experience complete immune reconstitution. The factors positively associated with the chance of the normalization of immune parameters were: age below 35 years old, high lymphocyte T CD4+ count and high HIV viral load at the beginning of antiretroviral treatment and starting the therapy during acute HIV infection.

The results imply that antiretroviral therapy may have both beneficial and adverse influences on cardiovascular risk and does not guarantee complete immune reconstitution. However, its absence leads to the impairment of the immune system and consequently to death. Early HIV diagnosis and introduction of antiretroviral therapy improves the chances of immune recovery. Moreover, all patients undergoing antiretroviral therapy should be regularly and precisely accessed in terms of cardiovascular disease.

Wstęp

Na początku pandemii HIV, gdy leczenie antyretrowirusowe nie było jeszcze dostępne, główną przyczyną zgonu wśród pacjentów zakażonych HIV były choroby wskaźnikowe AIDS. Szybki postęp w rozwoju leczenia antyretrowirusowego oraz coraz większa dostępność tej terapii umożliwiły całkowitą supresję wiremii HIV, odbudowę układu immunologicznego, ograniczenie transmisji zakażenia, zmniejszenie częstości występowania chorób oportunistycznych oraz liczby zgonów z powodu AIDS. Obecnie leczenie antyretrowirusowe jest standardowym postępowaniem u wszystkich pacjentów z rozpoznanym zakażeniem HIV, dzięki czemu przewidywana długość życia osób leczonych jest zbliżona do populacji ogólnej [1].

Wraz ze zwiększeniem długości życia pacjentów zauważono, że wśród osób zakażonych HIV leczonych antyretrowirusowo często spotykamy choroby cywilizacyjne - niezwiązane z AIDS, a będące efektem wydłużenia życia tych pacjentów np. choroby układu sercowo-naczyniowego. Obecnie szacuje się, że osoby zakażone HIV mają o 50% do 100% wyższą zapadalność i śmiertelność z powodu chorób układu krążenia [2]. Wśród najczęściej występujących można wyróżnić nadciśnienie tętnicze, hipercholesterolemię oraz podwyższone stężenie glukozy we krwi. Pacjenci zakażeni HIV mają również wyższe ryzyko udaru niedokrwiennego, zaburzeń rytmu serca, niewydolności serca i nagłej śmierci sercowej niż ich rówieśnicy bez zakażenia HIV [2,3]. Wśród wielu przyczyn tego zjawiska znajdują się między innymi: długotrwała ekspozycja na HIV, długotrwała supresja układu immunologicznego, a także przewlekły stan zapalny [4]. Ponadto, pacjenci zakażeni HIV są częściej narażeni na działanie niezależnych czynników ryzyka sercowo-naczyniowego, jak palenie tytoniu czy nadużywanie alkoholu [5]. Dodatkowym niezależnym czynnikiem ryzyka choroby sercowo-naczyniowej jest przewlekłe zakażenie HCV, a szacuje się, że ko-infekcja HIV/HCV występuje u ponad 10% pacjentów zakażonych HIV, podczas gdy w populacji ogólnej wynosi ok. 1-3% [6-8].

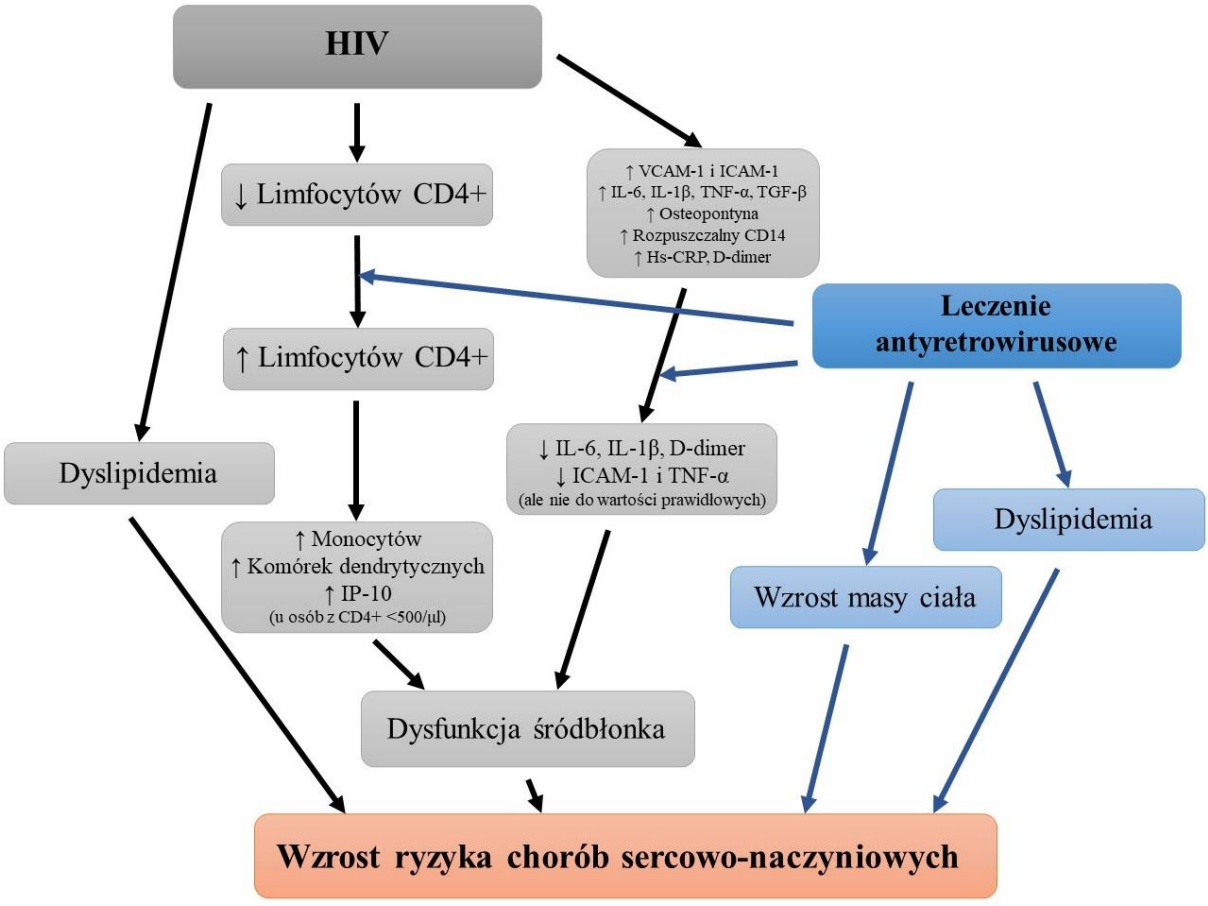
Zakażenie HIV nie jest jedynym czynnikiem wpływającym na układ sercowo-naczyniowy [9]. Leczenie antyretrowirusowe, poza zahamowaniem replikacji wirusa, może również mieć potencjalnie niekorzystne skutki dla układu krążenia. To działanie różni się w zależności od stosowanej grupy leków, a także od konkretnego preparatu.

Obecnie najczęściej stosowanymi lekami są inhibitory integrazy (InSTI), nukleoz(t)ydowe inhibitory odwrotnej transkryptazy (NRTI), nienukleoz(t)ydowe inhibitory odwrotnej transkryptazy (NNRTI) oraz inhibitory proteazy (PI). Nowe leki charakteryzują się wysoką skutecznością i niską toksycznością [10]. Istnieją jednak doniesienia o ich potencjalnej szkodliwości na układ sercowo-naczyniowy. Najczęstszym działaniem niepożądanym inhibitorów integrazy jest wzrost masy ciała, otyłość oraz choroby bezpośrednio związane ze wzrostem masy ciała [11]. Większym ryzykiem wzrostu masy ciała obarczone są osoby przyjmujące biktegrawir i dolutegrawir niż elwitegrawir [12]. Jednak pomimo wzrostu masy ciała, pacjenci przyjmujący inhibitory integrazy mają mniejsze ryzyko zawału serca, udaru niedokrwiennego, konieczności pomostowania aortalno-wieńcowego lub przezskórnej interwencji wieńcowej niż osoby przyjmujące leki innych grup [13].

Potencjalne działania niepożądane NRTI na układ sercowo naczyniowy wynikają z oksydacyjnego stresu mitochondrialnego i związanej z mitofagią toksyczności dla śródbłonka naczyń [14]. U osób otrzymujących NRTI zaobserwowano również kumulację β-galaktozydazy w komórkach śródbłonka, zredukowane procesy utleniania zależne od adenozyno-5'-trifosforanu oraz zwiększoną produkcję reaktywnych form tlenu [15]. Poszczególne preparaty charakteryzują się różnym profilem bezpieczeństwa. Niekiedy nawet forma leku może mieć duże znaczenie w kontekście działań niepożądanych, co można zaobserwować szczególnie na podstawie dwóch form tenofowiru - disoproksylu tenofowiru (TDF) i alafenamidu tenofowiru (TAF). TAF cechuje się niższym ryzykiem nefrotoksyczności, jednak opisywano wzrost stężenia cholesterolu całkowitego i cholesterolu LDL, a także BMI po zmianie leczenia z TDF na TAF [12,16].

Dorawiryna, najczęściej stosowany NNRTI, wydaje się mieć korzystny profil metaboliczny. Badania wykazały spadek stężenia cholesterolu całkowitego, cholesterolu LDL i trójglicerydów po zmianie leczenia na dorawirynę [17]. Inhibitory proteazy natomiast wydają się mieć raczej niekorzystny wpływ na układ sercowo-naczyniowy, wyrażony przede wszystkim pod postacią hipercholesterolemii. Do mechanizmów odpowiedzialnych za to zjawisko należy indukowanie syntezy reaktywnych form tlenu i upośledzanie funkcji mitochondriów [18].

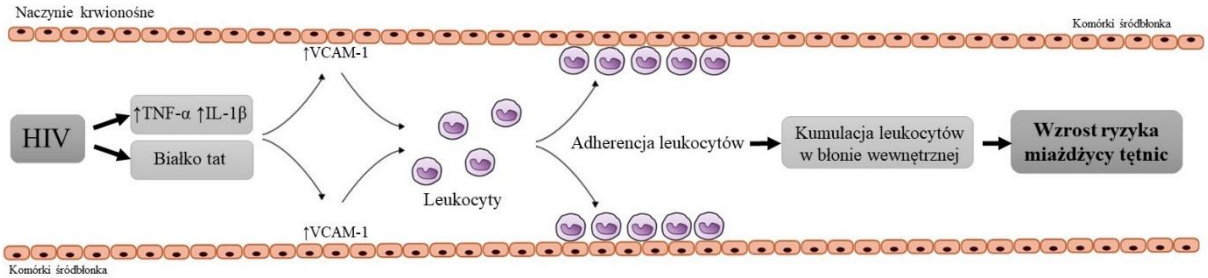
Jednym z ważnych czynników wpływających na ryzyko sercowo-naczyniowe jest przetrwały stan zapalny. Przewlekłe zakażenia wirusowe, w tym również zakażenie HIV, stymulują uwalnianie czynników prozapalnych, takich jak cząstki adhezyjne śródbłonka naczyniowego (VCAM-1 i ICAM-1), interleukiny (zwłaszcza IL-6 i IL-1β), czynnik martwicy nowotworu alfa (TNF-α), transformujący czynnik wzrostu nowotworów beta (TGF-β), osteopontyna, rozpuszczalny CD14, wysokoczułe białko C reaktywne (hs-CRP) czy D-dimer. Wszystkie te czynniki nasilają stan zapalny i wydzielane przewlekle, mogą prowadzić do wyczerpania immunologicznego, które z kolei stymuluje uszkodzenie naczyń krwionośnych i różnych narządów [19]. Choroby związane z przetrwałym stanem zapalnym, na które szczególnie narażone są osoby zakażone HIV, to przede wszystkim choroby układu sercowo-naczyniowego, ale także nowotwory, choroby płuc, choroby neurodegeneracyjne, zespół kruchości i wiele innych [20]. Na Rycinie 1 przedstawiono wpływ zakażenia HIV oraz leczenia antyretrowirusowego na wzrost ryzyka sercowo-naczyniowego.



Rycina 1. Wpływ zakażenia HIV i leczenia antyretrowirusowego na wzrost ryzyka sercowo-naczyniowego.

Wykazano, że leczenie antyretrowirusowe powoduje zmniejszenie procesów prozapalnych stymulowanych przez HIV. Badania oceniające stężenia IL-6, IL-1β, D-dimeru, ICAM-1 i TNF-α wykazują znaczący spadek stężenia tych biomarkerów u pacjentów leczonych antyretrowirusowo w porównaniu z pacjentami nieleczonymi. Co jednak istotne, pomimo leczenia antyretrowirusowego, stężenia biomarkerów utrzymują się na wyższym poziomie niż w populacji osób niezakażonych HIV [21-23]. Możliwym wytłumaczeniem tego zjawiska jest niewystarczające przenikanie leków do niektórych rezerwuarów, w których zachodzi replikacja HIV w organizmie, jak np. układ nerwowy, przewód pokarmowy czy węzły chłonne, co podtrzymuje utrzymywanie się stanu zapalnego [24].

Molekuła adhezyjna-1 komórki naczyniowej (VCAM-1), to białko adhezyjne, które ulega ekspresji pod wpływem działania cytokin, zarówno w obrębie dużych, jak i małych naczyń krwionośnych. W komórkach śródbłonka ekspresja VCAM-1 jest stymulowana przez białko Tat, TNF-α i IL-1β i odbywa się poprzez receptory toll-podobne, co następnie nasila stan zapalny i stymuluje migrację leukocytów [25]. Przewlekle wzmożona ekspresja VCAM-1 prowadzi do kumulacji leukocytów w błonie wewnętrznej naczyń krwionośnych, co jest ważnym czynnikiem wpływającym na rozwój miażdżycy naczyń [26]. VCAM-1 jest biomarkerem uszkodzenia śródbłonka naczyń stosowanym w wielu badaniach klinicznych do oceny ryzyka sercowo-naczyniowego [27]. Wykazano, że stężenia VCAM-1 są wyższe u osób zakażonych HIV niż w populacji ogólnej, co może być jednym z możliwych powodów podwyższonego ryzyka sercowo-naczyniowego w tej grupie pacjentów [28]. Rycina 2 przedstawia wpływ zakażenia HIV na wzrost ryzyka miażdżycy tętnic poprzez stymulację ekspresji VCAM-1.



Rycina 2. Wpływ zakażenia HIV na wzrost ryzyka miażdżycy tętnic poprzez stymulację ekspresji VCAM-1 na komórkach śródbłonka naczyń.

Celem pierwszej publikacji naukowej należącej do cyklu, opublikowanej przez czasopismo *Viruses* pod tytułem ***„VCAM-1 as a Biomarker of Endothelial Function***

among HIV-Infected Patients Receiving and Not Receiving Antiretroviral Therapy" (tłum. *„VCAM-1 jako biomarker funkcji śródbłonka u pacjentów zakażonych HIV otrzymujących i nieotrzymujących leczenia antyretrowirusowego"*) Viruses. 2022 Mar 11;14(3):578. doi: 10.3390/v14030578. PMID: 35336985; PMCID: PMC8955345 była ocena dysfunkcji śródbłonka poprzez pomiar stężenia VCAM-1 we krwi obwodowej osób zakażonych HIV otrzymujących i nieotrzymujących leczenia antyretrowirusowego. Badanie wykazało, że osoby zakażone HIV, leczone ponad rok miały znacząco niższe stężenia VCAM-1 niż osoby, które nie były dotychczas leczone antyretrowirusowo.

Nieleczone zakażenie HIV prowadzi także do postępującego uszkodzenia funkcji układu immunologicznego, które manifestuje się przede wszystkim pod postacią obniżenia liczby limfocytów T pomocniczych CD4+ oraz obniżenia stosunku limfocytów T CD4+ do limfocytów T CD8+ [29]. Leczenie antyretrowirusowe zapobiega utracie limfocytów T CD4+, a także pośrednio, poprzez supresję replikacji HIV, pozwala na odbudowę układu immunologicznego [30]. Odbudowa jest szczególnie istotna dla pacjentów z zaawansowanym niedoborem odporności, gdyż pozwala ona zminimalizować ryzyko wystąpienia chorób oportunistycznych. Centers for Disease Control and Prevention (CDC) uznaje osiągnięcie liczby limfocytów T CD4+ $\geq$ 500 komórek/µL i stosunku limfocytów T CD4+:CD8+ $\geq$ 1 jako jeden z celów leczenia zakażenia HIV [31]. Jednak ten cel udaje się osiągnąć zaledwie u około 30% pacjentów zakażonych HIV [32].

Celem kolejnej pracy będącej elementem cyklu, pod tytułem ***„Evaluation of Clinical Biomarkers Related to CD4 Recovery in HIV-Infected Patients—5-Year Observation"*** (tłum. *„Analiza biomarkerów klinicznych związanych z odbudową liczby limfocytów CD4 u pacjentów zakażonych HIV – pięcioletnia obserwacja"*) Viruses. 2022 Oct 18;14(10):2287. doi: 10.3390/v14102287. PMID: 36298842; PMCID: PMC9607521 była ocena czynników wpływających na odbudowę immunologiczną po włączeniu leczenia antyretrowirusowego. Analizie poddane były czynniki kliniczne, w tym: wiek, płeć, schemat leczenia antyretrowirusowego, czynniki biochemiczne, jak stadium zakażenia HIV, współwystępowanie chorób oportunistycznych, choroby współistniejące niezwiązane z AIDS oraz współistnienie przewlekłych zakażeń wirusami HCV lub HBV oraz wyniki badań laboratoryjnych: liczba limfocytów T CD4+ i wiremia HIV w momencie rozpoznania. Badanie wykazało, że wiek poniżej 35 lat, wyższa liczba limfocytów T CD4+ i wysoka wiremia HIV w momencie włączenia leczenia

antyretrowirusowego oraz rozpoczęcie leczenia w fazie ostrej choroby retrowirusowej to pozytywne czynniki predykcyjne normalizacji liczby limfocytów T CD4+ oraz stosunku limfocytów T CD4+:CD8+. Żaden schemat leczenia antyretrowirusowego nie wykazał wyższości nad innym w kontekście odbudowy immunologicznej, co jest zgodne z najnowszymi doniesieniami naukowymi na ten temat [33].

W kontekście odbudowy układu immunologicznego szczególną grupą pacjentów są osoby nieodpowiadające immunologicznie na leczenie antyretrowirusowe (ang. immunological non-responders). W 2008 r. The Department of Health and Human Services (DHHS) przyjmuje, że są to pacjenci, u których liczba limfocytów T CD4+ utrzymuje się pomiędzy 350 a 500 komórek/μL po 4-7 latach skutecznego wirusologicznie leczenia antyretrowirusowego [34]. Osoby te mają większe ryzyko rozwoju nie tylko chorób oportunistycznych, ale także chorób niezależnych od AIDS, w tym zwłaszcza zespołu metabolicznego, chorób układu sercowo-naczyniowego, chorób wątroby, nefropatii, nowotworów niedefiniujących AIDS oraz zaburzeń neuropoznawczych [35]. Mechanizmy tych zjawisk nie są do końca poznane, jednak wykazano, że osoby, u których nie dochodzi do wzrostu liczby limfocytów CD4+ pod wpływem leczenia antyretrowirusowego wykazują większą aktywację monocytów i komórek dendrytycznych, a także wyższe stężenia proteiny 10 indukowanej interferonem gamma (IP-10) [36]. Następnie IP-10 promuje migrację leukocytów, co może skutkować powstawaniem zmian miażdżycowych, a w efekcie prowadzić do zawału serca, przewlekłego zespołu wieńcowego, epizodów niedokrwiennych czy incydentów zakrzepowo-zatorowych [37].

Osoby zakażone HIV, podobnie jak populacja ogólna, narażone są również na inne czynniki ryzyka chorób sercowo-naczyniowych. Wykazano, że u zakażonych HIV choroby układu krążenia rozwijają się średnio 10 lat wcześniej niż w populacji ogólnej [38]. Odmiennie niż w pozostałej populacji, u zakażonych HIV ważnym czynnikiem ryzyka sercowo-naczyniowego jest płeć żeńska, która wiąże się z 1,5-2 razy wyższym ryzykiem rozwoju chorób układu krążenia niż płeć męska [39]. Do innych czynników ryzyka należy palenie papierosów, przewlekłe infekcje wirusowe (HCV, HBV, CMV czy EBV), otyłość, hipercholesterolemia, nadciśnienie tętnicze i zaburzenia gospodarki węglowodanowej. Celem pracy poglądowej ***„Human Immunodeficiency Virus as a Risk Factor for Cardiovascular Disease”*** (tłum. „*Wirus nabytego niedoboru odporności jako czynnik ryzyka sercowo-naczyniowego”*) wydanej na łamach czasopisma *Cardiovascular*

Toxicology, DOI: 10.1007/s12012-023-09815-4 było zgromadzenie aktualnych informacji na temat modyfikowalnych i niemodyfikowalnych czynników ryzyka sercowo-naczyniowego w populacji osób zakażonych HIV, z uwzględnieniem wpływu leczenia antyretrowirusowego, znaczenia spadku liczby limfocytów T CD4+ i odbudowy immunologicznej.

Odbudowa immunologiczna to jeden z celów leczenia antyretrowirusowego, a występowanie chorób sercowo-naczyniowych to jeden z największych problemów medycznych, z którymi mierzą się obecnie pacjenci zakażeni HIV poddawani terapii antyretrowirusowej. Oba te problemy są wieloczynnikowe, a mechanizmy i czynniki ryzyka wszystkich zjawisk nie zostały jeszcze odkryte. Najnowsze badania analizują wpływ różnorodnych cytokin i aktywności enzymów (jak interleukiny, TNF-α, czy aktywność kaspaz) na komórki śródbłonka, naczyniowe komórki mięśni gładkich oraz limfocyty T CD4+, co może przyczyniać się do wzrostu ryzyka chorób układu krążenia lub zwiększać podatność limfocytów T CD4+ na pyroptozę. [40, 41] Mimo obiecujących wyników, badania wciąż są na wczesnym etapie i wymagają wnikliwej analizy.

Założenia i cel pracy

Celem przeprowadzonych badań była ocena wpływu leczenia antyretrowirusowego na ryzyko sercowo-naczyniowe, wyrażone za pomocą oceny funkcji śródbłonka naczyń oraz poszukiwanie czynników klinicznych i biochemicznych wpływających na odbudowę immunologiczną po włączeniu leczenia antyretrowirusowego.

Pierwszy etap badań dotyczył oceny funkcji śródbłonka poprzez pomiar stężenia VCAM-1 we krwi odwodowej. Celem pracy ***„VCAM-1 as a Biomarker of Endothelial Function among HIV-Infected Patients Receiving and Not Receiving Antiretroviral Therapy”*** (tłum. *„VCAM-1 jako biomarker funkcji śródbłonka u pacjentów zakażonych HIV otrzymujących i nieotrzymujących leczenia antyretrowirusowego”*) Viruses. 2022 Mar 11;14(3):578. doi: 10.3390/v14030578. PMID: 35336985; PMCID: PMC8955345 była obserwacja potencjalnych różnic w stężeniu VCAM-1 u pacjentów uprzednio leczonych i nieleczonych antyretrowirusowo. Wśród pacjentów leczonych antyretrowirusowo dokonano również porównania stężenia VCAM-1 w zależności od długości leczenia. Dodatkowym celem pracy była analiza czynników wpływających na zmiany stężenia VCAM-1, jak wiek, ko-infekcje HCV lub HBV czy palenie papierosów w badanej populacji. W pracy naukowej oprócz stężenia VCAM-1 oceniano również różnice w liczbie limfocytów T CD4+, stężeniach cholesterolu całkowitego, LDL, HDL i trójglicerydów w zależności od stosowania leczenia antyretrowirusowego oraz, u osób leczonych, od jego długości. Badanie zostało zatwierdzone przez Komisję Bioetyczną Warszawskiego Uniwersytetu Medycznego (AKBE/128/2021).

Kolejnym etapem badań była obserwacja odbudowy immunologicznej po włączeniu leczenia antyretrowirusowego u osób zakażonych HIV. Celem pracy była obserwacja zmian liczby limfocytów CD4+ oraz stosunku limfocytów CD4+:CD8+ przez pięć lat od momentu włączenia leczenia antyretrowirusowego. Dodatkowym celem była ocena, w jaki sposób poszczególne czynniki, jak wiek, płeć, choroby oportunistyczne, choroby niezależne od AIDS, zakażenie HCV i HBV, a także liczba limfocytów T CD4+, wiremia HIV i faza zakażenia HIV w momencie włączenia leczenia wpływają na szansę normalizacji parametrów immunologicznych pod wpływem leczenia antyretrowirusowego. Badanie zostało zatwierdzone przez komisję bioetyczną

(AKBE/188/2023), a wyniki zostały opublikowane w pracy ***„Evaluation of Clinical Biomarkers Related to CD4 Recovery in HIV-Infected Patients—5-Year Observation”*** (tłum. *„Analiza biomarkerów klinicznych związanych z odbudową liczby limfocytów CD4 u pacjentów zakażonych HIV – pięcioletnia obserwacja”*) Viruses. 2022 Oct 18;14(10):2287. doi: 10.3390/v14102287. PMID: 36298842; PMCID: PMC9607521.

Celem pracy poglądowej ***„Human Immunodeficiency Virus as a Risk Factor for Cardiovascular Disease”*** (tłum. „*Wirus nabytego niedoboru odporności jako czynnik ryzyka sercowo-naczyniowego”*) Cardiovascular Toxicology. 2024 Jan;24(1):1-14. doi: 10.1007/s12012-023-09815-4. Epub 2023 Nov 20. PMID: 37982976; PMCID: PMC10838226 było zgromadzenie aktualnych informacji na temat modyfikowalnych i niemodyfikowalnych czynników ryzyka sercowo-naczyniowego w populacji osób zakażonych HIV, z uwzględnieniem parametrów stanu zapalnego i zmian ich stężenia, wpływu leczenia antyretrowirusowego oraz liczby limfocytów T CD4+. Założeniem pracy było zebranie najnowszej wiedzy dotyczącej mechanizmów ryzyka sercowo-naczyniowego u osób zakażonych HIV.

Kopie opublikowanych prac

„VCAM-1 as a Biomarker of Endothelial Function among HIV-Infected Patients Receiving and Not Receiving Antiretroviral Therapy” (tłum. *„VCAM-1 jako biomarker funkcji śródbłonka u pacjentów zakażonych HIV otrzymujących i nieotrzymujących leczenia antyretrowirusowego”*) Viruses, 2022, 14, 578. DOI: 10.3390/v14030578

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VCAM-1 as a Biomarker of Endothelial Function among HIV-Infected Patients Receiving and Not Receiving Antiretroviral Therapy

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Abstract: The Human Immunodeficiency Virus and retroviral therapy are both known risk factors for cardiovascular disease. It remains an open question whether HIV or ARV leads to increased arterial inflammation. The objective of this study was to investigate the changes in endothelial activation by measuring VCAM-1 levels among HIV-infected patients who were and were not treated with antiretroviral therapy. It is a retrospective study that included 68 HIV-infected patients, 23 of whom were never antiretroviral-treated, 15 who were ART-treated for no longer than a year, and 30 who were ART-treated for longer than a year. Blood samples were collected for biochemical analysis of the concentration of VCAM-1. The results show a statistically lower VCAM-1 level ($p = 0.007$) in patients treated with ART longer than a year (1442 ng/mL) in comparison to treatment-naïve patients (2392 ng/mL). The average VCAM-1 level in patients treated no longer than a year (1552 ng/mL) was also lower than in treatment-naïve patients, but with no statistical significance ($p = 0.096$). Long-term antiretroviral therapy was associated with the decline of VCAM-1 concentration. That may suggest the lowering of endothelial activation and the decreased risk of the development of cardiovascular disease among ARV-treated patients. However, VCAM-1 may not be a sufficient factor itself to assess this, since simultaneously there are a lot of well-known cardiovascular-adverse effects of ART.

Keywords: VCAM-1; HIV; cardiovascular; endothelium; marker

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1. Introduction

It is estimated that up to 38 million people in the world are infected with the Human Immunodeficiency Virus (HIV) [1]. Antiretroviral therapy is the best option for sustaining viral suppression and reducing HIV-related mortality. In Poland, over 12 thousand people living with HIV (PLHIV) are receiving antiretroviral treatment [2].

HIV is known to be a risk factor for cardiovascular disease (CVD) [3]. A cardiovascular disease is a group of circulatory system disorders whose underlying cause is most often atherosclerosis [4]. In general, population mortality rates from CVD are decreasing, whereas among HIV–infected patients they are even increasing [5]. Recent studies show that people living with HIV have a higher risk of CVD, particularly heart failure and stroke [6]. Even patients who receive effective therapy are still prone to increased arterial inflammation and impaired smooth muscle function [7], which promote atherosclerosis and plaque formation. These changes in blood vessels play a significant role in the development of cardiovascular disease [8]. HIV-infected patients undergoing antiretroviral therapy (ART) have a 50% to 200% higher risk of heart failure than the matched HIV-negative community [9]. These results remain the same even after the correction of standard risk factors such as age, gender, and smoking status [10,11].

Vascular cell adhesion molecule-1 (VCAM-1) is a protein that functions as a cell adhesion molecule [12]. When cytokines stimulate the endothelial cells, the VCAM-1 gene is expressed on both large and small blood vessels [13]. This phenomenon occurs during the inflammatory process. VCAM-1 triggers endothelial signaling through NADPH oxidase-generated reactive oxygen species. This leads to the opening of intercellular passageways for the migration of leukocytes [14]. However, quiescent endothelial cells do not induce VCAM-1 expression by themselves [15]. The response to cytokines and chemokines concerns not only endothelial cells but also cardiac cells, especially fibroblasts. They respond to several chemoattractants released during cardiac injury, which involves damage-associated molecular patterns. This, through many pathways such as PI3K/AKT or NF-KB, induces VCAM-1 expression, which therefore leads to the leukocytes' recruitment [16].

Another important factor that initiates an inflammatory response is inflammasomes. The NLRP3 (NLR-family pyrin domain-containing protein 3) is a very characteristic inflammasome, whose assembly leads to caspase 1-dependent release of the cytokines IL-1β and IL-18 and pyroptosis, which is a programmed cell death. VCAM-1 is one of the triggers that induces the NLRP3 inflammasome. Vascular endothelium plays an important role in the regulation of inflammation progression, and therefore in cardiovascular implications, such as cardiovascular disease or metabolic syndrome. There are studies which investigate the influence of NLRP3 inflammasome-targeting drugs on endothelial dysfunction. This requires further studies; however, there are presumptions that the inhibition of the NLRP3 inflammasome could contribute to the improvement of endothelial functions [17].

Due to these properties, VCAM-1 is a diagnostic biomarker used in many clinical studies to estimate endothelial dysfunction, which is a risk factor for cardiovascular diseases [18].

This study aimed to assess the possible differences in endothelial activation in HIV-infected patients who were and were not treated with antiretroviral therapy. This was performed by the evaluation of the association between VCAM-1 concentration and the duration of antiretroviral therapy.

2. Materials and Methods

2.1. Patients

We studied the population of 68 adult patients. All data comes from the Department of Infectious and Tropical Diseases and Hepatology, the Medical University of Warsaw from the period of 2009–2014. The inclusion criteria were HIV infection, regardless of the stage of illness and time of treatment and age 18 or older. The exclusion criteria were the occurrence of ischaemic heart disease and undergoing lipid-lowering therapy.

2.2. Assessments

All patients underwent physical examination and laboratory testing. Blood samples were collected for total cholesterol, HDL-cholesterol, LDL-cholesterol, triglyceride level, CD4, CD8 count and percentage, and HIV viral load. VCAM-1 concentration was measured in serum with the usage of The Quantikine® Human VCAM-1/CD106 Immunoassay. This method is a 2-h solid-phase ELISA that employs an enzyme-linked monoclonal antibody specific for human VCAM-1. Any VCAM-1 present in samples is sandwiched by that monoclonal antibody and the immobilized antibody. Then a substrate solution is added and color develops in proportion to the amount of VCAM-1. The lower limit of the VCAM-1/CD106 Immunoassay is 6.3 ng/mL [19].

2.3. Statistical Analysis

The ANOVA test was used to evaluate the difference in mean value among quantitative variables. The Tukey HSD test was used as a post hoc test for the assessment of statistical significance in variables among two groups of patients. The *p*-value was set at 0.05. All statistical analyses were performed using Python 3.7 software.

3. Results

3.1. Patients

Among 68 patients, 23 were ART-naïve (18 men, 5 women), 15 were receiving antiretroviral therapy shorter than a year (12 men, 3 women), and 30 were treated longer than a year (23 men, 7 women). The characteristics of the patients are presented in Table 1.

Table 1. Basic characteristics of the patients.

Characteristics of the Patients	ART-Naïve Patients	≤1 Year of ART Therapy	>1 Year of ART Treatment	*p*
Age (years)	34.3 (25–49)	34.4 (21–74)	45.6 (30–70)	0.000
VCAM-1 (ng/mL)	2392 (534–5198)	1552 (662–3364)	1442 (246–7166)	0.008
Total cholesterol (mmol/L)	3.66 (1.28–4.94)	4.36 (2.74–7.18)	4.42 (2.17–6.25)	0.032
LDL-cholesterol (mmol/L)	1.94 (0.47–4.48)	2.53 (1.36–4.94)	2.53 (1.22–4.44)	0.041
HDL-cholesterol (mmol/L)	1.09 (0.4–2.1)	1.22 (0.68–1.9)	1.39 (0.32–2.9)	0.108
Triglyceride (mmol/L)	1.61 (0.82–2.9)	1.61 (0.93–2.57)	1.93 (0.88–5.29)	0.305
CD4 (cells/μL)	212 (6–482)	282 (27–704)	413 (103–791)	0.003
CD4 (%)	24.6 (3–58)	25.5 (6–56)	34 (11–67)	0.056
CD8 (cells/μL)	537 (71–1391)	831 (65–1770)	921 (78–2666)	0.041
CD8 (%)	73.7 (41–92)	71.1 (45–89)	67 (40–91)	0.233
CD4:CD8	0.398 (0.04–0.9)	0.399 (0.07–1.26)	0.597 (0.09–1.7)	0.117
Viral load (copies/mL)	901,160 (0–10,000,000)	83,557 (0–746,695)	11,499 (0–226,006)	0.031
Co-infections	2 patients–HBV 5 patients–HCV 2 patients–HBV/HCV	2 patients–HBV 2 patients–HCV 2 patients–HBV/HCV	5 patients–HBV 11 patients-HCV	0.047
Smoking cigarettes	16	9	22	0.185
Length of therapy (weeks)	0	3–52	76–988	0.000
Average length of therapy (weeks)	0	21.6	300.4	0.000
Median length of therapy (weeks)	0	20	222.5	0.000

3.2. Antiretroviral Therapy

Table 2 presents the data concerning the composition of antiretroviral therapy used among examined patients. Of the group of 23 naïve patients, 9 were newly diagnosed with HIV, and 14 were not treated before.

Table 2. Antiretroviral therapy among patients.

Applied Antiretroviral Therapy	(*n*)
Nucleoside Reverse Transcriptase Inhibitors (NRTI)	92
Protease Inhibitors (PI)	39 [1]
Non-nucleoside Reverse Transcriptase Inhibitors (NNRTI)	9
Integrase Inhibitors (II)	2

[1] Four without ritonavir as a booster.

3.3. Measurement of VCAM-1 Concentration in Healthy Volunteers

The mean VCAM-1 concentration among 36 healthy volunteers was 557 ng/mL. The results varied from 349 to 991 ng/mL and the standard deviation was 139.6 ng/mL [16]. No negative samples were obtained from healthy volunteers. Figure 1 presents the comparison of the results among the healthy volunteers and examined groups of patients.

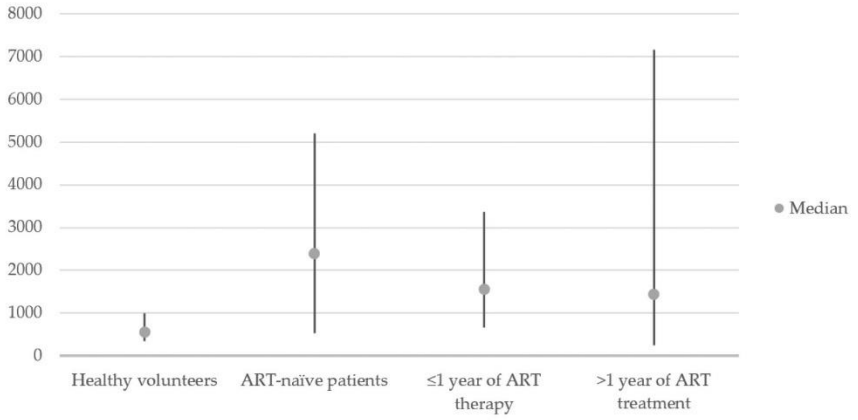

Figure 1. Distribution of VCAM-1 concentrations in healthy volunteers and examined groups of patients.

3.4. VCAM-1 Concentration in Examined Groups of Patients

The ANOVA test was performed to assess the difference among VCAM-1 concentrations in patients with different durations of the antiretroviral therapy. Since it showed statistical significance ($p = 0.008$), we performed further analysis. There were no negative samples among the examined patients. Table 3 shows the results.

Table 3. Post hoc tests assessing VCAM-1 concentrations and length of antiretroviral therapy using Tukey HSD test.

Compared Groups -Length of the Antiretroviral Therapy (Years)	Average Differential in VCAM-1 Concentration (ng/mL)	*p*
Naïve vs. treated ≤1 year	840 (−180–1800)	0.096
≤1 year vs. treated >1 year	200 (−740–1120)	0.871
Naïve vs. treated >1 year	1040 (240–1840)	0.007

3.5. Coinfections

HIV infection was not the only chronic viral infection among our patients. In total, 31 out of 68 analyzed patients were coinfected. In total there were, 9 patients with the Hepatitis B Virus (HBV), 18 patients with the Hepatitis C Virus (HCV), 4 patients with both HBV and HCV. Therefore, we assessed the relationship between coinfection and VCAM-1 concentration. The results are shown in Table 4.

Table 4. The results of the ANOVA test assessing coinfections and VCAM-1 concentration.

	No Coinfection	HCV Coinfection	HBV Coinfection	HCV and HBV Coinfections	*p*
VCAM-1 concentration	1453.8 (246–3826)	2497.8 (628–7166)	2265.4 (664–5198)	2207.6 (1122–3130)	0.047

Since the *p*-value was <0.05, we performed the Tukey HSD test for further investigation and presented the results in Table 5.

Table 5. The results of the Tukey HSD test comparing coinfections and VCAM-1 concentration.

Compared Groups of Patients-Coinfections	Average Differential in VCAM-1 Concentration (ng/mL)	*p*
No coinfections vs. HCV coinfection	1044 (9.2–2078.8)	0.047
No coinfections vs. HBV coinfection	811.6 (−503.2–2126.4)	0.371
No coinfections vs. HCV and HBV coinfections	753.8 (−1110.6–2618)	0.689
HCV coinfection vs. HBV coinfection	−232.4 (−1694.4–1229.6)	0.9
HCV coinfection vs. HCV and HBV coinfections	−290.2 (−2261.2–1680.6)	0.9
HBV coinfection vs. HCV and HBV coinfections	−57.8 (−2189–2073.4)	0.9

We observed statistical significance in the VCAM-1 serum concentration among patients with HCV coinfection and patients who were not coinfected. The remaining coinfections were not statistically significant.

3.6. Age Correlation

The average age of patients from our three groups differed, especially between the naïve patients (the average of 34.3 years) and patients treated longer than a year (the average of 45.6 years). There was also a difference in the VCAM-1 level in those two groups, so we decided to examine the correlation between our patients' age and the level of VCAM-1, which appeared to be statistically insignificant. We located the results in Table 6.

Table 6. The correlation between patients' age and VCAM-1 level.

The Group of Patients–Length of Antiretroviral Therapy	r-Value	*p*-Value
All patients	−0.14	0.244
ARV-naive patients	0.20	0.334
ARV $\leq$ 1 year	0.06	0.818
ARV > 1 year	−0.08	0.672

3.7. Smoking Cigarettes

We analyzed the habit of smoking among our patients. We classified smoking patients as those who were smoking at least 20 cigarettes per day for a minimum of 5 years. In treatment-naïve patients, 16 were smoking and 7 were not. Of patients treated for less than a year, nine were smoking and six were not. In addition, among patients receiving ART for longer than a year, 22 were smoking and 8 were not. Among 47 out of 68 analyzed patients declared tobacco use, which stated 69%. The results of the statistical analysis concerning smoking are presented in Table 7.

Table 7. The statistical significance of smoking and VCAM-1 concentration.

	VCAM-1 Concentration in Smoking Patients	VCAM-1 Concentration in Non-Smoking Patients	*p*
VCAM-1 concentration	2017.2 (246–7166)	1529.2 (528–5198)	0.185

There was no significance in VCAM-1 concentration among patients who were or were not smoking cigarettes.

4. Discussion

Our findings point out that endothelial activation concerns many people living with HIV. This activation was indicated especially among people who were HIV-positive and were not receiving antiretroviral treatment. That may suggest that this group of people could be the most susceptible to the development of cardiovascular disease. Our results

have been supported by studies that show that there exists an association between untreated HIV infection and an increased risk of CVD [20] and that a vascular inflammatory process that is reflected by a pattern of endothelial activation occurs through untreated HIV infection [21]. On the other hand, a higher risk of CVD and lipid metabolism disorders are the known adverse effects of ART [22]. At present, there are a lot of controversial debates about whether HIV-infected patients receiving antiretroviral therapy are more prone to developing CVD than those who are treatment-naïve [23]. Some studies show that patients receiving antiretroviral therapy (ART), including protease inhibitors (PI), more often develop lipodystrophy, dyslipidemia, direct mitochondrial DNA damage, and insulin resistance than HIV-positive individuals who are treatment-naïve [24]. The duration of exposure to ART as well as the drug class appeared to be the important factors in myocardial infarction. Among our patients, thirty-seven were treated with ART, including protease inhibitors, and we still observed the lowering of VCAM-1 serum concentration in comparison to treatment-naïve patients. In some studies, the levels of VCAM-1, which is the marker of endothelium activation, were lowered by short-term ART [25]. In contrast to those reports, the analysis of our patients does not show a statistically important difference between the group of patients treated less than a year and the group of non-treated patients. The reason for this situation is probably that the number of patients is different in both groups (15 patients treated for no longer than a year in comparison to 23 naïve patients). To summarize, it is evident that the initiation of effective ART ameliorates vascular inflammation but is not able to fully correct it. Research supports the hypothesis that the HIV viral load may directly result in an atherogenic milieu in untreated HIV infection [18], which can also be suggested in our analysis.

4.1. VCAM-1 Concentration According to Patients' Age

Since our patients were at different ages, it is essential to consider this. Chronic inflammation, which comprises the endothelium, is associated with HIV infection [26] and the natural aging process without HIV infection [27]. However, endothelial dysfunction occurs earlier and is accelerated in HIV-positive subjects [28]. The correlation between our patients' age and the level of VCAM-1 concentration appeared to be statistically insignificant. Other studies seem to support these results by showing that there is no elevated frailty caused by the HIV-associated inflammation that could be raveled in similarly aged uninfected individuals [29].

4.2. Smoking Cigarettes and VCAM-1 Concentration

Another important subject is tobacco usage, since 69% of our patients were smoking at least 20 cigarettes per day for a minimum of 5 years. Globally, smoking is more common among people who are HIV-infected than in the general population [30]. However, we did not observe a statistically significant difference in VCAM-1 serum concentration among patients who were and were not smokers. That could be the result of the small population of non-smoking patients in our study. Studies show that smoking is associated with an elevated cardiovascular risk, including coronary artery disease, peripheral vascular disease, ischaemic heart disease, atherosclerosis, myocardial infarction, and stroke [31]. Tobacco use, as well as HIV infection itself, were associated with increased endothelial biomarker levels [32].

4.3. Impact of Coinfections on VCAM-1 Concentration

HIV, HBV, and HCV are the most common chronic viral infections documented worldwide [33]. The causes of this phenomenon have similar ways of spreading, which are blood and blood products, sharing of needles to inject drugs, and sexual activity [34]. In our patients, co-infections were mostly occurring in the group of individuals that were ART-treated for longer than a year (16 patients).

Chronic Hepatitis C (CHC) patients are more likely to suffer from both liver disease and cardiovascular disease (CVD). Studies have shown a higher spread of type 2 diabetes

mellitus (DM), insulin resistance, and hepatic steatosis, which are known CVD risk factors in HCV-positive patients compared to uninfected individuals [35]. Furthermore, recent studies have shown that HCV infection is a direct risk factor for subclinical and clinical cardiovascular disease (CVD) [36] and is directly connected to arteriosclerosis. HCV RNA sequences have been found in the plaque tissues of patients who underwent carotid revascularization. It shows that HCV RNA sequences seem to play a local effect on the endothelium [37], which can be the reason for increased levels of VCAM-1 in patients with liver diseases such as liver cirrhosis or chronic hepatitis C (CHC). Among our patients, there were 18 individuals infected with both HIV and HCV, and the level of VCAM-1 concentration was significantly increased in this group of patients. Some studies have corresponded with a slight increase in CVD among subjects with HIV-HCV co-infection in contrast to those without HCV co-infection [38], and our study supports this thesis.

On the other hand, HBV was not related to the time of CVD occurrence [38]. The natural mileage of HBV infection is meaningfully altered by HIV co-infection, as it is more aggressive with higher HBV DNA levels and lower inflammatory activity [39]. We also did not observe a significantly higher concentration of VCAM-1 among patients with HBV co-infection. It is also known that HIV, HBV, and HCV co-infection are connected with liver-related deaths [40]. Only four of our patients were co-infected with both HCV and HBV, and therefore we cannot conclude about the CVD occurrence in this population.

4.4. Dyslipidemia and Endothelial Dysfunction

Studies suggest that there is a higher prevalence of dyslipidemia among HIV-infected patients [41]. Dyslipidemia concerns both ART-treated and non-treated patients and is associated with a higher risk of stroke and myocardial infarction [10]. Since it is closely connected to CVD, we decided to evaluate the differences in total cholesterol, triglycerides, HDL-cholesterol, LDL-cholesterol among patients who are treatment-naïve, and ART-treated for shorter and longer than a year. Both total cholesterol and LDL-cholesterol levels appeared significantly higher among patients undergoing antiretroviral therapy than in treatment-naïve individuals, whereas HDL-cholesterol and triglycerides were statistically insignificant. However, despite the elevation of the concentration of total cholesterol and LDL-cholesterol, the VCAM-1 concentration was significantly lower among the treated patients.

4.5. Severity of HIV Infection

Our study showed statistical significance in CD4, CD8 count, and viral load among the three groups of patients. These results are predictable since effective antiretroviral therapy leads to an increase in CD4 count and a decrease in VL [42]. However, studies discussing the topics of CD4 count, viral load, and endothelial dysfunction are not unambiguous. Some researchers show that more viral copies may have a negative impact on the endothelium [43]. Others claim that neither CD4 count, nor HIV viral load, are the predictors of endothelial dysfunction [44].

4.6. VCAM-1 Targeting Molecules

Evidence suggests that VCAM-1 is associated with multiple disorders such as cardiovascular disease, cancer, rheumatoid arthritis, and asthma. There are many molecules that trigger the expression of VCAM-1. One of the most characteristic VCAM-1 inducing cytokines is TNF-α [45]. It is a member of the TNF/TNFR cytokine superfamily, which is involved in the maintenance of the immune system but also plays an important role in chronic inflammation [46]. Studies also point out that alpha D beta 2, a member of beta 2 integrins, can support eosinophil adhesion to VCAM-1. Moreover, alpha D beta 2 binds to VCAM-1 and can also support lymphoid cell adhesion to VCAM-1 [47]. One study developed the theranostic nanocarriers decorated with VCAM-1 antibodies that seem to localize the endothelial senescence and prevent pro-senescent endothelial responses [48].

Nutraceuticals, which are dietary supplements, are currently gaining attention due to their therapeutic potential [49]. There is research suggesting that some nutraceuticals could improve vascular function. There is a study that tested the use of hyaluronic acid hydrogel of Quercetin on human thyroid cancer cells. Quercetin showed an anti-inflammatory effect via a CD44-dependent interaction with thyroid cancer cells [50]. Since CD44 is known to bind VCAM-1, which decreases tumor growth [51], it is possible that Quercetin could reduce the expression of VCAM-1 and therefore endothelial activation.

4.7. The Role of Hyaluronic Acid in the Imrpovement of Endothelial Function

Hyaluronic acid is a natural polysaccharide that commonly occurs in human bodies. Moreover, it is being used as a pharmaceutical, mostly in ophthalmology. Hyaluronic acid can be cross-linked or conjugated with multiple biomacromolecules. Studies report the importance of interactions of hyaluronic acid with the CD44 receptor in pathological processes such as cancer. Research shows that controlled release of proteins and pharmaceuticals from hyaluronic acid resulted in many benefits in cancer treatment, which included, for example, an enhanced therapeutic effect with minimum toxicity [52]. Another study showed that cross-linked hyaluronic acid sub-micron particles can recognize cancer tissue and can be used to deliver bioactives in a specific and controlled manner to cancerous tissue [53]. The CD44 receptor is crucial in HIV infection. It enhances the infection in CD4(+) T cells. Studies are being developed to assess the role of hyaluronic acid in the mucosal transmission of HIV. Hyaluronic acid seems to reduce HIV infection during the interaction of HIV with CD4(+) in a CD44-dependent manner. It could be relevant to HIV mucosal transmission in general [54]. Since the CD44 receptor also binds VCAM-1, it could also be helpful in reducing endothelial activation. The role of hyaluronic acid in this process is not clear, and it remains an open topic for further studies.

4.8. Limitations of the Study

The main limitation of our study was the small study population. Moreover, we did not perform the calculation and justification of the sample size selected. All of our patients were HIV-positive, since we were not able to compare our results to HIV-negative individuals.

5. Conclusions

In our study, we observed that the concentration of VCAM-1, which is the marker of endothelial activation, was statistically lower in HIV-infected patients on antiretroviral therapy for longer than a year than in HIV-infected patients who were not receiving the therapy. That means that effective antiretroviral therapy may potentially inhibit the influence of the virus on the endothelium. This leads us to the conclusion that it is possible that long-term antiretroviral therapy may be associated with a lower risk of cardiovascular disease than untreated HIV infection. However, the lowering of VCAM-1 concentration may not be a sufficient factor to assess the improvement of endothelial function. It requires further studies since, simultaneously, there are a lot of well-known cardiovascular adverse effects of ART, including dyslipidemia and hyperglycemia.

6. Patents

This section is not mandatory but may be added if there are patents resulting from the work reported in this manuscript.

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References

1. UNAIDS. Fact Sheet—Latest Statistics on the Status of the AIDS Epidemic. 2019. Available online: https://www.unaids.org/en/resources/fact-sheet (accessed on 31 January 2020).
2. National AIDS Centre. Basic Epidemiologic Information. 2019. Available online: https://aids.gov.pl/hiv_aids/450-2-2/ (accessed on 31 January 2020).
3. Rosenson, R.S.; Hubbard, D.; Monda, K.L.; Reading, S.R.; Chen, L.; Dluzniewski, P.J.; Burkholder, G.A.; Muntner, P.; Colantonio, L.D. Excess Risk for Atherosclerotic Cardiovascular Outcomes among US Adults with HIV in the Current Era. *J. Am. Hear. Assoc.* **2020**, *9*, e013744. [CrossRef] [PubMed]
4. Kralj, V.; Bilos, I.B. Morbidity and mortality from cardiovascular diseases. *Cardiol. Croat.* **2013**, *8*, 373–378. [CrossRef]
5. Feinstein, M.J.; Bahiru, E.; Achenbach, C.; Longenecker, C.T.; Hsue, P.; So-Armah, K.; Freiberg, M.S.; Lloyd-Jones, D. Patterns of Cardiovascular Mortality for HIV-Infected Adults in the United States: 1999 to 2013. *Am. J. Cardiol.* **2016**, *117*, 214–220. [CrossRef] [PubMed]
6. Alonso, A.; Barnes, A.E.; Guest, J.L.; Shah, A.; Shao, I.Y.; Marconi, V. HIV Infection and Incidence of Cardiovascular Diseases: An Analysis of a Large Healthcare Database. *J. Am. Hear. Assoc.* **2019**, *8*, e012241. [CrossRef]
7. Grinspoon, S. Novel mechanisms and anti-inflammatory strategies to reduce cardiovascular risk in human immunodeficiency virus. *Trans. Am. Clin. Clim. Assoc.* **2018**, *129*, 140–154.
8. Hsue, P.Y.; Giri, K.; Erickson, S.; MacGregor, J.S.; Younes, N.; Shergill, A.; Waters, D.D. Clinical Features of Acute Coronary Syndromes in Patients with Human Immunodeficiency Virus Infection. *Circulation* **2004**, *109*, 316–319. [CrossRef]
9. Butt, A.A.; Chang, C.-C.; Kuller, L.; Goetz, M.B.; Leaf, D.; Rimland, D.; Gibert, C.L.; Oursler, K.K.; Rodriguez-Barradas, M.C.; Lim, J.; et al. Risk of Heart Failure with Human Immunodeficiency Virus in the Absence of Prior Diagnosis of Coronary Heart Disease. *Arch. Intern. Med.* **2011**, *171*, 737–743. [CrossRef]
10. Freiberg, M.S.; Chang, C.C.H.; Kuller, L.H.; Skanderson, M.; Lowy, E.; Kraemer, K.L.; Butt, A.A.; Goetz, M.B.; Leaf, D.; Oursler, K.A.; et al. HIV Infection and the Risk of Acute Myocardial Infarction. *JAMA Intern. Med.* **2013**, *173*, 614–622. [CrossRef]
11. Womack, J.A.; Chang, C.H.; So-Armah, K.; Alcorn, C.; Baker, J.V.; Brown, S.T.; Budoff, M.; Butt, A.; Gibert, C.; Goetz, M.; et al. HIV Infection and Cardiovascular Disease in Women. *J. Am. Hear. Assoc.* **2014**, *3*, e001035. [CrossRef]
12. Cybulsky, M.; Fries, J.W.; Williams, A.J.; Sultan, P.; Eddy, R.L.; Byers, M.G.; Shows, T.B.; Gimbrone, M.A., Jr.; Collins, T. The human VCAM1 gene is assigned to chromosome 1p31–p32. *Cytogenet. Genome Res.* **1991**, *58*, 1852.
13. VCAM1 Vascular Cell Adhesion Molecule 1. Available online: https://www.ncbi.nlm.nih.gov/gene/7412 (accessed on 31 January 2020).
14. Cook-Mills, J.M.; Marchese, M.E.; Abdala-Valencia, H. Vascular Cell Adhesion Molecule-1 Expression and Signaling during Disease: Regulation by Reactive Oxygen Species and Antioxidants. *Antioxid. Redox Signal.* **2011**, *15*, 1607–1638. [CrossRef] [PubMed]
15. Jefferson, A.; Ruparelia, N.; Choudhury, R.P. Exogenous Microparticles of Iron Oxide Bind to Activated Endothelial Cells but, Unlike Monocytes, Do Not Trigger an Endothelial Response. *Theranostics* **2013**, *3*, 428–436. [CrossRef] [PubMed]
16. Olivares-Silva, F.; Landaeta, R.; Aránguiz, P.; Bolivar, S.; Humeres, C.; Anfossi, R.; Vivar, R.; Boza, P.; Muñoz, C.; Pardo-Jiménez, V.; et al. Heparan sulfate potentiates leukocyte adhesion on cardiac fibroblast by enhancing Vcam-1 and Icam-1 expression. *Biochim. Biophys. Acta Mol. Basis Dis.* **2018**, *1864*, 831–842. [CrossRef] [PubMed]
17. Bai, B.; Yang, Y.; Wang, Q.; Li, M.; Tian, C.; Liu, Y.; Aung, L.H.H.; Li, P.-F.; Yu, T.; Chu, X.-M. NLRP3 inflammasome in endothelial dysfunction. *Cell Death Dis.* **2020**, *11*, 776. [CrossRef]
18. Figueras-Aloy, J.; Gómez-López, L.; Rodríguez-Miguélez, J.-M.; Salvia-Roiges, M.D.; Jordán-García, I.; Ferrer-Codina, I.; Carbonell-Estrany, X.; Jiménez-González, R. Serum Soluble ICAM-1, VCAM-1, L-Selectin, and P-Selectin Levels as Markers of Infection and their Relation to Clinical Severity in Neonatal Sepsis. *Am. J. Perinatol.* **2007**, *24*, 331–338. [CrossRef]
19. Quantikine®ELISA Human VCAM-1/CD106 Immunoassay Catalog Number DVC00, SVC00, PDVC00; For the Quantitative Determination of Human Vascular Cell Adhesion Molecule-1 (VCAM-1) Concentrations in Cell Culture Supernates, Serum, and Plasma. 2017, R&D Systems 1-2,9. Available online: https://www.antibodies-online.com/kit/366645/Vascular+Cell+Adhesion+Molecule+1+VCAM1+ELISA+Kit/ (accessed on 14 February 2022).
20. Kingery, J.R.; Alfred, Y.; Smart, L.; Nash, E.; Todd, J.; Naguib, M.R.; Downs, J.; Kalluvya, S.; Kataraihya, J.B.; Peck, R. Short-term and long-term cardiovascular risk, metabolic syndrome and HIV in Tanzania. *Heart* **2016**, *102*, 1200–1205. [CrossRef]

21. O'Halloran, J.; Dunne, E.; Gurwith, M.; Lambert, J.S.; Sheehan, G.; Feeney, E.; Pozniak, A.; Reiss, P.; Kenny, D.; Mallon, P. The effect of initiation of antiretroviral therapy on monocyte, endothelial and platelet function in HIV-1 infection. *HIV Med.* **2015**, *16*, 608–619. [CrossRef]
22. Sutton, S.S.; Magagnoli, J.; Cummings, T.H.; Hardin, J.W.; Edun, B.; Beaubrun, A. Chronic kidney disease, cardiovascular disease, and osteoporotic fractures in patients with and without HIV in the US Veteran's Affairs Administration System. *Curr. Med. Res. Opin.* **2019**, *35*, 117–125. [CrossRef]
23. Wang, T.; Yi, R.; Green, L.A.; Chelvanambi, S.; Seimetz, M.; Clauss, M. Increased cardiovascular disease risk in the HIV-positive population on ART: Potential role of HIV-Nef and Tat. *Cardiovasc. Pathol.* **2015**, *24*, 279–282. [CrossRef]
24. Lambert, C.; Sandesara, P.; Hirsh, B.; Shaw, L.; Lewis, W.; Quyyumi, A.; Schinazi, R.; Post, W.; Sperling, L. HIV, highly active antiretroviral therapy and the heart: A cellular to epidemiological review. *HIV Med.* **2016**, *17*, 411–424. [CrossRef]
25. Francisci, D.; Giannini, S.; Baldelli, F.; Leone, M.; Belfiori, B.; Guglielmini, G.; Malincarne, L.; Gresele, P. HIV type 1 infection, and not short-term HAART, induces endothelial dysfunction. *AIDS* **2009**, *23*, 589–596. [CrossRef] [PubMed]
26. Hsu, D.C.; Sereti, I. Serious Non-AIDS Events: Therapeutic Targets of Immune Activation and Chronic Inflammation in HIV Infection. *Drugs* **2016**, *76*, 533–549. [CrossRef] [PubMed]
27. Kaluz, S.; Tan, C.; Van Meir, E.G. Taking a HIF pill for old age diseases? *Aging* **2018**, *10*, 290–292. [CrossRef] [PubMed]
28. Friis-Moller, N.; Reiss, P.; Sabin, C.; Weber, R.; Monforte, A.D.; El-Sadr, W.; Thiebaut, R.; De Wit, S.; Kirk, O.; Fontas, E.E.; et al. Class of Antiretroviral Drugs and the Risk of Myocardial Infarction. *N. Engl. J. Med.* **2007**, *356*, 1723–1735. [CrossRef]
29. Wallet, M.A.; Buford, T.W.; Joseph, A.-M.; Sankuratri, M.; Leeuwenburgh, C.; Pahor, M.; Manini, T.M.; Sleasman, J.W.; Goodenow, M.M. Increased inflammation but similar physical composition and function in older-aged, HIV-1 infected subjects. *BMC Immunol.* **2015**, *16*, 43. [CrossRef]
30. Jaquet, A.; Dabis, F. Smoking status and HIV in low-income and middle-income countries. *Lancet Glob. Health* **2017**, *5*, e557–e558. [CrossRef]
31. Shah, R.S.; Cole, J.W. Smoking and stroke: The more you smoke the more you stroke. *Expert Rev. Cardiovasc. Ther.* **2010**, *8*, 917–932. [CrossRef]
32. Ahlström, M.; Knudsen, A.; Ullum, H.; Gerstoft, J.; Kjaer, A.; Lebech, A.-M.; Hasbak, P.; Obel, N. Association between smoking status assessed with plasma-cotinine and inflammatory and endothelial biomarkers in HIV-positive and HIV-negative individuals. *HIV Med.* **2018**, *19*, 679–687. [CrossRef]
33. McCarron, B.; Main, J.; Thomas, H.C. HIV and hepatotropic viruses: Interactions and treatments. *Int. J. STD AIDS* **1997**, *8*, 739–746. [CrossRef]
34. Horvath, J.; Raffanti, S.P. Clinical Aspects of the Interactions between Human Immunodeficiency Virus and the Hepatotropic Viruses. *Clin. Infect. Dis.* **1994**, *18*, 339–347. [CrossRef]
35. Babiker, A.; Hassan, M.; Muhammed, S.; Taylor, G.; Poonia, B.; Shah, A.; Bagchi, S. Inflammatory and cardiovascular diseases biomarkers in chronic hepatitis C virus infection: A review. *Clin. Cardiol.* **2019**, *43*, 222–234. [CrossRef] [PubMed]
36. Petta, S.; Maida, M.F.; Macaluso, F.S.; Barbara, M.; Licata, A.; Craxì, A.; Cammà, C. Hepatitis C Virus Infection Is Associated with Increased Cardiovascular Mortality: A Meta-Analysis of Observational Studies. *Gastroenterology* **2016**, *150*, 145–155. [CrossRef] [PubMed]
37. Boddi, M.; Abbate, R.; Chellini, B.; Giusti, B.; Solazzo, V.; Sofi, F.; Pratesi, G.; Pratesi, C.; Gensini, G.; Zignego, A.L. HCV infection facilitates asymptomatic carotid atherosclerosis: Preliminary report of HCV RNA localization in human carotid plaques. *Dig. Liver Dis.* **2007**, *39*, S55–S60. [CrossRef]
38. Gillis, J.; the OHTN Cohort Study Group; Smieja, M.; Cescon, A.; Rourke, S.B.; Burchell, A.N.; Cooper, C.; Raboud, J.M. Risk of cardiovascular disease associated with HCV and HBV co-infection among antiretroviral-treated HIV-infected individuals. *Antivir. Ther.* **2014**, *19*, 309–317. [CrossRef]
39. Guha, S.K.; Sarkar, J.; Saha, D.; Bandyopadhyay, B.; Saha, B.; Kedia, D.; Mazumder, D.G.; Chakravarty, R. Baseline characteristics of HIV & hepatitis B virus (HIV/HBV) co-infected patients from Kolkata, India. *Indian J. Med. Res.* **2016**, *143*, 636–642. [CrossRef]
40. Smith, C.C.; Sabin, C.; Lundgren, J.; Thiebaut, R.; Weber, R.; Monforte, A.D.; Kirk, O.; Friis-Moller, N.; Phillips, A.N.; Reiss, P.; et al. Factors associated with specific causes of death amongst HIV-positive individuals in the D:A:D study. *AIDS* **2010**, *24*, 1537–1548. [CrossRef] [PubMed]
41. Mikhail, N.E. Lipid Abnormalities in Patients Infected with Human Immunodeficiency Virus. *Endocr. Pract.* **2008**, *14*, 492–500. [CrossRef]
42. Mussini, C.; Lorenzini, P.; Cozzi-Lepri, A.; Lapadula, G.; Marchetti, G.; Nicastri, E.; Cingolani, A.; Lichtner, M.; Antinori, A.; Gori, A.; et al. CD4/CD8 ratio normalisation and non-AIDS-related events in individuals with HIV who achieve viral load suppression with antiretroviral therapy: An observational cohort study. *Lancet HIV* **2015**, *2*, e98–e106. [CrossRef]
43. Blum, A.; Hadas, V.; Burke, M.; Yust, I.; Kessler, A. Viral load of the human immunodeficiency virus could be an independent risk factor for endothelial dysfunction. *Clin. Cardiol.* **2005**, *28*, 149–153. [CrossRef]
44. Dubé, M.P.; Shen, C.; Mather, K.J.; Waltz, J.; Greenwald, M.; Gupta, S.K. Relationship of Body Composition, Metabolic Status, Antiretroviral Use, and HIV Disease Factors to Endothelial Dysfunction in HIV-Infected Subjects. *AIDS Res. Hum. Retrovir.* **2010**, *26*, 847–854. [CrossRef]
45. Kong, D.-H.; Kim, Y.K.; Kim, M.R.; Jang, J.H.; Lee, S. Emerging Roles of Vascular Cell Adhesion Molecule-1 (VCAM-1) in Immunological Disorders and Cancer. *Int. J. Mol. Sci.* **2018**, *19*, 1057. [CrossRef] [PubMed]

46. Balkwill, F. TNF-α in promotion and progression of cancer. *Cancer Metastasis Rev.* **2006**, *25*, 409–416. [CrossRef] [PubMed]
47. Van der Vieren, M.; Crowe, D.T.; Hoekstra, D.; Vazeux, R.; Hoffman, P.A.; Grayson, M.H.; Bochner, B.S.; Gallatin, W.M.; Staunton, D.E. The leukocyte integrin αDβ2 binds VCAM-1: Evidence for a binding interface between I domain and VCAM-1. *J. Immunol.* **1999**, *163*, 1984–1990.
48. Belcastro, E.; Rehman, A.U.; Remila, L.; Park, S.-H.; Gong, D.S.; Anton, N.; Auger, C.; Lefebvre, O.; Goetz, J.G.; Collot, M.; et al. Fluorescent nanocarriers targeting VCAM-1 for early detection of senescent endothelial cells. *Nanomed. Nanotechnol. Biol. Med.* **2021**, *34*, 102379. [CrossRef]
49. Sachdeva, V.; Roy, A.; Bharadvaja, N. Current Prospects of Nutraceuticals: A Review. *Curr. Pharm. Biotechnol.* **2020**, *21*, 884–896. [CrossRef] [PubMed]
50. Quagliariello, V.; Armenia, E.; Aurilio, C.; Rosso, F.; Clemente, O.; de Sena, G.; Barbarisi, M.; Barbarisi, A. New Treatment of Medullary and Papillary Human Thyroid Cancer: Biological Effects of Hyaluronic Acid Hydrogel Loaded with Quercetin Alone or in Combination to an Inhibitor of Aurora Kinase. *J. Cell. Physiol.* **2016**, *231*, 1784–1795. [CrossRef]
51. Gomez, K.E.; Wu, F.; Keysar, S.B.; Morton, J.J.; Miller, B.; Chimed, T.-S.; Le, P.N.; Nieto, C.; Chowdhury, F.N.; Tyagi, A.; et al. Cancer cell CD44 mediates macrophage/monocyte-driven regulation of head and neck cancer stem cells. *Cancer Res.* **2020**, *80*, 4185–4198. [CrossRef]
52. Bayer, I.S. Hyaluronic Acid and Controlled Release: A Review. *Molecules* **2020**, *25*, 2649. [CrossRef]
53. Rosso, F.; Quagliariello, V.; Tortora, C.; Di Lazzaro, A.; Barbarisi, A.; Iaffaioli, R.V. Cross-linked hyaluronic acid sub-micron particles: In vitro and in vivo biodistribution study in cancer xenograft model. *J. Mater. Sci. Mater. Med.* **2013**, *24*, 1473–1481. [CrossRef]
54. Li, P.; Fujimoto, K.; Bourguingnon, L.; Yukl, S.; Deeks, S.; Wong, J.K. Exogenous and endogenous hyaluronic acid reduces HIV infection of CD4 + T cells. *Immunol. Cell Biol.* **2014**, *92*, 770–780. [CrossRef]

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Evaluation of Clinical Biomarkers Related to CD4 Recovery in HIV-Infected Patients—5-Year Observation

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Abstract: Human Immunodeficiency Virus infection leads to the impairment of immune system function. Even long-term antiretroviral therapy uncommonly leads to the normalization of CD4 count and CD4:CD8 ratio. The aim of this study was to evaluate possible clinical biomarkers which may be related to CD4 and CD4:CD8 ratio recovery among HIV-infected patients with long-term antiretroviral therapy. The study included 68 HIV-infected patients undergoing sustained antiretroviral treatment for a minimum of 5 years. Clinical biomarkers such as age, gender, advancement of HIV infection, coinfections, comorbidities and applied ART regimens were analyzed in relation to the rates of CD4 and CD4:CD8 increase and normalization rates. The results showed that higher rates of CD4 normalization are associated with younger age ($p = 0.034$), higher CD4 count ($p = 0.034$) and starting the therapy during acute HIV infection ($p = 0.012$). Higher rates of CD4:CD8 ratio normalization are correlated with higher CD4 cell count ($p = 0.022$), high HIV viral load ($p = 0.006$) and acute HIV infection ($p = 0.013$). We did not observe statistically significant differences in CD4 recovery depending on gender, HCV/HBV coinfections, comorbidities and opportunistic infections. The obtained results advocate for current recommendations of introducing antiretroviral therapy as soon as possible, preferably during acute HIV infection, since it increases the chances of sufficient immune reconstruction.

Keywords: HIV; CD4; CD4:CD8; antiretroviral therapy; immune reconstruction

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1. Introduction

Human Immunodeficiency Virus (HIV) infection affects more than 38 million people in the world. In 2021, there were approximately 1.5 million new HIV-infection diagnoses [1]. In 2020, among all newly diagnosed patients, more than 50% were late presenters, with CD4 counts below 350 cells per mm^3, and more than 30% from the whole cohort had CD4 counts below 200 cells per mm^3 [2].

Untreated HIV infection leads to the progressive and continuous impairment of the immune system function [3]. The successive loss of peripheral blood CD4+ T cells can result in the development of opportunistic infections [4]. However, effective antiretroviral therapy (ART) can prevent the drop in CD4 count and to some extent restore the CD4 cell level [5]. Current studies suggest that people living with HIV (PLWH) who are undergoing antiretroviral treatment have similar life expectancy to the non-infected population, despite the higher incidence of comorbidities [6].

The CD4 cell count $\geq$ 500 cells/μL and CD4:CD8 ratio $\geq$ 1 are currently considered normal range and remain the target of immune reconstitution in HIV-infected patients [7]. Patients with a persistence of lower CD4 and CD4:CD8 ratios despite antiretroviral therapy were named as inadequate immunological responders or immunological non-responders (INRs). Being an immunological non-responder may result in increased risk of progression

to AIDS and non-AIDS events, and therefore higher rates of mortality [8]. The mechanisms for this phenomenon may be multifactorial [9]. Clinical risk factors for impaired CD4 recovery have not been established; however, older age, male gender, low CD4 cell count and low CD4:CD8 ratio at the time of diagnosis have been associated with worse immunological response to antiretroviral treatment [10].

Since the reasons for diverse immune reconstruction in HIV-infected patients are still not fully known and explained, we aimed to investigate this matter. We believe it is vital to search for clinical biomarkers which could be the predictors of immunologic reconstitution. Knowing these could possibly increase the chances of enhanced immune reconstruction. Moreover, it could help clinicians identify those patients at risk of maintaining low CD4 cell counts and CD4:CD8 ratios and extend the supervision over them in terms of the progression of HIV infection and development of opportunistic infections. We could also identify individuals being at risk of non-AIDS events and provide multi-specialized care to prevent this. For those reasons, our study aimed to evaluate possible clinical biomarkers which may be related to CD4 cell count and CD4:CD8 ratio recovery among HIV-infected patients with sustained long-term antiretroviral therapy.

2. Materials and Methods

2.1. Patients

The population of HIV-infected adult patients admitted to our department was analyzed in the period 2011–2022. The inclusion criteria were: HIV infection, persistent ART therapy for 5 years from the time of onset, reaching undetectable HIV viral load after a maximum of 1 year since the initiation of antiretroviral treatment and aged 18 or older. The exclusion criteria were the history of discontinuing ART at any time and any detectable HIV viral load after 1 year since beginning antiretroviral therapy.

2.2. Assessments

The analyzed patients underwent physical examination and laboratory testing. All patients were assessed in terms of HCV/HBV coinfections, comorbidities and opportunistic infections. The schemes of antiretroviral treatment applied during a 5-year observation period were studied. Blood samples were repetitively collected for CD4 count, CD4:CD8 ratio and HIV viral load. The first post-baseline examination was performed between 3 and 6 months after the introduction of ART, the second detection was after one year since the beginning of ART and the next examinations were performed yearly.

The immunophenotyping analyses included measurement of the absolute count of T lymphocyte (CD3+) subsets: CD4+ (helper/inducer), CD8+ (suppressor/cytotoxic) and CD4:CD8 ratio were determined using flow cytometry method with the application of three-color direct immunofluorescence reagents—TriTEST™ (BD Biosciences, North Ryde, Australia). A volume of 50 µL of whole blood samples collected the same day for EDTA anticoagulant were incubated in the darkness with the addition of 20 µL of fluorescence-conjugated monoclonal antibodies labelled appropriately: CD4-fluorescein isothiocyanate fluorescein (FITC)/CD8-phycoerythrin (PE)/CD3-peridinin chlorophyll protein (PerCP). Each sample stained with reagents was mixed with microbeads in a TruCOUNT™ tube and prepared according to manufacturer lyse/no-wash procedure. Data were acquired and analyzed using BD MultiSET™ software on the multicolor, dual-laser BD FACSCalibur analyzer. After data collection from 15,000 events, a specific region was set on SSC-H low/CD3-PerCP high+ cells population considered to be T lymphocytes. The gating strategy was based on the selection of the appropriate area on two parameter histograms for distribution of cell populations due to labelled markers CD3-PerCP/CD4-FITC and CD4-FITC/CD8-PE, by using the software provided. The ratio of fluorescent cells to TruCOUNT beads multiplied by the known concentration of beads in the tube was automatically recalculated by the built-in algorithm to the CD3+, CD4+ and CD8+ T-lymphocytes as absolute numbers of lymphocytes per microliter of blood analyzed.

HIV viral load was assessed by the Abbott RealTime HIV-1 assay using an in vitro reverse transcription-polymerase chain reaction (RT-PCR) assay with homogenous real-time fluorescent detection for the quantitation of Human Immunodeficiency Virus type 1 (HIV-1) on the automated m2000 System in human plasma. The assay used RT-PCR26 to generate amplified product from the RNA genome of HIV-1 in clinical specimens. The amount of HIV-1 target sequence was measured through the use of fluorescent labeled oligonucleotide probes on the Abbott m2000rt™ instrument. The range of the performed test was 40 to 10,000,000 copies/mL. We adopted HIV viral load < 40 copies/mL as undetectable.

We analyzed CD4 cell count and CD4:CD8 ratio growth and normalization over the course of 5 years of sustained antiretroviral therapy. CD4 cell count $\geq$ 500 cells/μL and CD4:CD8 ratio $\geq$ 1 was considered the normal range according to CDC Guidelines for Performing CD4+ T-Cell Determinations in Persons Infected with Human Immunodeficiency Virus [7]. The patients with CD4 $\geq$ 500 cells/μL and CD4:CD8 $\geq$ 1.0 at baseline were included in the examination of CD4 cell count and CD4:CD8 ratio growth and excluded from the analysis of CD4 cell count and CD4:CD8 ratio normalization.

We assessed the change of CD4 cell count during a 5-year observation. We adopted the parameter ΔCD4 cell count which signifies the difference in baseline CD4 count and CD4 count after 5 years of sustained antiretroviral therapy, while ΔCD4:CD8 ratio signifies the difference in baseline CD4:CD8 ratio and CD4:CD8 ratio after 5 years of ART. In the tables, we presented the mean and standard deviation of ΔCD4 cell count and ΔCD4:CD8 ratio if the variable had a normal distribution or used the median and interquartile range if the variable did not have a normal distribution.

The study group characteristics, CD4 cell count increase (ΔCD4 cell count) and CD4:CD8 ratio increase (ΔCD4:CD8 ratio) were reported in means and minimum–maximum values (range). The data contained in the boxplots were reported in median, interquartile range and minimum–maximum values (range).

2.3. Statistical Analysis

The Shapiro–Wilk test was performed for the verification of the normality of the distributions in the analyzed variables. A Student's t-test or Mann–Whitney U test were used to evaluate the difference in mean value in continuous variables, while $\chi 2$ or Fisher exact tests were performed for categorical variables. The Kruskal–Wallis ANOVA test was used to evaluate the difference in mean values among more than two quantitative variables. The *p*-value was set at 0.05. The analysis of variance for repeated measures with multiple factors and a generalized linear model with repeated measures showing the relationship between ΔCD4 cell count, ΔCD4:CD8 ratio, CD4 cell count normalization and CD4:CD8 ratio normalization and confounding factors was performed. All statistical analyses were performed using Python 3.7 software and the Statistica 13.1 program (StatSoft Poland, Kraków, Poland).

2.4. Ethics Approval

Ethical approval and written informed consent were waived by the Bioethics Committee of Medical University of Warsaw because of the retrospective nature of the study. Instead, the Bioethics Committee of Medical University of Warsaw approved the use of oral consent, which was documented in patients' medical records. All analyzed patients' data were fully anonymized. The study followed the principles of the Declaration of Helsinki.

3. Results

3.1. Study Group

We analyzed a group of 68 patients (61 men, 7 women) with diagnosed HIV infection. All patients had antiretroviral therapy introduced and continued uninterruptedly for a minimum of 5 years. At the beginning of antiretroviral treatment, 10 out of 68 patients (14.71%) had CD4 cell count $\geq$ 500 cells/μL and 2 out of 68 patients had CD4:CD8 ratio $\geq$ 1 (2.94%). The baseline characteristics of the patients are shown in Table 1.

Table 1. Study group characteristics.

Variable (n = 68)	Mean (Range)
Age	
Age of HIV diagnosis (years)	36.21 (18–65)
Age of initiation of ART (years)	36.78 (18–65)
CD4 count and CD4:CD8 ratio	
CD4 count at the initiation of ART (cells/μL)	260.67 (5–1081)
CD4:CD8 ratio at the initiation of ART (proportion)	0.33 (0.01–1.09)
CD4 count after 5 years of ART (cells/μL)	596.93 (136–1223)
CD4:CD8 ratio after 5 years of ART (proportion)	1.04 (0.19–4.95)
HIV viral load	
HIV viral load at the initiation of ART (copies/mL)	1,526,393 (106–26,600,930)
HIV viral load after 5 years of ART (copies/mL)	undetectable

Among patients, 8 had acute HIV infection, 8 had HCV coinfection, 3 had HBV coinfection and 22 had chronic comorbidities. Among the comorbidities were: hypercholesterolemia (n = 8), hypertension (n = 5), depression (n = 3), liver cirrhosis (n = 3), bipolar affective disorder (n = 1), Klinefelter syndrome (n = 1), chronic kidney disease (n = 1), Crohn's disease (n = 1), ulcerative colitis (n = 1), type 2 diabetes (n = 1), atopic dermatitis (n = 1), adrenal insufficiency (n = 1), granulomatosis with polyangiitis (n = 1), sarcoidosis (n = 1) and Hodgkin's lymphoma (n = 1).

In the analyzed group of patients, there were 25 people with AIDS-defining diseases. Among them, 11 people had more than one disease at the time: pneumocystis jirovecii pneumonia (n = 11), tuberculosis (n = 5), atypical mycobacterial disease (n = 5), esophageal candidiasis (n = 5), HIV encephalopathy (n = 3), CNS toxoplasmosis (n = 3), disseminated cytomegalovirus (CMV) disease (n = 2), Kaposi sarcoma (n = 2), cryptosporidiosis (n = 1) and cervical cancer (n = 1).

3.2. Antiretroviral Therapy

All of the analyzed patients were receiving a three-drug antiretroviral therapy regimen at some stage of the therapy. There most prevalent schemes were protease inhibitor-based therapy (one protease inhibitor (PI) plus two nucleotide analog reverse transcriptase inhibitors (NRTI)), non-nucleotide analog reverse transcriptase inhibitor-based therapy (one non-nucleotide analog reverse transcriptase inhibitor plus two nucleotide analog reverse transcriptase inhibitors) and integrase inhibitor-based therapy (one integrase inhibitor (InSTI) plus two nucleotide analog reverse transcriptase inhibitors (NRTI) or one integrase inhibitor plus one nucleotide analog reverse transcriptase inhibitor).

Among the cohort of patients, 33 individuals underwent the regimen change during a 5-year observation period (29 people underwent one regimen change and 3 people had two regimen changes). By the change of regimen, we assumed switching between groups: InSTI-based therapy, PI-based therapy and NNRTI-based therapy. Switching the pharmaceuticals with the same class of antiretrovirals was not considered a change of regimen. Table 2 presents the number of patients treated with different antiretroviral regimens.

Table 2. Applied regimens of antiretroviral therapy.

Treatment Regimen	Occurrence *n* (%)
2 NRTI + PI	47 (69.12)
2 NRTI + NNRTI	31 (45.59)
2 NRTI + InSTI	18 (26.47)
1 NRTI + InSTI	7 (10.29)

3.3. CD4 Recovery in All Patients

CD4 cell count recovery (ΔCD4) among all patients during the 5-year observation period after the introduction of antiretroviral therapy is presented in Figure 1. The growth CD4:CD8 ratio growth (ΔCD4:CD8) among our cohort of patients is shown in Figure 2. In the analyzed group of patients, 41 out of 68 (60.29%) had CD4 cell count $\geq$ 500 cells/μL and 30 out of 68 (44.12%) had CD4:CD8 ratio $\geq$ 1 after 5 years of sustained antiretroviral treatment. Since 10 patients had CD4 cell count $\geq$ 500 cells/μL and 2 patients had CD4:CD8 ratio $\geq$ 1 at baseline, 21 out of 58 patients (36.21%) managed to reach CD4 cell count $\geq$ 500 cells/μL and 28 out of 66 patients (42.42%) managed to reach CD4:CD8 ratio $\geq$ 1 during 5 years of ART.

3.4. CD4 Recovery Depending on Clinical Biomarkers

We analyzed whether clinical biomarkers such as age, gender, baseline CD4 count, baseline HIV viral load, acute HIV infection, HCV/HBV coinfections, comorbidities and opportunistic infections impacted the rate of CD4 cell count recovery and CD4:CD8 ratio recovery. The results are presented in Table 3.

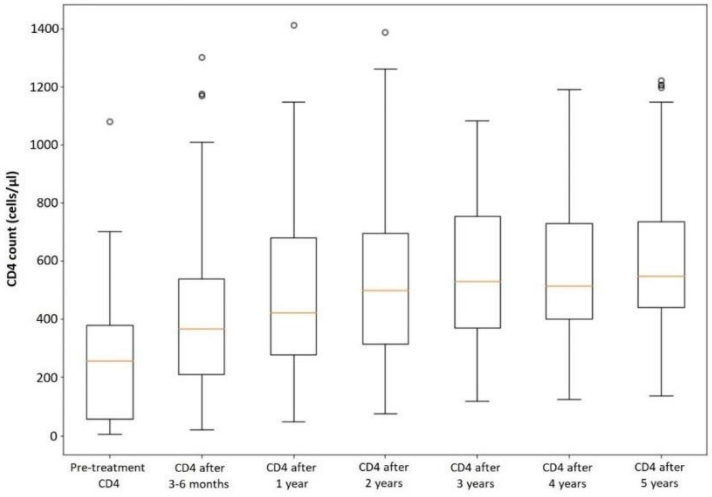

Figure 1. CD4 count recovery among all patients. The hollow circle stands for the outlier.

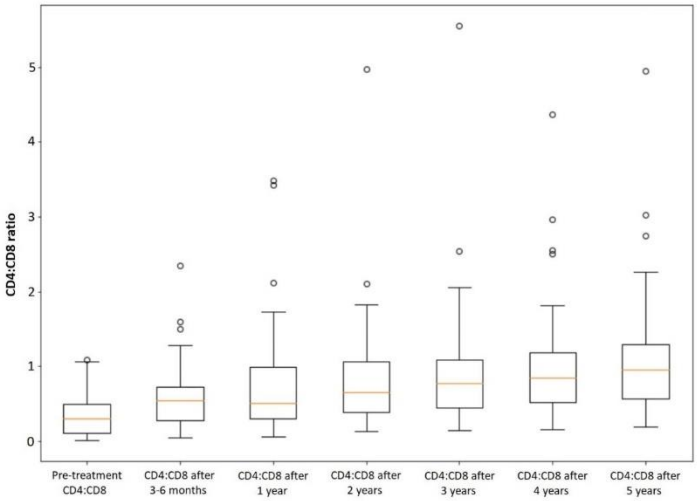

Figure 2. CD4:CD8 ratio growth among all patients. The hollow circle stands for the outlier.

Table 3. CD4 cell count and CD4:CD8 ratio recovery depending on clinical biomarkers.

Group of Patients	CD4 Cell Count Increase (ΔCD4 Cell Count)	*p*	CD4:CD8 Ratio Increase (ΔCD4:CD8 Ratio)	*p*
Age of initiation of ART < 35 (n = 33)	349.85 (247.74)	0.640	0.64 (0.41)	0.264
Age of initiation of ART ≥ 35 (n = 35)	323.14 (220.88)		0.50 (0.50)	
Male gender (n = 61)	320.00 (225.06)	0.093	0.54 (0.52)	0.097
Female gender (n = 7)	476.43 (271.45)		1.11 (0.94)	
CD4 < 200 cells/μL (n = 30)	347.50 (218.50)	0.087	0.43 (0.50)	0.018
CD4 ≥ 200 cells/μL (n = 38)	302.45 (246.30)		0.65 (0.50)	
CD4 < 350 cells/μL (n = 46)	339.00 (197.00)	0.082	0.53 (0.47)	0.181
CD4 ≥ 350 cells/μL (n = 22)	274.36 (282.96)		0.63 (0.74)	
HIV viral load < 1 mln copies/mL (n = 52)	319.48 (236.42)	0.292	0.54 (0.55)	0.284
HIV viral load ≥ 1 mln copies/mL (n = 16)	390.13 (219.58)		0.70 (0.56)	
Acute HIV infection (n = 8)	384.50 (53.00)	0.150	0.75 (0.74)	0.294
No acute HIV infection (n = 60)	331.13 (245.31)		0.54 (0.54)	
HCV/HBV coinfection (n = 12)	321.67 (268.91)	0.815	0.58 (0.36)	0.292
No HCV/HBV coinfection (n = 56)	339.20 (227.09)		0.58 (0.53)	
Any comorbidity (n = 22)	344.41 (239.01)	0.199	0.51 (0.59)	0.412
No comorbidities (n = 46)	311.5 (232.25)		0.62 (0.38)	

Table 3. *Cont.*

Group of Patients	CD4 Cell Count Increase (ΔCD4 Cell Count)	*p*	CD4:CD8 Ratio Increase (ΔCD4:CD8 Ratio)	*p*
Any opportunistic infection and CD4 < 200 cells/μL (*n* = 23)	364.00 (216.00)	0.490	0.43 (0.49)	0.303
No opportunistic infection and CD4 < 200 cells/μL (*n* = 7)	354.00 (169.55)		0.59 (0.36)	

The gray numbers indicate mean (standard deviation); The bule number indicate median (interquartile range).

We investigated how many people belonging to each group have managed to reach CD4 cell count normalization. The results are shown in Table 4.

Table 4. CD4 cell count and CD4:CD8 normalization depending on clinical biomarkers.

Group of Patients	CD4 Count Normalization (≥500 Cells/μL) (*n*/%)	*p*	CD4:CD8 Ratio Normalization (≥1) (*n*/%)	*p*
Age of initiation of ART < 35 (*n* = 28)	20 (71.43%)	0.034	16 (57.14%)	0.063
Age of initiation of ART ≥ 35 (*n* = 30)	11 (36.67%)		7 (23.33%)	
Male gender (*n* = 52)	28 (53.85%)	1.000	20 (38.46%)	0.673
Female gender (*n* = 6)	3 (50.00%)		3 (50.00%)	
CD4 < 200 cells/μL (*n* = 30)	11 (36.67%)	0.034	6 (20.00%)	0.022
CD4 ≥ 200 cells/μL (*n* = 28)	20 (71.43%)		17 (60.71%)	
CD4 < 350 cells/μL (*n* = 46)	22 (47.83%)	0.115	16 (34.78%)	0.006
CD4 ≥ 350 cells/μL (*n* = 12)	9 (75.00%)		7 (58.33%)	
HIV viral load < 1 mln copies/mL (*n* = 45)	24 (53.33%)	1.000	16 (35.55%)	0.006
HIV viral load ≥ 1 mln copies/mL (*n* = 13)	7 (53.85%)		7 (53.85%)	
Acute HIV infection (*n* = 7)	7 (100.00%)	0.012	6 (85.71%)	0.013
No acute HIV infection (*n* = 51)	24 (47.06%)		17 (33.33%)	
HCV/HBV coinfection (*n* = 9)	6 (66.66%)	0.481	3 (33.33%)	0.686
No HCV/HBV coinfection (*n* = 49)	25 (51.02%)		20 (40.82%)	
Any comorbidity (*n* = 18)	9 (50.00%)	0.083	7 (38.88%)	0.061
No comorbidities (*n* = 40)	22 (55.00%)		16 (40.00%)	
Any opportunistic infection and CD4 <200 cells/μL (*n* = 23)	8 (34.78%)	1.000	3 (13.04%)	0.336
No opportunistic infection and CD4 < 200 cells/μL (*n* = 7)	3 (42.86%)		3 (42.86%)	

Age below 35 years old, high CD4 count at the beginning of antiretroviral therapy and acute HIV infection showed to be positive factors of CD4 cell count normalization in 5 years. In terms of CD4:CD8 ratio normalization during 5-year antiretroviral therapy, high baseline CD4 count, high HIV viral load and acute HIV infection appeared to have statistical significance. Figure 3 presents CD4 count and CD4:CD8 ratio recovery over 5 years of sustained ART.

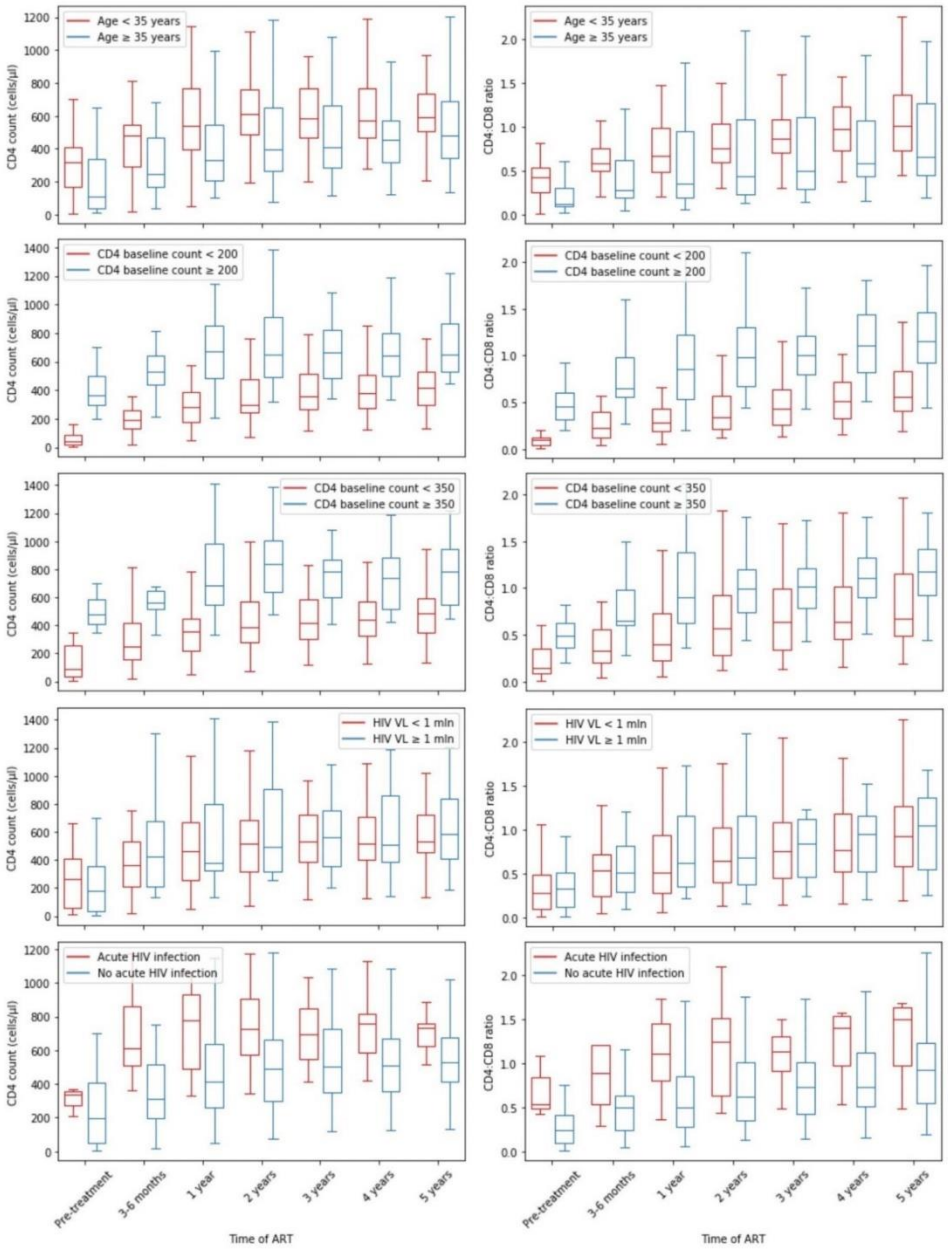

Figure 3. CD4 and CD4:CD8 recovery during 5 years of ART depending on clinical biomarkers.

We performed an analysis of variance for ΔCD4 cell count, ΔCD4:CD8 ratio, CD4 cell count normalization and CD4:CD8 ratio normalization. We obtained statistically significant p values for ΔCD4:CD8 ratio ($p < 0.001$), CD4 cell count normalization ($p = 0.024$) and CD4:CD8 ratio normalization ($p = 0.016$) and a statistically insignificant p value for ΔCD4 cell count ($p = 0.072$). A generalized linear model with repeated measures showing the relationship between ΔCD4 cell count, ΔCD4:CD8 ratio, CD4 cell count normalization and CD4:CD8 ratio normalization and confounding factors was also performed. For qualitative variables, we analyzed men vs. women, individuals with no acute HIV infection vs. patients with acute HIV infection, people with HCV/HBV coinfection vs. people without coinfections, patients with no comorbidities vs. patients with comorbidities and individuals with no opportunistic infections vs. people with the diagnosis of opportunistic infection. The results are presented in Table 5 and Figure 4.

Table 5. The results of a generalized linear model for confounding factors for ΔCD4 cell count and ΔCD4:CD8 ratio and normalization.

Variable	CD4 Cell Count Increase (ΔCD4 Cell Count)		CD4:CD8 Ratio Increase (ΔCD4:CD8 Ratio)		CD4 Cell Count Normalization		CD4:CD8 Ratio Normalization	
	b′	***p***	**b′**	***p***	**b′**	***p***	**b′**	***p***
Age of initiation of ART	−0.247	0.082	−0.160	0.104	−0.331	0.018	−0.083	0.540
Gender	0.177	0.197	0.023	0.801	0.048	0.703	0.176	0.163
Baseline CD4 cell count	−0.286	0.089	0.342	0.003	0.212	0.220	0.077	0.651
HIV viral load	−0.077	0.677	0.151	0.196	−0.023	0.886	0.012	0.938
Acute HIV infection	0.185	0.310	0.259	0.030	0.290	0.084	0.238	0.150
HCV/HBV coinfection	−0.043	0.763	−0.017	0.854	0.099	0.439	−0.038	0.765
Comorbidities	0.066	0.665	0.044	0.644	0.029	0.824	−0.004	0.973
Opportunistic infections	0.136	0.449	−0.219	0.082	0.072	0.685	−0.331	0.065

b′—coefficient estimate on the scale of the linear predictor.

In a generalized linear model with repeated measures, we also analyzed quantitative variables: baseline CD4 cell count, age of initiation of ART and HIV viral load at the start of antiretroviral therapy. We observed statistically significant p values for CD4 cell count increase in younger patients ($p = 0.020$) and lower baseline CD4 cell count ($p < 0.001$) and a statistically insignificant p value for HIV viral load ($p = 0.696$). In terms of CD4:CD8 ratio growth, we did not obtain statistically significant p values for age ($p = 0.756$), baseline CD4 count ($p = 0.628$) and HIV viral load ($p = 0.939$).

3.5. CD4 Recovery Depending on the Regimens of Antiretroviral Therapy

We analyzed three groups of patients depending on the applied antiretroviral therapy regimen. The first group of patients were undergoing protease inhibitor-based therapy (2 NRTI + 1 PI), the second group were receiving integrase inhibitor-based therapy (2 NRTI + 1 InSTI or 1 NRTI + 1 InSTI) and the third group were having non-nucleotide analog reverse transcriptase inhibitor-based therapy (2 NRTI + 1 NNRTI or 2 NRTI + 2 NNRTI). Table 6 presents CD4 cell count and CD4:CD8 ratio recovery among patients receiving different antiretroviral regimens. Table 7 shows the number of people who reached CD4 cell count and CD4:CD8 ratio normalization among patients receiving different antiretroviral regimens.

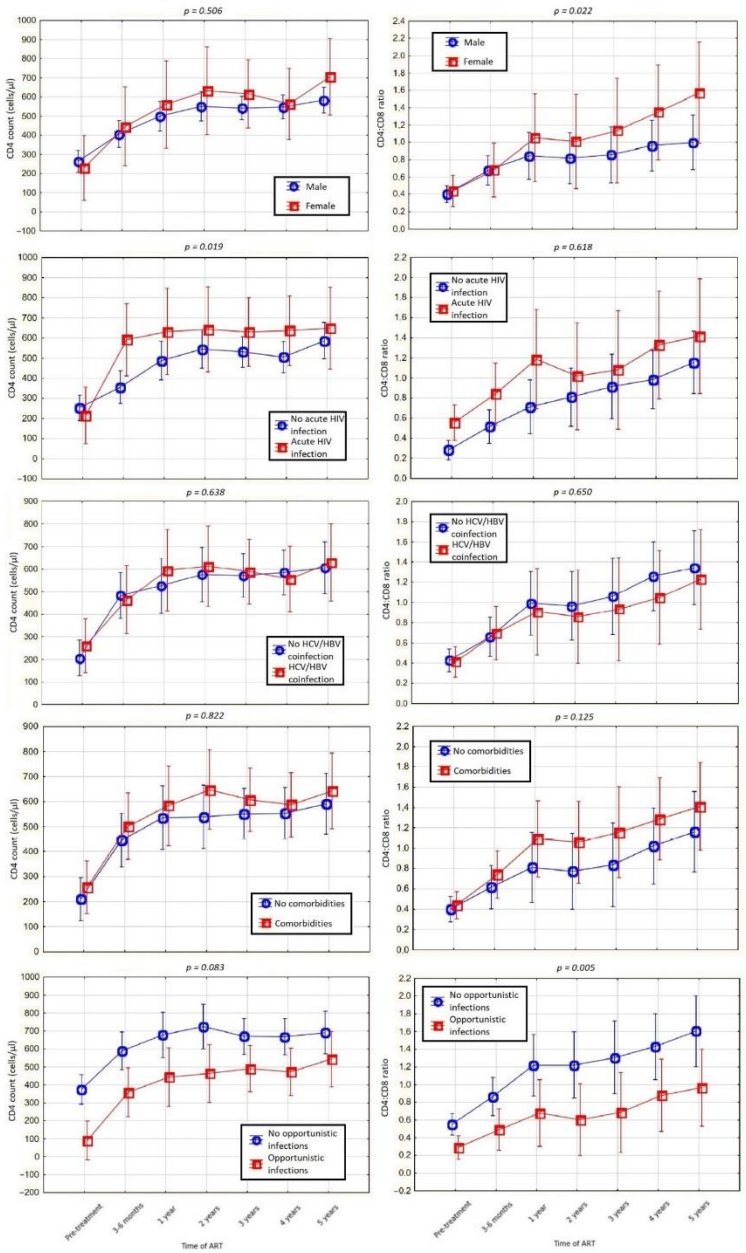

Figure 4. The results of a generalized linear model with repeated measures for CD4 cell count and CD4:CD8 ratio.

Table 6. CD4 cell count and CD4:CD8 recovery depending on the applied ART regimen.

Variable	PI-Based Therapy (n = 47)	InSTI-Based Therapy (n = 23)	NNRTI-Based Therapy (n = 31)	p
CD4 cell count increase	345.89 (228.52)	337.43 (265.16)	328.61 (254.71)	0.784
CD4:CD8 ratio increase	0.59 (0.61)	0.69 (0.51)	0.50 (0.51)	0.433

The gray numbers indicate mean (standard deviation); The bule number indicate median (interquartile range).

Table 7. CD4 cell count and CD4:CD8 normalization depending on the applied ART regimen.

Variable	PI-Based Therapy (n = 39)	InSTI-Based Therapy (n = 19)	NNRTI-Based Therapy (n = 26)	p
CD4 count normalization (≥500 cells/μL) (n/%)	21 (53.85)	12 (52.17)	13 (50.00)	0.251
CD4:CD8 ratio normalization (≥1) (n/%)	15 (38.46)	12 (52.17)	11 (42.31)	0.150

We did not observe statistically significant differences in the CD4 cell count and CD4:CD8 ratio increase and in the number of people who have managed to reach CD4 cell count and CD4:CD8 ratio normalization depending on applied antiretroviral treatment.

We presented the CD4 count and CD4:CD8 ratio recovery over 5 years in three groups of patients: receiving InSTI-based therapy, PI-based therapy and NNRTI-based therapy in Figure 5.

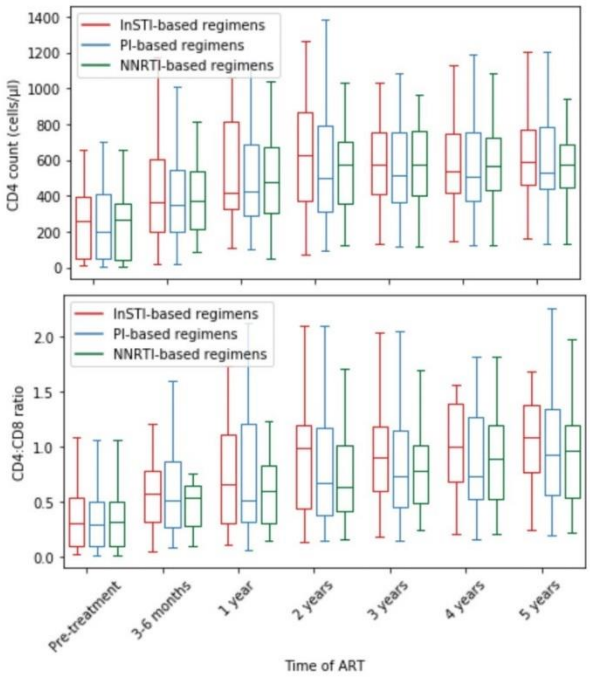

Figure 5. CD4 cell count and CD4:CD8 recovery depending on the applied ART regimen.

We also evaluated whether the changes in the antiretroviral treatment regimen had an impact on CD4 cell count and CD4:CD ratio increase and CD4 count and CD4:CD8 ratio normalization. The results of CD4 cell count and CD4:CD ratio increase are shown in Table 8. The results of CD4 cell count and CD4:CD8 ratio normalization are presented in Table 9.

Table 8. CD4 cell count and CD4:CD8 recovery depending on changes in the ART regimen.

Variable	No Changes of Regimen (n = 36)	1 or 2 Changes of Regimen (n = 32)	p
CD4 cell count increase	319.94 (207.28)	354.28 (260.96)	0.159
CD4:CD8 ratio increase	0.59 (0.37)	0.62 (0.55)	0.209

The gray numbers indicate mean (standard deviation); The bule number indicate median (interquartile range).

Table 9. CD4 cell count and CD4:CD8 normalization depending on changes in the ART regimen.

Variable	No Changes of Regimen (n = 32)	1 or 2 Changes of Regimen (n = 26)	p
CD4 count normalization (≥500 cells/μL) (n/%)	17 (53.13)	14 (53.85)	0.564
CD4:CD8 ratio normalization (≥1) (n/%)	10 (31.25)	13 (50.00)	0.128

There were no significant differences in CD4 cell count increase, CD4:CD8 ratio increase, CD4 count normalization and CD4:CD8 ratio normalization among patients who had the antiretroviral therapy regimen changed and among those who had one scheme applied.

4. Discussion

Immune reconstruction in HIV-infected patients is a process of rebuilding the immune system after the introduction of combined antiretroviral therapy. Centers for Disease Control and Prevention (CDC) consider CD4 cell count ≥ 500 cells/μL and CD4:CD8 ratio ≥ 1 as the normal range and one of the goals of HIV treatment [7]. Immune system recovery is most important in an advanced stage of the disease due to the increased risk for the development of opportunistic infections and neoplasms [4]. Studies show that even long-term antiretroviral treatment uncommonly leads to the normalization of CD4 count and CD4:CD8 ratio [11,12]. There are reports suggesting that CD4:CD8 ratio may better reflect immune dysfunction in well-controlled HIV infection than CD4+ cell count alone [13,14].

Among patients included in our study, 58 (85.29%) had CD4 cell count < 500 cells/μL and CD4:CD8 ratio < 1 at the initiation of ART. In those people, 36.21% managed to reach CD4 cell count ≥ 500 cells/μL and 42.42% gained CD4:CD8 ratio ≥ 1 during 5 years of ART. Among 46 patients (67.65%) with CD4 cell count < 350 cells/μL, 22 (47.83%) managed to reach CD4 ≥ 500 cells/μL and 16 (34.78%) gained CD4:CD8 ratio ≥ 1. Among 30 patients (44.12%) with CD4 < 200 cells/μL, 11 (36.67%) reached CD4 cell count normalization and 6 (20.00%) gained CD4:CD8 ratio normalization. In a study with a 4-year follow-up, 44% of patients normalized CD4 cell count and only 33% normalized CD4:CD8 ratio [15]. In other reports, the follow-up was shorter than 5 years. There was a study conducted in which during the median 2.6 years of observation, 28% of patients normalized CD4:CD8 ratio [16]. In another study with a median observation period of 2.77 years, only 7.2% of people normalized CD4:CD8 ratio [17]. Mussini et al. estimated the probability of ratio normalization during 5 years of ART and obtained a result of 29.4% [18]. That may suggest that longer antiretroviral treatment is associated with higher CD4:CD8 ratio normalization rates.

In our study, we analyzed whether various factors may have an impact on CD4 and CD4:CD8 ratio recovery. We evaluated biomarkers such as age, gender, CD4 cell count and CD4:CD8 ratio at the beginning of the therapy, HIV viral load at the beginning of ART, the existence of acute HIV infection, HCV or HBV coinfections, comorbidities or opportunistic infections.

4.1. CD4 Recovery Depending on Patient's Age

We analyzed two groups of HIV-infected patients: those younger than 35 years old and those who were 35 years old or older at the start of antiretroviral treatment. We observed that there were significantly more people who managed to reach CD4 cell count $\geq$ 500 cells/μL in the group of younger patients. Apart from that, CD4:CD8 ratio normalization was also attained by more people in the younger patients' group; however, that difference did not appear statistically significant.

There are studies which seem to support the thesis that younger age may be a positive predictor in terms of CD4 recovery. Yu et al., who also compared CD4 growth among patients $\geq$35 years old and <35 years old, obtained similar results: younger patients reached higher CD4 cell count during long-term antiretroviral treatment than older patients [19]. Chen et al. also evaluated that older patients ($\geq$50 years old) gained lower median maximal CD4 cell count on antiretroviral therapy than patients who were younger than 50 years old [20]. There are more studies supporting that hypothesis [21]. There is also research involving children and adolescents with HIV infection indicating that in in this age group, older age is also associated with slower CD4:CD8 ratio recovery [22,23].

In contrast, there are also reports saying that age is not associated with CD4 recovery. An African cohort that included almost 3000 people living with HIV did not show differences in CD4 recovery among patients $\geq$50 and <50 years old when they started ART [24].

4.2. The Impact of Baseline CD4 Count on CD4 Recovery

Among the analyzed individuals, the proportion of patients with CD4 cell count < 350 cells/μL at the beginning of antiretroviral therapy accounted for 67.65% of the population, which is a high rate of late diagnoses. However, our study was conducted among patients diagnosed since 2011 and treated for a minimum of 5 years. In Poland before 2016, the antiretroviral treatment was not widely available to all HIV-infected patients [25]. Moreover, the study group might be biased, since the study was conducted among patients diagnosed in the hospital's department, usually constituting more advanced stages of the infection than patients diagnosed in the outpatients' clinic.

In our study, we analyzed CD4 recovery among patients with CD4 cell count $\geq$ 200 cells/μL and <200 cells/μL and also with CD4 $\geq$ 350 cells/μL and <350 cells/μL at the beginning of ART. Significantly, more patients with CD4 cell count $\geq$ 200 cells/μL obtained CD4 cell count and CD4:CD8 ratio normalization than patients with CD4 < 200 cells/μL. In the comparison of patients with CD4 $\geq$ 350 cells/μL and <350 cells/μL at ART initiation, we also observed a similar relationship; however, statistical significance was obtained only in terms of CD4:CD8 ratio normalization.

Other studies also seem to indicate that higher baseline CD4 cell count is associated with higher rates of patients who gain CD4:CD8 ratio normalization. There are researchers suggesting that starting ART with CD4:CD8 ratio > 0.5 is related with a greater likelihood of normalizing CD4:CD8 ratio [26]. Another study shows that significantly more people with baseline CD4 count < 100 cells/μL did not reach CD4 count $\geq$ 500 cells/μL in more than 6 years of observation [27]. A similar relationship of higher baseline CD4 count and CD4 recovery acceleration is also found in children [23].

On the contrary, there are studies which indicate that lower baseline CD4 cell count is associated with higher potential for improvement; therefore, patients with lower baseline CD4 count have a greater rate of CD4 count recovery [28]. That study contained 10 years of

observation, so it is possible that patients with low baseline CD4 count need more time for CD4 recovery. The observation of our cohort continues.

4.3. CD4 Recovery in Patients Who Start ART with Acute HIV Infection

In our cohort, there were 8 patients who had antiretroviral therapy introduced during acute HIV infection. Among them, 7 individuals (87.50%) had CD4 cell count < 500 cells/μL and CD4:CD8 ratio < 1 at the point of HIV infection diagnosis. Those 7 patients all achieved CD4 cell count normalization within 5 years of ART. During the observation, 6 out of 7 patients (85.71%) also managed to reach CD4:CD8 ratio normalization. The rates of CD4 and CD4:CD8 normalization in patients diagnosed in later stages of the disease were much lower (47.06% and 33.33% of patients, respectively). Those differences appear statistically significant.

There are many studies which state that early initiation of ART in HIV-infected patients is very important. Researchers confirm that antiretroviral treatment introduced in acute HIV infection is effective in CD4 cell count recovery [29] and has the beneficial role in CD4 cells recovery and rates of CD4:CD8 ratio normalization [30]. Apart from CD4 recovery, early ART enables the clearance of other cells infected by HIV, such as in gut-associated lymphoid tissue and lymph nodes [31]. Early ART helps in bone marrow and peripheral B cells recovery, which are also affected by HIV infection [32,33].

There are also reports suggesting that immune recovery is comparable in primary and chronic HIV infection [34] or that suboptimal CD4 recovery occurs uncommonly evenly when introducing ART in acute HIV infection [35]. However, those studies also highlight the importance of early initiation of the treatment, since it is beneficial.

4.4. HIV Viral Load and CD4 Recovery

Our analyses show that HIV viral load $\geq$ 1 million copies/mL at the beginning of antiretroviral therapy, which concerned 16 patients (23.53%), was associated with higher rates of CD4:CD8 ratio normalization. We did not observe a similar relationship concerning CD4 cell count normalization. Among analyzed patients, similar to other studies, there were both patients with acute HIV infection (5, 31.25%) and advanced HIV infection (11, 68.75%). There are little data concerning the impact of HIV viral load on CD4 recovery; however, there are some studies which seem to support our results. Muscatello et al. demonstrated that there may be a relationship between higher HIV viral load and CD4 recovery [36]. In that study, high HIV viral load at ART initiation was associated with acute HIV infection, and that may be the reason for the obtained results. Another study, which did not analyze patients with acute HIV infection, connected high viral load with an increased risk of AIDS and, thus, inferior rates of CD4 recovery [37]. Since our population of patients with high HIV viral load included both patients with acute HIV infection and patients with advanced HIV infection, the results are difficult to compare with those of other researchers.

4.5. CD4 Recovery Depending on Patient's Gender

Our study involved 68 HIV-infected patients, with 61 patients male and 7 female. We did not obtain statistically significant results, likely due to the inequality of the two groups. We observed that during 5 years of antiretroviral treatment, CD4 cell count and CD4:CD8 ratio increased more among women than men (the mean ΔCD4 among women was 476.43 cells/μL vs. men 320.00 cells/μL, while the mean ΔCD4:CD8 among women was 1.11 vs. men 0.66). Moreover, a larger percentage of women reached CD4:CD8 ratio normalization after 5 years (50.00% of women, 38.46% of men). We did not observe that relationship in CD4 cell count normalization (50.00% of women, 53.85% of men).

There are few current studies concerning the influence of gender on CD4 recovery. A French study reports that being a female may have beneficial impact on immune recovery, especially during long-term antiretroviral treatment, which may give women additional protection from adverse clinical events and premature ageing [38]. Another study suggested

that there are no significant differences in CD4 recovery among men and women in chronic HIV infection [39]. Meditz et al. reported similar rates of CD4 cell count growth between men and women, noting that women had an elevated risk of HIV/AIDS-related events [40]. Moreover, there are studies suggesting that women's poor CD4 recovery may be associated with vitamin D insufficiency [41].

4.6. The Influence of HCV/HBV Coinfection on CD4 Normalization

In our study group, 8 individuals were coinfected with HCV and 3 with HBV. The increase in CD4 cell count and CD4:CD8 ratio during 5 years of ART was higher among patients without HCV or HBV infection than in individuals with coinfections. The rates of CD4:CD8 normalization were also higher in the group of patients without HCV/HBV coinfection; however, rates of CD4 cells normalization were greater in the population of patients with coinfections. These results were not statistically significant.

Different studies suggest that HCV coinfection may have a negative impact on CD4 recovery among HIV/HCV-coinfected patients [42]. HCV may accelerate the depletion of CD4 cells because of the accumulation of dysfunctional immune activation during chronic viral infection [43]. Moreover, studies show that the introduction of direct-acting antiviral agents (DAA) leads to rapid decrease of CD4 cell count at the beginning of DAA therapy. The decrease may last even after the achievement of sustained virological response (SVR), but HCV clearance may induce improvement [44]. On the other hand, there are studies suggesting that HCV clearance after DAA treatment does not seem to have an impact on CD4 cell recovery [45]. On the contrary, researchers suggest that in HIV-infected children who are also vertically infected with HCV, HCV coinfection does not have a negative effect in long-term CD4 recovery compared to children with HIV infection alone [46].

Researchers studying HBV/HIV coinfection suggest that the presence of HBcAb seems to be associated with reduced CD4:CD8 ratio growth [47]. Another study suggests that in HIV/HBV-coinfected HBeAg-negative patients, immune recovery is continuously lower than in HBeAg-positive and HIV-mono-infected individuals [48]. One African study showed the acceleration of CD4 cell count recovery in HBV/HIV-coinfected patients with high HBV DNA viral load after ART initiation; however, it did not lead to increased rates of CD4:CD8 ratio normalization [49]. In children with HIV/HBV coinfection, similarly to HCV infection, CD4 increase was similar than in patients with HIV infection alone [50].

In general, recent studies suggest that in patients with HIV infection coinfected with HCV or HBV, CD4 recovery rates are lower than in patients with HIV mono-infection [51].

4.7. Comorbidities and CD4 Recovery

Among 68 HIV-infected patients in our study group, 22 (32.35%) were also diagnosed with other comorbidities. The most common chronic illnesses were: hypercholesterolemia, hypertension, depression and liver cirrhosis. Patients with comorbidities had slightly lower rates of CD4 cell count and CD4:CD8 ratio normalization; however, those differences were not statistically significant.

There are little data describing the impact of comorbidities on CD4 recovery in HIV-infected patients. Some studies suggest that dyslipidemia in HIV-infected patients may result in worse CD4 recovery outcomes [52,53]. Another study shows that high levels of HDL particles, HDL cholesterol and larger sizes of LDL particles have a better CD4 recovery than patients with high ratios of non-HDL lipoprotein particles [54]. Moreover, the administration of both statins and fibrates in HIV-related dyslipidemia do not seem to act significantly on clinical immune response in patients receiving antiretroviral treatment [55]. Hypertension, together with CD4 recovery, is suggested to be an epiphenomenon of the improvement of the HIV infection state, not the influencing factor [56]. Depression seems to be a risk factor for incomplete short-term HIV viral suppression among HIV-infected patients, and therefore poor CD4 cell count recovery [57]. Little is known whether liver cirrhosis affects CD4 cell count recovery; however, there are studies indicating that HIV/HCV-coinfected patients with lower CD4 recovery rates show more intense destructive processes

in the liver than successfully recovered subjects [58], while also higher rates of CD4 recovery may lead to transient liver injury in patients with HIV/HCV coinfection, due to activation of the immune process [59].

4.8. The Impact of the Presence of AIDS-Defining Diseases on CD4 Recovery

In our study group, there were 25 patients with at least one opportunistic infection. We compared their rates of CD4 recovery to patients with Acquired Immunodeficiency Syndrome (AIDS) without opportunistic infections (CD4 cell count < 200/µL—7 patients). We observed lower CD4 cell count and CD4:CD8 ratio normalization rates among patients in the first group during 5 years of antiretroviral treatment (CD4 cell count normalization: 34.78% vs. 42.86%, respectively; CD4:CD8 ratio normalization: 13.04% vs. 42.86%, respectively). These differences were not statistically significant, probably due to the small sample size.

The available data examining the impact of the occurrence of opportunistic infections on CD4 recovery after antiretroviral therapy introduction are lacking. There are studies indicating that the development of AIDS is associated with poorer rates of CD4 and CD4:CD8 normalization than among patients diagnosed in earlier stages of HIV infection [10,60]. Many researchers acknowledge the importance of immunological non-responders (INR)—who are HIV-infected individuals failing to achieve the normalization of CD4 cell counts despite persistent virological suppression. That phenomenon may concern even up to 40% of people living with HIV [61]. These patients have an increased risk of progression to AIDS and non-AIDS events and present higher rates of mortality than HIV-infected individuals with adequate immune reconstitution [62]. The risk factors for INR are lower nadir CD4 T cell count, lower CD4:CD8 ratios and a lower naïve/memory CD4 cell ratio [58]. The predictor of long-term immunologic recovery in advanced HIV patients can be the CD4 slope during the first year of antiretroviral treatment [63]. Our study showed a plateau of CD4 cell count and CD4:CD8 ratio after approximately 1 year of antiretroviral treatment.

4.9. Applied Regimen of ART Influencing CD4 Recovery

Our analyses included patients hospitalized in 2011–2022; therefore, it is difficult to assess the therapeutic approach in all individuals. During the 11-year period, the therapeutic options were expanding and the recommendations regarding introducing various medications were changing. Thus, we decided to analyze the treatment regimens by the groups of applied therapeutics. The prevalent ART regimens in analyzed patients were: protease inhibitor-based therapy (47 patients), integrase inhibitor-based therapy (23 patients) and non-nucleotide analog reverse transcriptase inhibitor-based therapy (31 patients). There were no statistically significant differences between ΔCD4 and ΔCD4:CD8 among patients belonging to these three groups. The CD4 and CD4:CD8 normalization rates between patients in these groups also did not reach statistical significance; however, in the group of patients treated with integrase inhibitor-based therapy, more individuals managed to gain CD4:CD8 normalization rates than in other groups. We also examined whether the changes of regimen had an impact on CD4 recovery, but did not uncover statistically significant differences between patients who underwent one or two changes of regimen and individuals who did not change the scheme of the therapy.

Some studies also suggest that InSTI-based regimens show a better immune recovery rate and the type of first-line ART can have an impact on immune reconstitution [64,65]. One study compared the efficacy of regimens including raltegravir and efavirenz in CD4 recovery, with raltegravir seeming to lead to faster CD4:CD8 ratio normalization [66]. It is probable that this relationship may exist only when introducing antiretroviral treatment in advanced HIV infection. When ART was introduced in acute or recent HIV infection, viral suppression and immunological recovery were excellent, with no differences between ART regimens [67]. The meta-analysis including 33 studies showed that there were no effective medications specific for improving CD4 cell count reconstitution [68].

4.10. Limitations of the Study

The main limitation of our study was a small study population. Some analyses included a differentiated number of individuals because of the retrospective nature of the study and the available data. Therefore, in a few analyses, it is difficult to obtain statistically significant results. Moreover, we did not perform the stratification of therapeutic regimens by the applied drugs. We also did not perform the calculation and justification of the sample size selected.

5. Conclusions

Our study, which evaluated CD4 recovery during 5 years of effective antiretroviral treatment, suggests that clinical biomarkers such as younger age, higher CD4 baseline cell count, higher HIV viral load at the initiation of ART and the introduction of ART at the point of acute HIV infection are positive predictors of immune reconstitution. We therefore advocate for current recommendations to introduce antiretroviral therapy as soon as possible, preferably during acute HIV infection, since it provides the highest rates of CD4 cell count and CD4:CD8 ratio normalization. To make this possible, it is valid to introduce common rapid antiretroviral therapy in all HIV-infected patients.

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References

1. Fact Sheet—Latest Statistics on the Status of the AIDS Epidemic. UNAIDS. 2021. Available online: https://www.unaids.org/en/resources/fact-sheet (accessed on 3 August 2022).
2. European Center for Disease Prevention and Control (ECDC)/WHO Regional Office for Europe. *HIV/AIDS Surveillance in Europe*; 2019 Data; ECDC: Stockholm, Sweden, 2020. Available online: https://www.ecdc.europa.eu/sites/default/files/documents/hiv-surveillance-report-2020.pdf (accessed on 3 August 2022).
3. Shenoy, N.; Ramapuram, J.; Shenoy, A.; Ahmed, J.; Srikant, N. Incidence of Opportunistic Infections among HIV-Positive Adults on Highly Active Antiretroviral Therapy in a Teaching Hospital, India: Prospective Study. *J. Int. Assoc. Provid. AIDS Care* **2017**, *16*, 309–311. [CrossRef]
4. Gaines, H.; von Sydow, M.A.; von Stedingk, L.V.; Biberfeld, G.; Böttiger, B.; Hansson, L.O.; Lundbergh, P.; Sönnerborg, A.B.; Wasserman, J.; Strannegård, O. Immunological changes in primary HIV-1 infection. *AIDS* **1990**, *4*, 995–1000. [CrossRef] [PubMed]
5. Sobrino-Vegas, P.; Moreno, S.; Rubio, R.; Viciana, P.; Bernardino, J.I.; Blanco, J.R.; Bernal, E.; Asensi, V.; Pulido, F.; del Amo, J.; et al. Impact of late presentation of HIV infection on short-, mid- and long-term mortality and causes of death in a multicenter national cohort: 2004–2013. *J. Infect.* **2016**, *72*, 587–596. [CrossRef] [PubMed]
6. Marcus, J.L.; Leyden, W.A.; Alexeeff, S.E.; Anderson, A.N.; Hechter, R.C.; Hu, H.; Lam, J.O.; Towner, W.J.; Yuan, Q.; Horberg, M.A.; et al. Comparison of Overall and Comorbidity-Free Life Expectancy Between Insured Adults with and Without HIV Infection, 2000-2016. *JAMA Netw. Open* **2020**, *3*, e207954. [CrossRef]
7. CDC. Revised Guidelines for Performing CD4+ T-Cell Determinations in Persons Infected with Human Immunodeficiency Virus (HIV). 1997. Available online: https://www.cdc.gov/mmwr/preview/mmwrhtml/00045580.htm (accessed on 10 August 2022).

8. Gazzola, L.; Tincati, C.; Bellistrì, G.M.; Monforte, A.D.; Marchetti, G.C. The Absence of $CD4^+$ T Cell Count Recovery Despite Receipt of Virologically Suppressive Highly Active Antiretroviral Therapy: Clinical Risk, Immunological Gaps, and Therapeutic Options. *Clin. Infect. Dis.* **2009**, *48*, 328–337. [CrossRef]
9. Gunda, D.W.; Kilonzo, S.B.; Kamugisha, E.; Rauya, E.Z.; Mpondo, B.C. Prevalence and risk factors of poor immune recovery among adult HIV patients attending care and treatment centre in northwestern Tanzania following the use of highly active antiretroviral therapy: A retrospective study. *BMC Res. Notes* **2017**, *10*, 197. [CrossRef] [PubMed]
10. Yang, X.; Su, B.; Zhang, X.; Liu, Y.; Wu, H.; Zhang, T. Incomplete immune reconstitution in HIV/AIDS patients on antiretroviral therapy: Challenges of immunological non-responders. *J. Leukoc. Biol.* **2020**, *107*, 597–612. [CrossRef]
11. Caby, F.; Guihot, A.; Lambert-Niclot, S.; Guiguet, M.; Boutolleau, D.; Agher, R.; Valantin, M.-A.; Tubiana, R.; Calvez, V.; Marcelin, A.-G.; et al. Determinants of a Low CD4/CD8 Ratio in HIV-1–Infected Individuals Despite Long-term Viral Suppression. *Clin. Infect. Dis.* **2016**, *62*, 1297–1303. [CrossRef]
12. Han, W.M.; Apornpong, T.; Kerr, S.J.; Hiransuthikul, A.; Gatechompol, S.; Do, T.; Ruxrungtham, K.; Avihingsanon, A. CD4/CD8 ratio normalization rates and low ratio as prognostic marker for non-AIDS defining events among long-term virologically suppressed people living with HIV. *AIDS Res. Ther.* **2018**, *15*, 13. [CrossRef]
13. Milanés-Guisado, Y.; Gutiérrez-Valencia, A.; Rodríguez, M.T.; Espinosa, N.; Viciana, P.; López-Cortés, L.F. Absolute CD4+ T cell count overstate immune recovery assessed by $CD4^+/CD8^+$ ratio in HIV-infected patients on treatment. *PLoS ONE* **2018**, *13*, e0205777. [CrossRef]
14. Serrano-Villar, S.; Deeks, S.G. CD4/CD8 ratio: An emerging biomarker for HIV. *Lancet HIV* **2015**, *2*, e76–e77. [CrossRef]
15. Lee, S.S.; Wong, N.S.; Wong, B.C.K.; Wong, K.H.; Chan, K.C.W. Combining CD4 recovery and CD4: CD8 ratio restoration as an indicator for evaluating the outcome of continued antiretroviral therapy: An observational cohort study. *BMJ Open* **2017**, *7*, e016886. [CrossRef]
16. Zhabokritsky, A.; Szadkowski, L.; Cooper, C.; Loutfy, M.; Wong, A.; McClean, A.; Hogg, R.S.; Walmsley, S.L.; Brumme, Z.; Burchell, A.N.; et al. Increased CD4: CD8 ratio normalization with implementation of current ART management guidelines. *J. Antimicrob. Chemother.* **2021**, *76*, 729–737. [CrossRef]
17. Leung, V.; Gillis, J.; Raboud, J.; Cooper, C.; Hogg, R.S.; Loutfy, M.R.; Machouf, N.; Montaner, J.S.G.; Rourke, S.B.; Tsoukas, C.; et al. Predictors of CD4: CD8 Ratio Normalization and Its Effect on Health Outcomes in the Era of Combination Antiretroviral Therapy. *PLoS ONE* **2013**, *8*, e77665. [CrossRef]
18. Mussini, C.; Lorenzini, P.; Cozzi-Lepri, A.; Lapadula, G.; Marchetti, G.; Nicastri, E.; Cingolani, A.; Lichtner, M.; Antinori, A.; Gori, A.; et al. CD4/CD8 ratio normalisation and non-AIDS-related events in individuals with HIV who achieve viral load suppression with antiretroviral therapy: An observational cohort study. *Lancet HIV* **2015**, *2*, e98–e106. [CrossRef]
19. Yu, H.L.; Yang, Y.C.; Zhao, Y.; Cao, D.D.; Jin, C.; Ye, R.H.; Cao, Y.F.; Liu, X.J.; Yao, S.T.; Chen, C.; et al. Prevalence and related factors of $CD4^+$ T lymphocytes immune recovery among adult HIV/AIDS on an-tiretroviral therapy in Dehong Dai and Jingpo Autonomous Prefecture. *Zhonghua Liu Xing Bing Xue Za Zhi* **2021**, *42*, 1050–1055. [CrossRef]
20. Chen, J.; Titanji, K.; Sheth, A.N.; Gandhi, R.; McMahon, D.; Ofotokun, I.; Weitzmann, M.N.; De Paris, K.; Dumond, J.B. The effect of age on $CD4^+$ T-cell recovery in HIV-suppressed adult participants: A sub-study from AIDS Clinical Trial Group (ACTG) A5321 and the Bone Loss and Immune Reconstitution (BLIR) study. *Immun. Ageing* **2022**, *19*, 4. [CrossRef]
21. Ezeamama, A.E.; Mupere, E.; Oloya, J.; Martinez, L.; Kakaire, R.; Yin, X.; Sekandi, J.N.; Whalen, C.C. Age, sex, and nutritional status modify the $CD4^+$ T-cell recovery rate in HIV–tuberculosis co-infected patients on combination antiretroviral therapy. *Int. J. Infect. Dis.* **2015**, *35*, 73–79. [CrossRef]
22. Han, W.M.; Apornpong, T.; Handoko, R.; Jantarabenjakul, W.; Gatechompol, S.; Ubolyam, S.; Kerr, S.J.; Avihingsanon, A.; Ruxrungtham, K.; Phanuphak, P.; et al. CD4/CD8 Ratio Recovery of Children and Adolescents Living with HIV With Virological Suppression: A Prospective Cohort Study. *J. Pediatr. Infect. Dis. Soc.* **2021**, *10*, 88–96. [CrossRef]
23. Simms, V.; Rylance, S.; Bandason, T.; Dauya, E.; McHugh, G.; Munyati, S.; Mujuru, H.; Rowland-Jones, S.L.; Weiss, H.A.; Ferrand, R.A. $CD4^+$ cell count recovery following initiation of HIV antiretroviral therapy in older childhood and adolescence. *AIDS* **2018**, *32*, 1977–1982. [CrossRef]
24. Bahemana, E.; The AFRICOS Study Group; Esber, A.; Dear, N.; Ganesan, K.; Parikh, A.; Reed, D.; Maganga, L.; Khamadi, S.; Mizinduko, M.; et al. Impact of age on CD4 recovery and viral suppression over time among adults living with HIV who initiated antiretroviral therapy in the African Cohort Study. *AIDS Res. Ther.* **2020**, *17*, 23. [CrossRef]
25. Polish AIDS Society. *Polish AIDS Society Recommendations*; PTN AIDS: Warszawa-Wrocław, Poland, 2015. Available online: http://www.ptnaids.pl/attachments/article/20/Rekomendacje%20PTN%20AIDS%202015.pdf (accessed on 21 September 2022).
26. Bellissimo, F.; Pinzone, M.R.; Celesia, B.M.; Cacopardo, B.; Nunnari, G. Baseline CD4/CD8 T-cell Ratio Predicts Prompt Immune Restoration upon cART Initiation. *Curr. HIV Res.* **2016**, *14*, 491–496. [CrossRef] [PubMed]
27. Mutoh, Y.; Nishijima, T.; Inaba, Y.; Tanaka, N.; Kikuchi, Y.; Gatanaga, H.; Oka, S. Incomplete Recovery of CD4 Cell Count, CD4 Percentage, and CD4/CD8 Ratio in Patients with Human Immunodeficiency Virus Infection and Suppressed Viremia During Long-term Antiretroviral Therapy. *Clin. Infect. Dis.* **2018**, *67*, 927–933. [CrossRef] [PubMed]
28. Bishop, J.D.; DeShields, S.; Cunningham, T.; Troy, S.B. CD4 Count Recovery After Initiation of Antiretroviral Therapy in Patients Infected with Human Immunodeficiency Virus. *Am. J. Med Sci.* **2016**, *352*, 239–244. [CrossRef] [PubMed]

29. Song, A.; Li, Z.; Luo, Z.; Lu, X.; Wang, R.; Liu, L.; Xia, W.; Wan, Z.; Zhang, T.; Su, B.; et al. Effects of Early and Delayed Antiretroviral Therapy on Plasma Anti-CD4 Autoreactive IgG and Its Association with $CD4^+$ T-Cell Recovery in Acute HIV-Infected Individuals. *Front. Pharmacol.* **2020**, *11*, 449. [CrossRef] [PubMed]
30. Little, S.J. Treatment of Acute HIV Infection and the Potential Role of Acutely HIV-Infected Persons in Cure Studies. *Top. Antivir. Med.* **2015**, *23*, 156–160.
31. Leyre, L.; Kroon, E.; Vandergeeten, C.; Sacdalan, C.; Colby, D.J.; Buranapraditkun, S.; Schuetz, A.; Chomchey, N.; de Souza, M.; Bakeman, W.; et al. Abundant HIV-infected cells in blood and tissues are rapidly cleared upon ART initiation during acute HIV infection. *Sci. Transl. Med.* **2020**, *12*, eaav3491. [CrossRef]
32. Badura, R.; Foxall, R.B.; Ligeiro, D.; Rocha, M.; Godinho-Santos, A.; Trombetta, A.C.; Sousa, A.E. Early ART in Acute HIV-1 Infection: Impact on the B-Cell Compartment. *Front. Cell. Infect. Microbiol.* **2020**, *10*, 347. [CrossRef]
33. Chan, P.; Kerr, S.J.; Kroon, E.; Colby, D.; Sacdalan, C.; Hellmuth, J.; Reiss, P.; Vasan, S.; Ananworanich, J.; Valcour, V.; et al. Cognitive trajectories after treatment in acute HIV infection. *AIDS* **2021**, *35*, 883–888. [CrossRef]
34. Gelpi, M.; Hartling, H.J.; Thorsteinsson, K.; Gerstoft, J.; Ullum, H.; Nielsen, S.D. Immune recovery in acute and chronic HIV infection and the impact of thymic stromal lymphopoietin. *BMC Infect. Dis.* **2016**, *16*, 591. [CrossRef]
35. Handoko, R.; Colby, D.J.; Kroon, E.; Sacdalan, C.; de Souza, M.; Pinyakorn, S.; Prueksakaew, P.; Munkong, C.; Ubolyam, S.; Akapirat, S.; et al. Determinants of suboptimal $CD4^+$ T cell recovery after antiretroviral therapy initiation in a prospective cohort of acute HIV-1 infection. *J. Int. AIDS Soc.* **2020**, *23*, e25585. [CrossRef]
36. Muscatello, A.; Nozza, S.; Fabbiani, M.; De Benedetto, I.; Ripa, M.; Dell'Acqua, R.; Antinori, A.; Pinnetti, C.; Calcagno, A.; Ferrara, M.; et al. Enhanced Immunological Recovery with Early Start of Antiretroviral Therapy During Acute or Early HIV Infection–Results of Italian Network of ACuTe HIV InfectiON (INACTION) Retrospective Study. *Pathog. Immun.* **2020**, *5*, 8–33. [CrossRef]
37. Marconi, V.C.; Grandits, G.; Okulicz, J.F.; Wortmann, G.; Ganesan, A.; Crum-Cianflone, N.; Polis, M.; Landrum, M.; Dolan, M.J.; Ahuja, S.K.; et al. Cumulative Viral Load and Virologic Decay Patterns after Antiretroviral Therapy in HIV-Infected Subjects Influence CD4 Recovery and AIDS. *PLoS ONE* **2011**, *6*, e17956. [CrossRef]
38. Novelli, S.; Delobel, P.; Bouchaud, O.; Avettand-Fenoel, V.; Fialaire, P.; Cabié, A.; Souala, F.; Raffi, F.; Catalan, P.; Weiss, L.; et al. Enhanced immunovirological response in women compared to men after antiretroviral therapy initiation during acute and early HIV-1 infection: Results from a longitudinal study in the French ANRS Primo cohort. *J. Int. AIDS Soc.* **2020**, *23*, e25485. [CrossRef]
39. Scully, E.P. Sex Differences in HIV Infection. *Curr. HIV/AIDS Rep.* **2018**, *15*, 136–146. [CrossRef]
40. Meditz, A.L.; MaWhinney, S.; Allshouse, A.; Feser, W.; Markowitz, M.; Little, S.; Hecht, R.; Daar, E.S.; Collier, A.C.; Margolick, J.; et al. Sex, Race, and Geographic Region Influence Clinical Outcomes Following Primary HIV-1 Infection. *J. Infect. Dis.* **2011**, *203*, 442–451. [CrossRef]
41. Aziz, M.; Livak, B.; Burke-Miller, J.; French, A.L.; Glesby, M.J.; Sharma, A.; Young, M.; Villacres, M.C.; Tien, P.C.; Golub, E.T.; et al. Vitamin D insufficiency may impair CD4 recovery among Women's Interagency HIV Study participants with advanced disease on HAART. *AIDS* **2013**, *27*, 573–578. [CrossRef]
42. Akhtar, A.; Fatima, S.; Saeed, H.; Soo, C.T.; Khan, A.H. HIV-HCV Coinfection: Prevalence and Treatment Outcomes in Malaysia. *Intervirology* **2022**, *65*, 87–93. [CrossRef]
43. Skinner, N.E.; Vergara, C.; El-Diwany, R.; Paul, H.; Skaist, A.; Wheelan, S.J.; Thomas, D.L.; Ray, S.C.; Balagopal, A.; Bailey, J.R. Decreased Activated $CD4^+$ T Cell Repertoire Diversity After Antiretroviral Therapy in HIV-1/HCV Coinfection Correlates with $CD4^+$ T Cell Recovery. *Viral Immunol.* **2021**, *34*, 622–631. [CrossRef]
44. Emmanuel, B.; El-Kamary, S.S.; Magder, L.S.; Stafford, K.A.; Charurat, M.E.; Poonia, B.; Chairez, C.; McLaughlin, M.; Hadigan, C.; Masur, H.; et al. Immunological recovery in T-cell activation after sustained virologic response among HIV positive and HIV negative chronic Hepatitis C patients. *Hepatol. Int.* **2019**, *13*, 270–276. [CrossRef]
45. Bandera, A.; Lorenzini, P.; Taramasso, L.; Cozzi-Lepri, A.; Lapadula, G.; Mussini, C.; Saracino, A.; Ceccherini-Silberstein, F.; Puoti, M.; Quiros-Roldan, E.; et al. The impact of DAA-mediated HCV eradication on $CD4^+$ and $CD8^+$ T lymphocyte trajectories in HIV/HCV coinfected patients: Data from the ICONA Foundation Cohort. *J. Viral Hepat.* **2021**, *28*, 779–786. [CrossRef]
46. McPhee, C.F.; Sainz, T.; Navarro, M.L. Recovery of CD4 T Cells in HIV/HCV Coinfected Children: Is it Really Impaired? *Pediatr. Infect. Dis. J.* **2018**, *37*, 278. [CrossRef]
47. Malagnino, V.; Cerva, C.; Teti, E.; Campogiani, L.; Compagno, M.; Biland, L.F.; Saderi, L.; Armenia, D.; Salpini, R.; Svicher, V.; et al. Poor CD4/CD8 ratio recovery in HBcAb-positive HIV patients with worse immune status is associated with significantly higher CD8 cell numbers. *Sci. Rep.* **2021**, *11*, 3965. [CrossRef] [PubMed]
48. Nie, Y.; Deng, X.; Lan, Y.; Li, L.; Cai, W.; Li, F.; Hu, F. Impact of HBeAg status on immune recovery in HIV/HBV co-infected patients after highly active antiretroviral therapy. *Infect. Genet. Evol.* **2021**, *91*, 104781. [CrossRef]
49. Houghtaling, L.; Moh, R.; Chekaraou, M.A.; Gabillard, D.; Anglaret, X.; Eholié, S.P.; Zoulim, F.; Danel, C.; Lacombe, K.; Boyd, A. $CD4^+$ T Cell Recovery and Hepatitis B Virus Coinfection in HIV-Infected Patients from Côte d'Ivoire Initiating Antiretroviral Therapy. *AIDS Res. Hum. Retrovir.* **2018**, *34*, 439–445. [CrossRef] [PubMed]
50. Peebles, B.K.; Nchimba, L.; Chilengi, R.; Moore, C.B.; Mubiana-Mbewe, M.; Vinikoor, M.J. Pediatric HIV–HBV Coinfection in Lusaka, Zambia: Prevalence and Short-Term Treatment Outcomes: Table 1. *J. Trop. Pediatr.* **2015**, *61*, 464–467. [CrossRef]

51. Kwofie, T.B.; Adigbli, D.; Osei-Yeboah, J.; Ativi, E.; Lokpo, S.Y. Hepatitis B and C infections in HIV-1 patients on combination antiretroviral therapy (cART) in Ghana: Implications for immunologic recovery, clinical response to treatment, and hepatotoxicity. *Heliyon* **2021**, *7*, e07172. [CrossRef] [PubMed]
52. Azzoni, L.; Foulkes, A.S.; Firnhaber, C.; Yin, X.; Crowther, N.J.; Glencross, D.; Lawrie, D.; Stevens, W.; Papasavvas, E.; Sanne, I.; et al. Metabolic and anthropometric parameters contribute to ART-mediated $CD4^+$ T cell recovery in HIV-1-infected individuals: An observational study. *J. Int. AIDS Soc.* **2011**, *14*, 37. [CrossRef] [PubMed]
53. Chan, D.P.-C.; Lee, M.-P.; Wong, N.-S.; Leung, R.K.-K.; Naftalin, C.M.; Lee, S.-S. Association of immune recovery with hyperlipidaemia and apolipoprotein gene polymorphisms following highly active antiretroviral therapy in a cohort of Chinese HIV patients. *BMJ Open* **2016**, *6*, e010998. [CrossRef]
54. Rodríguez-Gallego, E.; Gómez, J.; Pacheco, Y.M.; Peraire, J.; Viladés, C.; Beltrán-Debón, R.; Mallol, R.; López-Dupla, M.; Veloso, S.; Alba, V.; et al. A baseline metabolomic signature is associated with immunological $CD4^+$ T-cell recovery after 36 months of antiretroviral therapy in HIV-infected patients. *AIDS* **2018**, *32*, 565–573. [CrossRef]
55. Manfredi, R.; Calza, L.; Chiodo, F. Prolonged statin administration does not act on the cell-mediated immunity of HIV-infected dyslipidemic patients treated with a steady and effective highly active antiretroviral therapy. A two-year prospective study of statin versus fibrate administration. *J. Biol. Regul. Homeost. Agents* **2006**, *20*, 1–9.
56. Bursztyn, M.; Israel, S. Is $CD4^+$ T-cell recovery—Associated with hypertension during initial antiretroviral therapy in human immunodeficiency virus patients? *J. Clin. Hypertens.* **2020**, *22*, 1563–1564. [CrossRef]
57. Aibibula, W.; Cox, J.; Hamelin, A.-M.; Moodie, E.E.M.; Anema, A.; Klein, M.B.; Brassard, P. The Canadian Co-infection Cohort Investigators Association between depressive symptoms, CD4 count and HIV viral suppression among HIV-HCV co-infected people. *AIDS Care* **2018**, *30*, 643–649. [CrossRef]
58. Shmagel, N.G.; Shmagel, K.V.; Saidakova, E.V.; Korolevskaya, L.B.; Chereshnev, V.A. Discordant response of $CD4^+$ T cells to antiretroviral therapy in HIV-infected patients coinfected with hepatitis C virus is accompanied by increased liver damage. *Dokl. Biochem. Biophys.* **2015**, *465*, 358–360. [CrossRef]
59. Kang, F.; Chen, W.; Zhang, X.; Nie, W.; Fu, J.; Xu, X.; Zhao, P.; Zhang, X.; Li, W.; Wang, F.-S.; et al. Transient Liver Injury Associated with the Early Recovery of HCV-Specific T-Cell Responses and HCV Rebound in HIV-1/HCV Coinfected Patients Undergoing Highly Active Antiretroviral Therapy. *JAIDS J. Acquir. Immune Defic. Syndr.* **2013**, *62*, 135–142. [CrossRef]
60. Bayarsaikhan, S.; Jagdagsuren, D.; Gunchin, B.; Sandag, T. Survival, CD4 T lymphocyte count recovery and immune reconstitution pattern during the first-line combination antiretroviral therapy in patients with HIV-1 infection in Mongolia. *PLoS ONE* **2021**, *16*, e0247929. [CrossRef]
61. Nakanjako, D.; Kiragga, A.N.; Musick, B.S.; Yiannoutsos, C.T.; Wools-Kaloustian, K.; Diero, L.; Oyaro, P.; Lugina, E.; Ssali, J.C.; Kambugu, A.; et al. Frequency and impact of suboptimal immune recovery on first-line antiretroviral therapy within the International Epidemiologic Databases to Evaluate AIDS in East Africa. *AIDS* **2016**, *30*, 1913–1922. [CrossRef]
62. Corbeau, P.; Reynes, J. Immune reconstitution under antiretroviral therapy: The new challenge in HIV-1 infection. *Blood* **2011**, *117*, 5582–5590. [CrossRef]
63. Kim, K.-H.; Yi, J.; Lee, S.H. The CD4 slope can be a predictor of immunologic recovery in advanced HIV patients: A case-control study. *Korean J. Intern. Med.* **2015**, *30*, 705–713. [CrossRef]
64. Fabbiani, M.; Borghetti, A.; Squillace, N.; Colafigli, M.; Taramasso, L.; Lombardi, A.; Rossetti, B.; Ciccullo, A.; Colella, E.; Picarelli, C.; et al. Integrase Inhibitors Use and Cytomegalovirus Infection Predict Immune Recovery in People Living with HIV Starting First-Line Therapy. *JAIDS J. Acquir. Immune Defic. Syndr.* **2021**, *86*, 119–127. [CrossRef]
65. De Salvador-Guillouet, F.; Sakarovitch, C.; Durant, J.; Risso, K.; Demonchy, E.; Roger, P.M.; Fontas, E. Antiretroviral Regimens and CD4/CD8 Ratio Normalization in HIV-Infected Patients during the Initial Year of Treatment: A Cohort Study. *PLoS ONE* **2015**, *10*, e0140519. [CrossRef]
66. Serrano-Villar, S.; Zhou, Y.; Rodgers, A.J.; Moreno, S. Different impact of raltegravir versus efavirenz on CD4/CD8 ratio recovery in HIV-infected patients. *J. Antimicrob. Chemother.* **2016**, *72*, 235–239. [CrossRef]
67. Ambrosioni, J.; Farrera, J.; De Lazzari, E.; Nicolás, D.; Manzardo, C.; Hernández-Meneses, M.M.; Mosquera, M.M.; Ligero, C.; Marcos, M.A.; Sánchez-Palomino, S.; et al. Immunological and virological efficacy of different antiretroviral regimens initiated during acute/recent HIV infection. *AIDS* **2020**, *34*, 2269–2274. [CrossRef]
68. Zhang, Y.; Jiang, T.; Li, A.; Li, Z.; Hou, J.; Gao, M.; Huang, X.; Su, B.; Wu, H.; Zhang, T.; et al. Adjunct Therapy for $CD4^+$ T-Cell Recovery, Inflammation and Immune Activation in People Living With HIV: A Systematic Review and Meta-Analysis. *Front. Immunol.* **2021**, *12*, 632119. [CrossRef] [PubMed]

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REVIEW

Human Immunodeficiency Virus as a Risk Factor for Cardiovascular Disease

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Abstract
The developments in HIV treatments have increased the life expectancy of people living with HIV (PLWH), a situation that makes cardiovascular disease (CVD) in that population as relevant as ever. PLWH are at increased risk of CVD, and our understanding of the underlying mechanisms is continually increasing. HIV infection is associated with elevated levels of multiple proinflammatory molecules, including IL-6, IL-1β, VCAM-1, ICAM-1, TNF-α, TGF-β, osteopontin, sCD14, hs-CRP, and D-dimer. Other currently examined mechanisms include CD4 + lymphocyte depletion, increased intestinal permeability, microbial translocation, and altered cholesterol metabolism. Antiretroviral therapy (ART) leads to decreases in the concentrations of the majority of proinflammatory molecules, although most remain higher than in the general population. Moreover, adverse effects of ART also play an important role in increased CVD risk, especially in the era of rapid advancement of new therapeutical options. Nevertheless, it is currently believed that HIV plays a more significant role in the development of metabolic syndromes than treatment-associated factors. PLWH being more prone to develop CVD is also due to the higher prevalence of smoking and chronic coinfections with viruses such as HCV and HBV. For these reasons, it is crucial to consider HIV a possible causal factor in CVD occurrence, especially among young patients or individuals without common CVD risk factors.

Keywords Antiretroviral Therapy · Human Immunodeficiency Virus · Cardiovascular Disease · Risk Factor

Introduction

Human Immunodeficiency Virus (HIV) infection is a chronic disease that is a known risk factor for CVD, a leading cause of mortality worldwide [1]. It is estimated that rates of morbidity and mortality from CVD are 50–100% higher in those with HIV than in a well-matched population without HIV infection [2]. Among the most prevalent cardiovascular conditions in people living with HIV (PLWH) are hypertension, hypercholesterolemia, low HDL-cholesterol, hypertriglyceridemia, and high serum glucose. Moreover, PLWH are more prone to experience ischemic stroke, arrhythmias, heart failure, myocardial infarction, and sudden cardiac death [2, 3]. The mechanisms leading to increased cardiovascular risk in PLWH include viral stimulation of pro-inflammatory molecules, CD4 + lymphocyte depletion, increased intestinal permeability, microbial translocation, and altered cholesterol metabolism [4]. Moreover, the higher prevalence of smoking and other chronic viral coinfections also play important roles in the altered pro-inflammatory status of PLWH [5, 6].

Antiretroviral therapy (ART), the treatment of choice, has significantly contributed to the management of HIV infection and therefore to the increase in life expectancy for PLWH [7]. However, it has been reported that ART can not only suppress the virus and restore immune system function but also may be harmful in terms of cardiovascular risk [8, 9]. Such unfavorable effects depend on the form of ART and the specific drugs applied; however, it is currently believed that antiretroviral therapy plays only a minor role in cardiovascular risk in comparison to HIV itself [9].

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Since cardiovascular disease is more prevalent among PLWH than in the general population of the same age, this study aims to explore the finding that early CVD development may be a symptom of HIV infection [10]. In PLWH, similarly to the general population, there are both unmodifiable and modifiable risk factors for CVD. Therefore, HIV infection should be considered in patients with early occurrence of dyslipidemia, hypertension, and high serum glucose levels, with a focus on individuals without the usual risk factors for CVD.

Unmodifiable CVD Risk Factors in PLWH

Age

In the general population, age ≥ 45 years for men and ≥ 55 years for women is considered one of the main unmodifiable risk factors of CVD [8]. Studies have reported that multiple chronic comorbidities, including CVD, occur in PLWH approximately a decade earlier than in the general population [10]. There are also reports of increased prevalence of early atherosclerosis and heart failure among PLWH and the beginning of excess heart age in early adulthood [11, 12]. Moreover, studies have suggested that cardiovascular manifestations of HIV infection, especially low HDL cholesterol and hypertriglyceridemia, may already occur in childhood [13]. As a result, the risk of death from CVD appears to be significantly higher among PLWH from 25 to 64 years for every 10-year age group, ranging from a 31% elevated risk among those aged 55–64 years to 202% among those aged 25–34 years in comparison to the general population [14].

Gender

It has been well documented that in the general population, the risk of CVD is higher in men than in premenopausal women [15]. Interestingly, studies have shown that women living with HIV have 1.5 to 2-fold higher cardiovascular disease risk than men living with HIV [16]. That difference is pronounced in premenopausal women and seems to diminish in old age [17]. The reasons for the increased CVD risk in women seem to be multivariate, and the traditional risk factors may occur more often among women than among men living with HIV. The prevalence of cigarette smoking is higher in women than in men among those living with HIV [18]. Moreover, women living with HIV are more likely to be overweight or obese and to gain weight following antiretroviral therapy compared to men [19, 20]. From a pathophysiological point of view, 59 differentially expressed genes were found in intermediate monocytes in women living with HIV, and these included known atherosclerosis genes such as the liver X receptor gene nuclear receptor subfamily 1 group H member 2 (NR1H2), Nexilin (NEXN), TNF Receptor Associated Factor 1 (TRAF1), Toll-like Receptor 7 (TLR7), and Galectin 3 Binding Protein (LGALS3BP) [21]. Women living with HIV experience menopause earlier than women in the general population, a phenomenon that is associated with an increased risk of visceral fat, reduced muscle mass, and changes in bone density, all of which are HIV-independent but are well-known risk factors for CVD [22].

Studies have shown that transgender women receiving gender-affirming hormonal therapy (GAHT) experience a gain in fat, a decline in lean body mass, and an increase in insulin resistance, all of which are risk factors for cardiovascular disease [23]. In contrast, these effects are not seen in transgender men, possibly because testosterone used as GAHT decreases body fat and increases lean body mass, effects that usually lead to unchanged body mass index (BMI) [24]. Cyproterone acetate, a widely used GAHT in transgender women, leads to an increase in body fat, especially in the android region [24]. Transgender PLWH may be additionally prone to CVD due to other complex factors such as drug-drug interactions with ART, social stressors, and stigma [25–27].

Race

In the general population, the prevalence of CVD is highest in Black individuals [28]. That association seems to occur also in PLWH. Studies have shown that CVD-related hospitalization rates in PLWH were 45% higher for African Americans than Whites [29]. One of the probable mechanisms explaining this epidemiology is that Black PLWH may be more amenable to inflammation since it has been observed that they experience over 50% higher D-dimer levels while having a detectable HIV viral load in comparison to Whites [30]. Data concerning the mechanism explaining this phenomenon are limited; however, in a study of the general population, several fibrinogen gene polymorphisms, including the Thr312Ala alpha chain variant and the FGG-10,034 C/T variant seem to be associated with 20% higher D-dimer concentrations and may partially explain the racial differences in D-dimer concentration [31].

The lowest hospitalization rates due to CVD have been observed in Asian men living with HIV. Compared to Black men living with HIV, Asians had a three-fold lower rate of hospitalization due to cardiovascular reasons [32]. Those inequalities may result from a lesser number of CVD risk factors among Asians. The cardiovascular health score defined by the American Heart Association comprises seven health factors and behaviors: dietary quality, smoking, physical activity, body mass index, blood pressure,

cholesterol, and blood glucose. The average is 0.24 points lower in Asians than Whites and 0.47 points lower than in Blacks [33].

Modifiable CVD Risk Factors in PLWH

CD4 + lymphocyte Depletion and Recovery

Untreated HIV infection is associated with a gradual depletion of memory CD4 + lymphocyte count, resulting in higher IL-2 levels and thus increasing the incidence of atherosclerosis and other related inflammatory diseases, including CVD [34]. A lower CD4 + lymphocyte count may be related to the impairment of endothelial function, since it has been reported that circulating microparticles, mostly platelets and endothelial particles, are strongly associated with arterial stiffness in PLWH with advanced immune suppression [35]. A low CD4 + level is also associated with elevated blood pressure, blood glucose, and triglycerides, and decreased HDL cholesterol [36]. There are also reports that PLWH having a CD4 + lymphocyte count < 350 cells/μl have a 30% higher likelihood of having a low HDL cholesterol concentration compared to those with CD4 cell counts > 350 cells/μl [37].

Studies have shown that PLWH with a low CD4 + lymphocyte count have higher proportions of T helper type 17 cells (Th17) and senescent cells, which are associated with higher cardiovascular risk [38]. Senescent cells are known to be connected to atherosclerosis and cardiac fibrosis, and sustained production of Th17 may be a pro-inflammatory factor [39, 40]. The prevalence of clonal hematopoiesis, which has been associated with higher cardiovascular mortality, is higher in PLWH with lower CD4 + lymphocyte counts and residual HIV transcriptional activity [41].

PLWH with lower CD4 + lymphocyte counts has been reported to have low cholesterol efflux and higher sensitivity to C-reactive protein (hs-CRP), both of which are CVD risk factors [42]. Cholesterol efflux capacity is a proinflammatory factor associated with atherosclerosis: a lower cholesterol efflux is negatively associated with the elevation of many proinflammatory molecules, including CRP, fibrinogen, interleukin-6 (IL-6), and serum amyloid A, and positively associated with cardiovascular mortality [43].

Concerning CD4 + lymphocyte count and cardiovascular risk, a special population of patients is those PLWH with poor immunological reconstruction (immunological non-responders; INRs), the patients with persistently lower CD4 + counts and CD4:CD8 ratios despite receiving effective antiretroviral therapy. INRs reportedly have higher rates of mortality due to cardiovascular disease [44]. However, after ART initiation, increases in HDL and LDL-cholesterol levels were observed in INRs, a result that makes it difficult to explicitly assess cardiovascular risk [45].

Microbial Translocation

Microbial translocation is a hallmark of HIV disease progression. It is defined as the movement of microorganisms or microbial products from the gastrointestinal mucosa into the systemic circulation [46]. The malfunctioning of the barrier leads to an enhanced microbial translocation that further leads to immune activation and inflammation, thereby increasing the risk of cardiovascular disease via pro-inflammatory mechanisms [47].

During infection, the depletion of CD4 + lymphocytes involves the Th17 CD4 + lymphocyte population, the role of which is to defend against various pathogens at mucosal barriers such as the gastrointestinal tract [48]. The depletion of these cells leads to an imbalance of the Th17/Treg ratio and enhanced production of cytokines, including IL-6, IL-17, IL-1β, IL-12, and IL-4, which disrupts epithelial junctions in the gastrointestinal mucosal barrier and therefore leads to increased microbial translocation [49, 50]. There are known markers of microbial translocation, including plasma levels of lipopolysaccharide and soluble CD14, which are bacterial products, and (1➔3)-β-D-Glucan, a fungal product, that are elevated in untreated PLWH [51, 52]. In addition to Th17 CD4 + lymphocytes, the decrease of mucosal-associated invariant T cells (MAIT) induced by chronic inflammation may contribute to increased susceptibility to microbial translocation. It has been suggested that HIV triggers highly activated MAITs to migrate to the colorectal mucosa where they are later subjected to bacteria-induced apoptosis [53]. This phenomenon is followed by increased levels of the proinflammatory cytokines IL-12 and IL-18 and thus an elevated risk of CVD [54].

Other indicators of intestinal damage are intestinal fatty acid-binding protein (I-FABP), zonulin, and regenerating islet-derived protein-3α (REG3α), all of which are considered intestinal permeability markers [55]. Both I-FABP and REG3α plasma levels are significantly elevated in PLWH not receiving ART, and they remain higher even after the introduction of ART compared to healthy controls [56, 57]. REG3α can be used to assess the degree of gut damage and systemic immune activation, and its plasma levels are positively correlated with other proinflammatory biomarkers such as IL-6, IL-8, CXCL13, and IDO-1, the fungal translocation product (1➔3)-β-D-Glucan, and the HIV viral load [58]. Zonulin levels are also elevated in PLWH, causing unclenching of the tight junctions between gut epithelial cells, leading to increases in permeability and macromolecule absorption [59]. I-FABP is involved in the uptake and transport of long-chain fatty acids from the intestinal lumen

and may be a marker for mucosal compromise or injury [60]. There are reports that both I-FABP and zonulin can be used to predict mortality in ART-treated PLWH [61].

HIV-related microbial translocation may also be the result of microbial dysbiosis, primarily expressed as decreased diversity or the outgrowth of potentially pathogenic bacteria [62]. A higher proportion of opportunistic pathogens may promote AIDS-related infections, and lower abundances of butyrate-producing bacteria may induce inflammatory bowel disease [63–65]. Likewise, microbial dysbiosis can lead to activation of the gut and peripheral T cells and increases in plasma pro-inflammatory factors such as TNF-α and soluble CD14 [44].

Dyslipidemia in PLWH

Since the beginning of the HIV epidemic, it has been reported that metabolic syndrome is twice as frequent in PLWH than in the general population [66]. This may be due to altered lipid metabolism causing low HDL cholesterol and hypertriglyceridemia, factors that are considered high-risk lipid profiles for atherosclerosis and cardiovascular disease [67]. Moreover, the prevalence of hypertriglyceridemia, lower HDL cholesterol, and glucose abnormalities are much more common in younger PLWH than in older healthy controls [68]. It has been reported that low HDL cholesterol and hypertriglyceridemia may already occur in children living with HIV [13].

Studies have shown that untreated HIV infection is associated with cardiovascular abnormalities, especially endothelial dysfunction and carotid intima-media thickening [69]. A possible reason for this is the synthesis of Tat by infected cells. The Tat protein elevates the expression levels of IFN-γ, TNF-α, IL-6, and IL-17 and therefore induces apoptosis of endothelial cells. This enables low-density lipoproteins to permeate the sub-endothelial space, thereby causing atherosclerotic lesions [70]. Thus, there is an association between HIV viral load and the risk of dyslipidemia, since the larger number of HIV copies promotes the expression of adhesive proteins and cytokines such as IFN-γ, Il-1β, IL-8, IL-15, and IL-17 [37]. Another possible explanation for the altered lipid metabolism in untreated HIV infection could be the impact of TNF-α decreasing the activity of adipose tissue lipoprotein lipase, an enzyme whose role is to hydrolyze the triacylglycerol component of chylomicrons and VLDL into non-esterified fatty acids and monoacylglycerols [71].

A relationship between lipid metabolism and CD4 + count has also been suggested: PLWH with lower CD4 + lymphocyte counts were reported to have lower concentrations of HDL cholesterol and higher levels of triglycerides than PLWH with higher CD4 + lymphocyte counts [37]. Apart from a low CD4 + lymphocyte count, a history of AIDS-defining events was also reported to be associated with higher total cholesterol and triglyceride concentrations; however, improvement over time has been observed, generally due to the use of lipid-lowering agents [72].

Hypertension in PLWH

The estimated prevalence of hypertension among PLWH varies from 4 to 50% depending on the country and on the quality of the available data [73]. Despite effective antihypertensive drugs, the achievement of blood pressure control in PLWH remains a challenge [74].

Pathophysiologic mechanisms of hypertension in PLWH are a combination of typical, well-known factors occurring in the general population and the chronic inflammation resulting from HIV infection. It has been reported that higher levels of IL-17 A, IFN-γ, IL-6, and CRP were significantly associated with hypertension in ART-treated PLWH [75]. Moreover, the levels of intermediate monocytes CD14 + 16 + were increased with higher HIV viral load, and this may lead to microbial translocation that drives systemic inflammation [76]. All of these factors enhance the activation of the renin-angiotensin-aldosterone system, a key factor in the development of hypertension [77]. Older age, high BMI, obesity, previous cardiovascular events, chronic kidney disease, a family history of hypertension, and dyslipidemia are traditional risk factors common in PLWH, all of which contribute to the development of hypertension [78].

Besides HIV infection, ART is another risk factor for hypertension in PLWH, since during ART the risk of hypertension is over 1.5-fold higher compared with ART-naïve patients [79]. The negative role of ART in arterial blood pressure involves protease inhibitors (PI) and integrase inhibitors (InSTI) [80]. The use of PIs is associated with carotid artery intima-media thickness and arterial stiffness progression, and InSTIs may promote weight gain and therefore increase the risk of hypertension [81, 82].

Glucose Metabolism in PLWH

It has been reported that PLWH have higher leptin concentrations, and this may increase central fat mass, worsen insulin sensitivity, and lead to higher glucose levels [83]. Another mechanism of altered glucose metabolism in PLWH may involve lower adiponectin levels that are associated with an increased risk of coronary stenosis [84]. ART may also negatively impact glucose metabolism, as a higher prevalence of insulin resistance was shown in PLWH receiving nucleoside reverse transcriptase inhibitors (NRTI) and PI treatment [85]. PLWH with diabetes mellitus have higher cardiovascular risk according to the Framingham equation

and the RAMA-EGAT score, and they more often develop cerebrovascular complications or chronic kidney disease than non-diabetic PLWH [86].

Type 2 diabetes mellitus poses a burden for PLWH, especially for women. It is estimated that the prevalence of diabetes mellitus among women living with HIV is 23% compared with 16% among men living with HIV [87]. Moreover, women living with HIV have a 1.31 greater odds of acquiring diabetes mellitus in comparison to women without HIV infection [87]. Type 2 diabetes, as it involves prolonged hyperglycemia and insulin resistance, impacts the formation of advanced glycation end products and overproduction of reactive oxygen species and activation of protein kinase C, further leading to chronic vascular inflammation resulting in the development of atherosclerotic cardiovascular disease [88].

Obesity in PLWH and Physical Activity

Obesity as a major metabolic syndrome is one of the traditional risk factors for CVD [89]. A study based on United States registries revealed that among PLWH, 19% of men and 42% of women are obese, while in the general United States population one in three adults is obese [90]. Currently, obesity is less prevalent in PLWH than in the general population; however, the BMI of PLWH has been increasing at a rate more than three times that of the HIV-negative population, and it is estimated that it can soon exceed that of the general population [91].

ART also has a crucial role in increasing the rate of obesity in PLWH [92]. Among widely used antiretroviral agents, weight gain is largely associated with InSTIs, especially bictegravir and dolutegravir [93]. The non-nucleoside reverse transcriptase inhibitors (NNRTIs) such as rilpivirine and NRTIs such as tenofovir alafenamide (TAF) also have a higher potential to cause weight gain than other drugs from these classes of ART [93]. A study analyzing mitochondrial DNA haplogroups in PLWH gaining weight on ART observed that the European haplogroup clade UK and the African haplogroup L3 were associated with significantly greater weight gain after switching to InSTI-based ART [94]. Studies have also suggested the role of direct ART interference with the melanocortin 4 receptor, since modulation of the melanocortin system can influence food intake and body weight. However, these results are currently debatable [95].

Physical activity plays a beneficial role in the reduction of CVD risk in both PLWH and the general population [96]. However, previous observational studies have demonstrated that the level of physical activity of PLWH is lower compared with the general population, a factor that may also have an impact on the increased prevalence of cardiovascular disease [97]. It has been reported that physical activity decreases the risk of CVD and increases the quality of life in PLWH [98].

Smoking

Smokers are more prone to develop heart failure, atrial fibrillation, venous thromboembolism, and ischemic episodes [6]. The mechanisms by which smoking increases CVD risk generally involve endothelial function, as smoking leads to the impairment of the endothelial cells' ability to perform repair mechanisms. This in turn results in increased levels of total and apoptotic circulating endothelial microparticles and progenitor cells in smokers [99]. Smoking also impairs endothelium-independent vasodilatation; nitrate-mediated and flow-mediated arterial dilation were lower in smokers than in the non-smoking population [100]. Furthermore, smoking leads to an increased expression of adhesion molecules and proinflammatory cytokines, including IL6, TNF-α, and IL1β [101]. These effects seem to concern not only traditional tobacco smoke; the use of alternative smoking products (e.g., e-cigarettes) was also associated with increased adhesion of monocytes to endothelial cells and increased ICAM-1 and VCAM-1 expression, although with a smaller effect size [102].

Smoking also plays a crucial role in CVD development among PLWH, since PLWH smoke two to three times more than the general population [103]. Smoking is a factor that shortens life expectancy, and in PLWH who smoke, mortality rates of three times those of nonsmokers without HIV infection have been observed. Moreover, tobacco use dramatically increases the mortality risk among PLWH [104]. Additionally, PLWH who smoke tobacco are less likely to quit. One of the possible reasons for the difficulty in quitting is the relatively higher nicotine metabolism in PLWH as measured by the nicotine metabolite ratio (NMR, 3-hydroxycotinine/cotinine). High nicotine metabolism is also responsible for a lower response to transdermal nicotine therapy [105].

Coinfections

Another important risk factor for CVD development and progression is the presence of coinfections of HIV with other viruses [106]. It has been estimated that approximately 10–15% of the mortalities in PLWH are due to liver diseases, primarily viral hepatitis [107]. Chronic HBV and HCV infections are prevalent among PLWH since the diseases are transmitted through similar routes as HIV [108]. Several reports have suggested that people with HIV/HCV coinfection have elevated levels of plasma inflammation and microbial translocation biomarkers, especially sCD14 and IL-6, compared to PLWH [109]. Patients with HIV/

HBV coinfection are more likely to have increased serum TNF-α, IL-6, IL-8, and IL-12p70 concentrations [110]. Additionally, it has been reported that mucosal-associated invariant T cells (MAIT) are depleted in chronic viral infections, a factor that may contribute to increased susceptibility to microbial translocation and therefore to elevated CVD risk [111].

Likewise, the herpesviruses CMV and EBV also appear to play important roles in the risk of CVD in PLWH. CMV can disrupt epithelial junctions in the gastrointestinal tract, thereby enhancing microbial translocation [112]. Chronic CMV infection is also associated with higher serum IL-6 levels and higher proportions of CMV pp65 (NLV)-specific CD8 + T cells [113]. HIV/EBV coinfection was found to be associated with higher IFN-γ, TGF-β1, and IL-2 expression levels [114].

Metabolic Effects of Antiretroviral Therapy

The most frequently used antiretroviral medication groups are integrase inhibitors, nucleoside reverse transcriptase inhibitors, non-nucleoside reverse transcriptase inhibitors, and protease inhibitors. According to the European AIDS Clinical Society, the majority of currently recommended regimens are based on InSTI. Another first-line treatment is based on doravirine (NNRTI) instead of InSTI. Common alternative regimens allow the usage of darunavir, a protease inhibitor. All recommended schemes of ART contain either one or two NRTIs [115]. Novel ARTs are marked by their high potency, low toxicity, and high effectiveness [116].

Integrase inhibitors are a relatively new class of ART that are supposed to have better efficacy, reduced treatment discontinuation, and higher genetic barrier to drug resistance than older classes of ART [117]. In terms of cardiovascular risk, InSTIs are generally associated with weight gain, obesity, and weight-related comorbidities [118]. Among InSTIs, PLWH taking bictegravir and dolutegravir are at greater risk of weight gain compared to elvitegravir [93]. The demographic factors associated with an increase in BMI are female gender and Black race [93]. Regardless of weight gain, the cardiovascular risk as assessed by the incidence of major adverse cardiac events such as myocardial infarction, ischemic stroke, coronary artery bypass grafting, and percutaneous coronary intervention appears to be decreased among patients receiving InSTI-based regimens in comparison to other classes of ART [119].

The potentially adverse cardiovascular effects of NRTIs are mitophagy-associated endothelial toxicity and mitochondrial oxidative stress [120]. The decrease in mitochondrial DNA copy number in late-passage human aortic endothelial cells and the elevation of senescence-associated β-galactosidase accumulation have been observed in PLWH receiving NRTIs [120]. Moreover, NRTI administration seems to induce increases in the production of reactive oxygen species, accumulation of β-galactosidase, and diminished ATP-linked respiration [121]. The safety profiles of TAF and tenofovir disoproxil (TDF), two widely used forms of tenofovir, show that even a change in the form of the same drug may result in a huge difference in adverse effects. TAF is generally associated with a better safety profile but a possible increase in cardiovascular risk after the switch from ART regimens containing TDF to TAF, especially via increases in total and LDL cholesterol and BMI [93, 122].

Doravirine has beneficial metabolic profiles and can reduce the risk of CVD. Studies have demonstrated decreases in total cholesterol, LDL cholesterol, and triglycerides after switching to doravirine from different regimens [123]. In contrast, PIs are generally considered to have unfavorable effects in terms of cardiovascular risk. The mechanisms responsible include the triggering of reactive oxygen species production, impaired mitochondrial function, and ubiquitin-proteasome system dysregulation, factors that can in turn initiate transcriptional changes that contribute to the perturbation of lipid metabolism [124].

The impact of HIV infection and antiretroviral therapy on chronic inflammation.

Proinflammatory Molecules

The molecules associated with HIV that promote inflammation and may lead to immune dysfunction are considered below.

High-sensitivity CRP, one of the most common markers of inflammation, is a well-known risk factor for CVD and a predictor of all-cause mortality [125]. Higher concentrations of hs-CRP in PLWH in comparison to the general population have been demonstrated. Increased levels of D-dimer, a marker of deterioration of CV condition and endothelial dysfunction, are also associated with increased HIV viral load, microbial translocation, immune activation, and mortality risk [126].

Interleukin-6 belongs to the interleukin-6 family, a group of cytokines that includes IL-6, IL-11, IL-27, ciliary neurotrophic factor, leukemia inhibitory factor, oncostatin M, cardiotrophin 1, and cardiotrophin-like cytokine [127]. IL-6 is a pro-inflammatory cytokine in which higher circulating levels are associated with HIV replication [128]. Increased levels of IL-6 are related to the development of CVD and can predict mortality due to CVD or CV events [71]. In HIV infection, IL-1β induces TNF-α and IL-6 expression, leading to sustained proinflammatory responses. HIV is also a factor in the production of IL-1β via transforming pro-IL-1β into bioactive IL-1β, a cytokine that is associated both with the progression to AIDS and higher CVD risk [129].

A detectable HIV viral load induces a higher TNF-α serum concentration that can initiate and accelerate apoptosis, atherogenesis, thrombosis, vascular remodeling, and oxidative stress and therefore increase cardiovascular risk [130, 131]. TGF-β is related to atherosclerosis-associated vascular inflammation, and the overexpression of TGF-β in PLWH promotes viral replication and plays an important role in the progression of HIV infection and associated diseases [132]. Chronic increase in osteopontin level, reported in PLWH, is another risk factor for CVD, since osteopontin plays a role in the secretion of multiple proinflammatory molecules, including IL-10, IL-12, IL-3, IFN-γ, and can also be used to predict major adverse cardiovascular events [133]. Elevated levels of sCD14 observed in PLWH have been associated with microbial translocation, increased immune activation, and a greater risk of mortality and morbidity due to CVD [134].

The expression of the adhesion molecules VCAM-1 and ICAM-1, which mediate inflammation and promote leukocyte migration, is stimulated by HIV-Tat-1 protein and proinflammatory cytokines such as TNF-α and IL-1β [135]. Toll-like receptors activate the expression of VCAM-1 and ICAM-1 in the endothelium, a response that is strongly associated with increased intimal leukocyte accumulation, an important factor in the pathogenesis of human atherosclerosis [136]. VCAM-1 is a diagnostic biomarker of endothelial dysfunction and vascular injury; together with ICAM-1, it has been used in many clinical studies to estimate the risk of CVD [137]. It has been reported that the expression of adhesion molecules in PLWH is significantly higher than in the general population [138].

The Impact of Antiretroviral Therapy on Pro-Inflammatory Biomarkers

ART is beneficial in terms of the decrease of the proinflammatory effect induced by HIV infection: studies evaluating the levels of IL-6, IL-1β, D-dimer ICAM-1, VCAM-1, and TNF-α showed a significant decrease in the concentration of those biomarkers in PLWH after receiving antiretroviral therapy. However, the levels of those biomarkers were still elevated in comparison to healthy controls [139–141]. Residual immune activation may continue in compartments such as the central nervous system, the gastrointestinal tract, or the lymph nodes, where ART penetrates insufficiently to completely suppress viral replication, resulting in residual systemic inflammation [142].

The summary of the mechanisms contributing to the elevated risk for cardiovascular disease among PLWH was presented in Fig. 1.

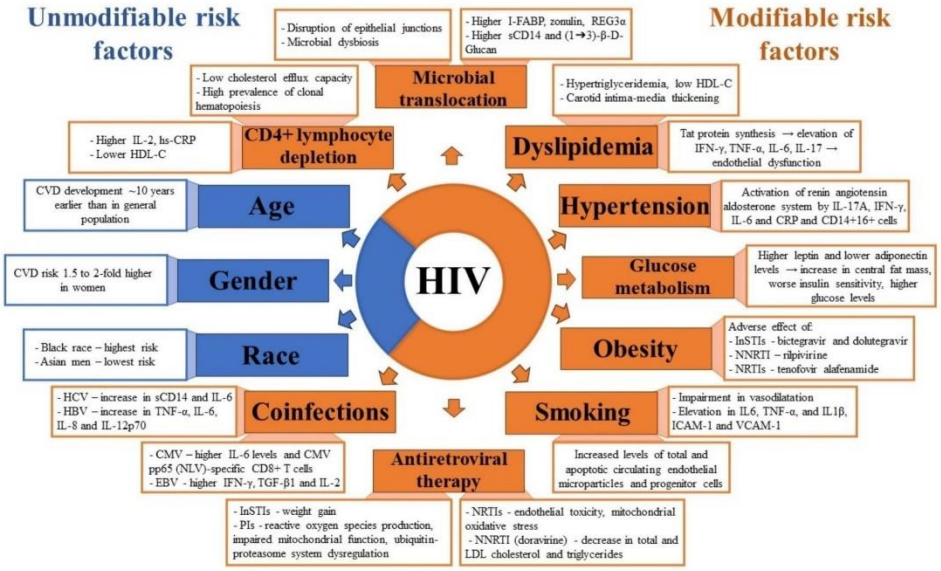

Fig. 1 Risk factors for cardiovascular disease among PLWH with underlying mechanisms

Conclusions

PLWH experience increased risk of cardiovascular disease, and the reasons for this are multivariate, including the impact of HIV infection itself, the adverse effects of antiretroviral therapy, the ambiguous effect of CD4 + cell count depletion and recovery, and other independent risk factors such as e smoking and chronic viral infections. Although HIV infection is an uncommon disease, clinicians should bear it in mind for people with early occurrence of cardiovascular disease. Dyslipidemia, hypertension, or high serum glucose levels, especially in young patients, should be considered in terms of HIV infection. The cooperation of specialists is crucial for providing the best medical care for people living with HIV and cardiovascular disease.

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Declarations

Competing Interests The authors declare no competing interests.

Ethics Approval This is a review article. The Bioethics Committee of Medical University of Warsaw has confirmed that no ethical approval was required.

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References

1. Benjamin, E. J., Blaha, M. J., Chiuve, S. E. (2017). Heart Disease and Stroke Statistics-2017 Update: A Report From the American Heart Association [published correction appears in *Circulation* 2017;135(10):e646] [published correction appears in *Circulation* 136(10:e196]. *Circulation 135*(10), e146-e603. https://doi.org/10.1161/CIR.0000000000000485
2. Freiberg, M. S., Chang, C. H., Skanderson, M., et al. (2017). Association between HIV Infection and the risk of Heart Failure with reduced ejection fraction and preserved ejection fraction in the antiretroviral therapy era: Results from the veterans Aging Cohort Study. *JAMA Cardiology*, *2*(5), 536–546. https://doi.org/10.1001/jamacardio.2017.0264
3. Perkins, M. V., Joseph, S. B., Dittmer, D. P., & Mackman, N. (2023). Cardiovascular Disease and Thrombosis in HIV Infection. *Arteriosclerosis, thrombosis, and vascular biology 43*(2), 175–191. https://doi.org/10.1161/ATVBAHA.122.318232
4. So-Armah, K., Benjamin, L. A., Bloomfield, G. S., et al. (2020). HIV and Cardiovascular Disease. *The Lancet HIV*, *7*(4), e279–e293. https://doi.org/10.1016/S2352-3018(20)30036-9
5. Jabeen, S., Rasheed, A., Jabeen, N., et al. (2020). Prevalence and association of HBV and HCV Infection with Cardiovascular Disease risk factors in a peri-urban population. *Journal of Pakistan Medical Association*, *70*(1), 58–63. https://doi.org/10.5455/JPMA.1542
6. Jeong, S. M., Yoo, J. E., Park, J., Jung, W., Lee, K. N., Han, K., Lee, C. M., Nam, K. W., Lee, S. P., & Shin, D. W. (2023). Smoking behavior change and risk of Cardiovascular Disease incidence and mortality in patients with type 2 Diabetes Mellitus. *Cardiovascular Diabetology*, *22*(1), 193. https://doi.org/10.1186/s12933-023-01930-4
7. Marcus, J. L., Leyden, W. A., Alexeeff, S. E., Anderson, A. N., Hechter, R. C., Hu, H., Lam, J. O., Towner, W. J., Yuan, Q., Horberg, M. A., & Silverberg, M. J. (2020). Comparison of overall and comorbidity-free life expectancy between insured adults with and without HIV Infection, 2000–2016. *JAMA Network open*, *3*(6), e207954. https://doi.org/10.1001/jamanetworkopen.2020.7954
8. Bishop, J. D., DeShields, S., Cunningham, T., & Troy, S. B. (2016). CD4 Count Recovery after initiation of antiretroviral therapy in patients infected with Human Immunodeficiency Virus. *The American Journal of the Medical Sciences*, *352*(3), 239–244. https://doi.org/10.1016/j.amjms.2016.05.032
9. Pambou, H. O. T., Gagneux-Brunon, A., Fossi, B. T., Roche, F., Guyot, J., Botelho-Nevers, E., Dupre, C., Bongue, B., & Nkenfou, C. N. (2022). Assessment of cardiovascular risk factors among HIV-infected patients aged 50 years and older in Cameroon. *AIMS public health 9*(3), 490–505. https://doi.org/10.3934/publichealth.2022034
10. Hsue, P. Y., Lo, J. C., Franklin, A., Bolger, A. F., Martin, J. N., Deeks, S. G., & Waters, D. D. (2004). Progression of Atherosclerosis as assessed by carotid intima-media thickness in patients with HIV Infection. *Circulation*, *109*(13), 1603–1608. https://doi.org/10.1161/01.CIR.0000124480.32233.8A
11. Erqou, S., Jiang, L., Choudhary, G., et al. (2021). Age at diagnosis of Heart Failure in United States Veterans with and without HIV Infection. *Journal of the American Heart Association*, *10*(11), e018983. https://doi.org/10.1161/JAHA.120.018983
12. Knudsen, A. D., Fuchs, A., Benfield, T., Gerstoft, J., Køber, L., Trøseid, M., Kofoed, K. F., & Nielsen, S. D. (2023). Coronary Artery Disease in Persons With Human Immunodeficiency Virus Without Detectable Viral Replication. *Open forum infectious diseases 10*(7), ofad298. https://doi.org/10.1093/ofid/ofad298
13. Tadesse, B. T., Foster, B. A., Chala, A., Chaka, T. E., Bizuayehu, T., Ayalew, F., Meskel, H., Tadesse, G., Jerene, S., Makonnen, D., & Aklillu, E., E (2019). HIV and cART-Associated Dyslipidemia among HIV-Infected Children. *Journal of Clinical Medicine*, *8*(4), 430. https://doi.org/10.3390/jcm8040430
14. Hanna, D. B., Ramaswamy, C., Kaplan, R. C. (2018). Trends in Cardiovascular Disease Mortality Among Persons With HIV in New York City, 2001–2012 [published correction appears in Clin Infect Dis. 2018;66(6):985]. *Clinical Infectious Diseases 63*(8), 1122–1129. https://doi.org/10.1093/cid/ciw470
15. Cybulska, B., Kłosiewicz-Latoszek, L., Szostak, W. (2022). Profilaktyka chorób scrowo-naczyniowych. 2022. Available online: https://www.mp.pl/interna/chapter/B16.II.2.3 (accessed on 02 February 2023).

16. Alonso, A., Barnes, A. E., Guest, J. L., Shah, A., Shao, I. Y., & Marconi, V. (2019). HIV Infection and incidence of Cardiovascular Diseases: An analysis of a large Healthcare Database. *Journal of the American Heart Association*, *8*(14), e012241. https://doi.org/10.1161/JAHA.119.012241
17. Kentoffio, K., Temu, T. M., Shakil, S. S., Zanni, M. V., & Longenecker, C. T. (2022). Cardiovascular Disease risk in women living with HIV. *Current Opinion in HIV and AIDS*, *17*(5), 270–278. https://doi.org/10.1097/COH.0000000000000756
18. Johnston, P. I., Wright, S. W., Orr, M., Pearce, F. A., Stevens, J. W., Hubbard, R. B., & Collini, P. J. (2021). Worldwide relative Smoking prevalence among people living with and without HIV. *AIDS (London England)*, *35*(6), 957–970. https://doi.org/10.1097/QAD.0000000000002815
19. Thompson-Paul, A. M., Wei, S. C., Mattson, C. L., Robertson, M., Hernandez-Romieu, A. C., Bell, T. K., & Skarbinski, J. (2015). Obesity among HIV-Infected adults receiving Medical Care in the United States: Data from the Cross-sectional Medical Monitoring Project and National Health and Nutrition Examination Survey. *Medicin*, *94*(27), e1081. https://doi.org/10.1097/MD.0000000000001081
20. Bares, S. H., Smeaton, L. M., Xu, A., Godfrey, C., & McComsey, G. A. (2018). HIV-Infected women Gain more weight than HIV-Infected men following the initiation of antiretroviral therapy. *Journal of Women's Health (2002)*, *27*(9), 1162–1169. https://doi.org/10.1089/jwh.2017.6717
21. Lin, J., Ehinger, E., Hanna, D. B., Qi, Q., Wang, T., Ghosheh, Y., Mueller, K., Anastos, K., Lazar, J. M., Mack, W. J., Tien, P. C., Berman, J. W., Cohen, M. H., Ofotokun, I., Gange, S., Liu, C., Heath, S. L., Tracy, R. P., Hodis, H. N., Landay, A. L., et al. (2023). HIV Infection and Cardiovascular Disease have both shared and distinct monocyte gene expression features: Women's interagency HIV study. *PloS One*, *18*(5), e0285926. https://doi.org/10.1371/journal.pone.0285926
22. Van Ommen, C. E., King, E. M., & Murray, M. C. M. (2021). Age at menopause in women living with HIV: A systematic review. *Menopause (New York N Y)*, *28*(12), 1428–1436. https://doi.org/10.1097/GME.0000000000001871
23. Klaver, M., de Blok, C. J. M., Wiepjes, C. M., Nota, N. M., Dekker, M. J. H. J., de Mutsert, R., Schreiner, T., Fisher, A. D., T'Sjoen, G., & den Heijer, M. (2018). Changes in regional body fat, lean body mass and body shape in trans persons using cross-sex hormonal therapy: Results from a multicenter prospective study. *European Journal of Endocrinology*, *178*(2), 163–171. https://doi.org/10.1530/EJE-17-0496
24. Suppakitjanusant, P., Ji, Y., Stevenson, M. O., Chantrapanichkul, P., Sineath, R. C., Goodman, M., Alvarez, J. A., & Tangpricha, V. (2020). Effects of gender-affirming hormone therapy on body mass index in transgender individuals: A longitudinal cohort study. *Journal of Clinical & Translational Endocrinology*, *21*, 100230. https://doi.org/10.1016/j.jcte.2020.100230
25. Lake, J. E., Wang, R., Barrett, B. W., Bowman, E., Hyatt, A. N., Debroy, P., Candelario, J., Teplin, L., Bodnar, K., McKay, H., Plankey, M., Brown, T. T., Funderburg, N., & Currier, J. S. (2022). Trans women have worse cardiovascular biomarker profiles than cisgender men Independent of hormone use and HIV serostatus. *AIDS (London England)*, *36*(13), 1801–1809. https://doi.org/10.1097/QAD.0000000000003346
26. Hiransuthikul, A., Himmad, L., Kerr, S. J., Janamnuaysook, R., Dalodom, T., Phanjaroen, K., Pankam, T., Kongkapan, J., Mills, S., Vannakit, R., Phanuphak, P., & Phanuphak, N. (2021). Drug-drug interactions among Thai Transgender Women living with human immunodeficiency undergoing feminizing hormone therapy and antiretroviral therapy: The iFACT Study. *Clinical Infectious Diseases*, *72*(3), 396–402. https://doi.org/10.1093/cid/ciaa038
27. Tun, W., Pulerwitz, J., Shoyemi, E., Fernandez, A., Adeniran, A., Ejiogu, F., Sangowawa, O., Granger, K., Dirisu, O., & Adedimeji, A. A. (2022). A qualitative study of how stigma influences HIV services for transgender men and women in Nigeria. *Journal of the International AIDS Society*, *25*(Suppl 1), e25933. https://doi.org/10.1002/jia2.25933
28. Vyas, A., Desai, R., Went, T. R., Wiltshire, D., Priyadarshni, S., Shalaby, M., & Khalife, W. (2023). Cardiovascular Disease Burden and Major Adverse Cardiac Events in Young Black Patients: A National Analysis of 2 cohorts 10 years apart (2017 Versus 2007). *Journal of the American Heart Association*, *12*(15), e029895. https://doi.org/10.1161/JAHA.123.029895
29. Oramasionwu, C. U., Morse, G. D., Lawson, K. A., Brown, C. M., Koeller, J. M., & Frei, C. R. (2013). Hospitalizations for Cardiovascular Disease in African americans and whites with HIV/AIDS. *Population Health Management*, *16*(3), 201–207. https://doi.org/10.1089/pop.2012.0043
30. O'Bryan, T. A., Agan, B. K., Tracy, R. P., Freiberg, M. S., Okulicz, J. F., So-Armah, K., Ganesan, A., Rimland, D., Lalani, T., Deiss, R. G., & Tramont, E. C. (2018). Brief report: Racial comparison of D-Dimer levels in US Male Military Personnel before and after HIV Infection and viral suppression. *Journal of Acquired Immune Deficiency Syndromes (1999)*, *77*(5), 502–506. https://doi.org/10.1097/QAI.0000000000001626
31. Lange, L. A., Reiner, A. P., Carty, C. L., Jenny, N. S., Cushman, M., & Lange, E. M. (2008). Common genetic variants associated with plasma fibrin D-dimer concentration in older European- and African-American adults. Journal of thrombosis and haemostasis. *JTH* *6*(4), 654–659. https://doi.org/10.1111/j.1538-7836.2008.02906.x
32. Davy-Mendez, T., Napravnik, S., Eron, J. J., Cole, S. R., Van Duin, D., Wohl, D. A., Gebo, K. A., Moore, R. D., Althoff, K. N., Poteat, T., Gill, M. J., Horberg, M. A., Silverberg, M. J., Nanditha, N. G. A., Thorne, J. E., & Berry, S. A., North American AIDS cohort collaboration on Research and Design (NA-ACCORD) of IeDEA (2021) racial, ethnic, and gender disparities in hospitalizations among persons with HIV in the United States and Canada, 2005–2015. *AIDS (London England)* *35*(8), 1229–1239. https://doi.org/10.1097/QAD.0000000000002876
33. Shah, N. S., Huang, X., Petito, L. C., Bancks, M. P., Ning, H., Cameron, N. A., Kershaw, K. N., Kandula, N. R., Carnethon, M. R., Lloyd-Jones, D. M., & Khan, S. S. (2023). Social and Psychosocial Determinants of Racial and Ethnic Differences in Cardiovascular Health in the United States Population. *Circulation* *147*(3), 190–200. https://doi.org/10.1161/CIRCULATIONAHA.122.061991
34. Wang, Y., Lifshitz, L., Silverstein, N. J., Mintzer, E., Luk, K., StLouis, P., Brehm, M. A., Wolfe, S. A., Deeks, S. G., & Luban, J. (2023). Transcriptional and chromatin profiling of human blood innate lymphoid cell subsets sheds light on HIV-1 pathogenesis. *The EMBO journal* e114153. Advance online publication. https://doi.org/10.15252/embj.2023114153
35. Kelly, C., Gurung, R., Kamng'ona, R., Sheha, I., Chammudzi, M., Jambo, K., Mallewa, J., Rapala, A., Heyderman, R., Mallon, P., Mwandumba, H., Khoo, S., & Klein, N. (2022). Circulating microparticles are increased amongst people presenting with HIV and advanced immune suppression in Malawi and correlate closely with arterial stiffness: A nested case control study. *Wellcome open Research*, *6*, 264. https://doi.org/10.12688/wellcomeopenres.17044.2
36. Saloner, R., Marquine, M. J., Sundermann, E. E., Hong, S., McCutchan, J. A., Ellis, R. J., Heaton, R. K., Grant, I., & Cherner, M. (2019). COMT Val158Met Polymorphism, Cardiometabolic Risk, and Nadir CD4 synergistically increase risk of neurocognitive impairment in men living with HIV. *Journal of Acquired Immune Deficiency Syndromes (1999)*, *81*(5), e148–e157. https://doi.org/10.1097/QAI.0000000000002083

37. Nosik, M., Belikova, M. G., Ryzhov, K., Avdoshina, D., Sobkin, A., Zverev, V., & Svitich, O. (2023). Unique Profile of Proinflammatory Cytokines in Plasma of Drug-Naïve Individuals with Advanced HIV/TB Co-Infection. *Viruses 15*(6), 1330. https://doi.org/10.3390/v15061330
38. Kundu, S., Freiberg, M. S., Tracy, R. P., So-Armah, K. A., Koethe, J. R., Duncan, M. S., Tindle, H. A., Beckman, J. A., Feinstein, M. J., McDonnell, W. J., Justice, A., Doyle, M. F., & Veterans Aging Cohort Study. (2022). Circulating T cells and Cardiovascular Risk in People with and without HIV Infection. *Journal of the American College of Cardiology*, *80*(17), 1633–1644. https://doi.org/10.1016/j.jacc.2022.08.756
39. Pham, L. M., Kim, E. C., Ou, W., Phung, C. D., Nguyen, T. T., Pham, T. T., Poudel, K., Gautam, M., Nguyen, H. T., Jeong, J. H., Yong, C. S., Park, S. Y., Kim, J. R., & Kim, J. O. (2021). Targeting and clearance of senescent foamy macrophages and senescent endothelial cells by antibody-functionalized mesoporous silica nanoparticles for alleviating aorta Atherosclerosis. *Biomaterials*, *269*, 120677. https://doi.org/10.1016/j.biomaterials.2021.120677
40. Lopez Krol, A., Nehring, H. P., Krause, F. F., Wempe, A., Raifer, H., Nist, A., Stiewe, T., Bertrams, W., Schmeck, B., Luu, M., Leister, H., Chung, H. R., Bauer, U. M., Adhikary, T., & Viseknuna, A. (2022). Lactate induces metabolic and epigenetic reprogramming of pro-inflammatory Th17 cells. *EMBO Reports*, *23*(12), e54685. https://doi.org/10.15252/embr.202254685
41. van der Heijden, W. A., van Deuren, R. C., van de Wijer, L., van den Munckhof, I. C. L., Steehouwer, M., Riksen, N. P., Netea, M. G., de Mast, Q., Vandekerckhove, L., de Voer, R. M., van der Ven, A. J., & Hoischen, A. (2022). Clonal hematopoiesis is Associated with low CD4 Nadir and increased residual HIV Transcriptional Activity in Virally suppressed individuals with HIV. *The Journal of Infectious Diseases*, *225*(8), 1339–1347. https://doi.org/10.1093/infdis/jiab419
42. Tort, O., Escribà, T., Egaña-Gorroño, L., de Lazzari, E., Cofan, M., Fernandez, E., Gatell, J. M., Martinez, E., Garcia, F., & Arnedo, M. (2018). Cholesterol efflux responds to viral load and CD4 counts in HIV + patients and is dampened in HIV exposed. *Journal of Lipid Research*, *59*(11), 2108–2115. https://doi.org/10.1194/jlr.M088153
43. Ritsch, A., Duerr, A., Kahler, P., Hunjadi, M., Stojakovic, T., Silbernagel, G., Scharnagl, H., Kleber, M. E., & März, W. (2020). Cholesterol Efflux Capacity and Cardiovascular Disease: The Ludwigshafen Risk and Cardiovascular Health (LURIC) Study. *Biomedicines 8*(11), 524. https://doi.org/10.3390/biomedicines8110524
44. Pacheco, Y. M., Jarrin, I., Rosado, I., Campins, A. A., Berenguer, J., Iribarren, J. A., Rivero, M., Muñoz-Medina, L., Bernal-Morell, E., Gutiérrez, F., & Leal, M., CoRIS (2015). Increased risk of non-AIDS-related events in HIV subjects with persistent low CD4 counts despite cART in the CoRIS cohort. *Antiviral Research*, *117*, 69–74. https://doi.org/10.1016/j.antiviral.2015.03.002
45. Masip, J., Jorba, R., López-Dupla, M., et al. (2022). Lipoprotein Profile in Immunological Non-responders PLHIV after antiretroviral therapy initiation. *International Journal of Molecular Sciences*, *23*(15), 8071. https://doi.org/10.3390/ijms23158071
46. Noguera-Julian, M., Rocafort, M., Guillén, Y., Rivera, J., Casadellà, M., Nowak, P., Hildebrand, F., Zeller, G., Parera, M., Bellido, R., Rodríguez, C., Carrillo, J., Mothe, B., Coll, J., Bravo, I., Estany, C., Herrero, C., Saz, J., Sirera, G., Torrela, A. (2016). Gut Microbiota Linked to Sexual Preference and HIV Infection. *EBioMedicine* 5, 135–146. https://doi.org/10.1016/j.ebiom.2016.01.032
47. Renault, C., Veyrenche, N., Mennechet, F., et al. (2022). Th17 CD4 + T-Cell as a preferential target for HIV reservoirs. *Frontiers in Immunology*, *13*, 822576. https://doi.org/10.3389/fimmu.2022.822576
48. Fert, A., Raymond Marchand, L., Salinas, W., & Ancuta, T. R., P (2022). Targeting Th17 cells in HIV-1 remission/cure interventions. *Trends in Immunology*, *43*, 580–594. https://doi.org/10.1016/j.it.2022.04.013
49. Singh, A., Vajpayee, M., Ali, S. A., & Chauhan, N. K. (2014). Cellular interplay among Th17, Th1, and Treg cells in HIV-1 subtype C Infection. *Journal of Medical Virology*, *86*(3), 372–384. https://doi.org/10.1002/jmv.23810
50. Bi, X., Ishizaki, A., Nguyen, L. V., Matsuda, K., Pham, H. V., Phan, C. T., Ogata, K., Giang, T. T., Phung, T. T., Nguyen, T. T., Tokoro, M., Pham, A. N., Khu, D. T., & Ichimura, H. (2016). Impact of HIV Infection and anti-retroviral therapy on the Immune Profile of and Microbial Translocation in HIV-Infected Children in Vietnam. *International Journal of Molecular Sciences*, *17*(8), 1245. https://doi.org/10.3390/ijms17081245
51. Tian, X., Xie, Y., Chen, J., Yin, W., Zhao, Y. L., Yao, P., Dong, M., Jin, C., & Wu, N. (2023). Increased Microbial translocation is a prognostic biomarker of different Immune responses to ART in people living with HIV. *Infection and Drug Resistance*, *16*, 3871–3878. https://doi.org/10.2147/IDR.S404384
52. Isnard, S., Royston, L., Scott, S. C., Mabanga, T., Lin, J., Fombuena, B., Bu, S., Berini, C. A., Goldberg, M. S., Finkelman, M., Brouillette, M. J., Fellows, L. K., Mayo, N. E., & Routy, J. P. (2023). Translocation of bacterial LPS is associated with self-reported cognitive abilities in men living with HIV receiving antiretroviral therapy. *AIDS Research and Therapy*, *20*(1), 30. https://doi.org/10.1186/s12981-023-00525-z
53. Xia, P., Xing, X. D., Yang, C. X., Liao, X. J., Liu, F. H., Huang, H. H., Zhang, C., Song, J. W., Jiao, Y. M., Shi, M., Jiang, T. J., Zhou, C. B., Wang, X. C., He, Q., Zeng, Q. L., Wang, F. S., & Zhang, J. Y. (2022). Activation-induced pyroptosis contributes to the loss of MAIT cells in chronic HIV-1 infected patients. *Military Medical Research*, *9*(1), 24. https://doi.org/10.1186/s40779-022-00384-1
54. Gaardbo, J. C., Trøsied, M., Stiksrud, B., Midttun, Ø., Ueland, P. M., Ullum, H., & Nielsen, S. D. (2015). Increased Tryptophan Catabolism Is Associated With Increased Frequency of CD161 + Tc17/MAIT Cells and Lower CD4 + T-Cell Count in HIV-1 Infected Patients on cART After 2 Years of Follow-Up. *Journal of acquired immune deficiency syndromes (1999) 70*(3), 228–235. https://doi.org/10.1097/QAI.0000000000000758
55. Seethaler, B., Basrai, M., Neyrinck, A. M., Nazare, J. A., Walter, J., Delzenne, N. M., & Bischoff, S. C. (2021). Biomarkers for assessment of intestinal permeability in clinical practice. American journal of physiology. *Gastrointestinal and liver physiology 321*(1), G11–G17. https://doi.org/10.1152/ajpgi.00113.2021
56. Isnard, S., Ramendra, R., Dupuy, F. P., Lin, J., Fombuena, B., Kokinov, N., et al. (2020). Plasma levels of c-type lectin REG3alpha and gut damage in people with human immunodeficiency virus. *Journal of Infectious Diseases*, *221*(1), 110–121. https://doi.org/10.1093/infdis/jiz423
57. Kentoffio, K., Temu, T. M., Shakil, S. S., Zanni, M. V., & Longenecker, C. T. (2022). Cardiovascular disease risk in women living with HIV. *Current Opinion in HIV and AIDS 17*(5), 270–278. doi: 0.1097/COH.0000000000000756.
58. Isnard, S., Ramendra, R., Dupuy, F. P., Lin, J., Fombuena, B., Kokinov, N., Kema, I., Jenabian, M. A., Lebouché, B., Costiniuk, C. T., Ancuta, P., Bernard, N. F., Silverman, M. S., Lakatos, P. L., Durand, M., Tremblay, C., Routy, J. P., & Aging Cohort Groups. (2020). Montreal Primary HIV Infection Study, the Canadian Cohort of HIV + Slow Progressors, and the Canadian HIV and Plasma Levels of C-Type Lectin REG3α and Gut Damage in People With Human Immunodeficiency Virus. *The Journal of infectious diseases 221*(1), 110–121. https://doi.org/10.1093/infdis/jiz423
59. Fasano, A. (2011). Zonulin and its regulation of intestinal barrier function: The biological door to inflammation, autoimmunity,

and cancer. *Physiological Reviews*, *91*(1), 151–175. https://doi.org/10.1152/physrev.00003.2008
60. Ockner, R. K., & Manning, J. A. (1974). Fatty acid-binding protein in small intestine. Identification, isolation, and evidence for its role in cellular fatty acid transport. *The Journal of Clinical Investigation*, *54*(2), 326–338. https://doi.org/10.1172/JCI107768
61. Hunt, P. W., Sinclair, E., Rodriguez, B., Shive, C., Clagett, B., Funderburg, N., et al. (2014). Gut epithelial barrier dysfunction and innate immune activation predict mortality in treated HIV Infection. *Journal of Infectious Diseases*, *210*(8), 1228–1238. https://doi.org/10.1093/infdis/jiu238
62. Jabłonowska, E., Strzelczyk, J., Piekarska, A., & Wójcik-Cichy, K. (2021). Gut microbiota diversity in HIV-Infected patients on successful antiretroviral treatment is linked to sexual preferences but not CD4 Nadir. *Archivum Immunologiae et therapiae experimentalis*, *69*(1), 14. https://doi.org/10.1007/s00005-021-00616-7
63. Zhang, Y., Xie, Z., Zhou, J., Li, Y., Ning, C., Su, Q., Ye, L., Ai, S., Lai, J., Pan, P., Liu, N., Liao, Y., Su, Q., Li, Z., Liang, H., Cui, P., & Huang, J. (2023). The altered metabolites contributed by dysbiosis of gut microbiota are associated with microbial translocation and immune activation during HIV Infection. *Frontiers in Immunology*, *13*, 1020822. https://doi.org/10.3389/fimmu.2022.1020822
64. Facchin, S., Vitulo, N., Calgaro, M., Buda, A., Romualdi, C., Pohl, D., Perini, B., Lorenzon, G., Marinelli, C., D'Incà, R., Sturniolo, G. C., & Savarino, E. V. (2020). Microbiota changes induced by microencapsulated sodium butyrate in patients with inflammatory bowel Disease. *Neurogastroenterology and Motility*, *32*(10), e13914. https://doi.org/10.1111/nmo.13914
65. Holle, J., Bartolomaeus, H., Löber, U., Behrens, F., Bartolomaeus, T. U. P., Anandakumar, H., Wimmer, M. I., Vu, D. L., Kuhring, M., Brüning, U., Maifeld, A., Geisberger, S., Kempa, S., Schumacher, F., Kleuser, B., Bufler, P., Querfeld, U., Kitschke, S., Engler, D., Kuhrt, L. D., et al. (2022). Inflammation in children with CKD linked to Gut Dysbiosis and Metabolite Imbalance. *Journal of the American Society of Nephrology: JASN*, *33*(12), 2259–2275. https://doi.org/10.1681/ASN.2022030378
66. Vargas-Pacherrez, D., Cotrim, H. P., Pires, L., Cunha, V., Coelho, V., Brites, C., & Daltro, C. (2020). Metabolic syndrome in HIV-patients in antiretroviral therapy. *Current HIV Research*, *18*(6), 388–395. https://doi.org/10.2174/1570162X18666200609115615
67. Padmapriyadarsini, C., Ramesh, K., Sekar, L., Ramachandran, G., Reddy, D., Narendran, G., Sekar, S., Chandrasekar, C., Anbarasu, D., Wanke, C., & Swaminathan, S. (2017). Factors affecting high-density lipoprotein cholesterol in HIV-infected patients on nevirapine-based antiretroviral therapy. *The Indian Journal of Medical Research*, *145*(5), 641–650. https://doi.org/10.4103/ijmr.IJMR_1611_15
68. Jumare, J., Dakum, P., Sam-Agudu, N., Memiah, P., Nowak, R., Bada, F., Oguama, U., Odonye, G., Adebiyi, R., Cairo, C., Kwaghe, V., Adebamowo, C., Abimiku, A., & Charurat, M. (2023). Prevalence and characteristics of metabolic syndrome and its components among adults living with and without HIV in Nigeria: A single-center study. *BMC Endocrine Disorders*, *23*(1), 160. https://doi.org/10.1186/s12902-023-01419-x
69. Low, H., Hoang, A., Pushkarsky, T., Dubrovsky, L., Dewar, E., Di Yacovo, M. S., Mukhamedova, N., Cheng, L., Downs, C., Simon, G., Saumoy, M., Hill, A. F., Fitzgerald, M. L., Nestel, P., Dart, A., Hoy, J., Bukrinsky, M., & Sviridov, D. (2019). HIV Disease, metabolic dysfunction and Atherosclerosis: A three year prospective study. *PloS One*, *14*(4), e0215620. https://doi.org/10.1371/journal.pone.0215620
70. Feeney, E. R., McAuley, N., O'Halloran, J. A., et al. (2013). The expression of cholesterol metabolism genes in monocytes from HIV-infected subjects suggests intracellular cholesterol accumulation. *The Journal of Infectious Diseases*, *207*(4), 628–637. https://doi.org/10.1093/infdis/jis723
71. Gori, E., Mduluza, T., Nyagura, M., Stray-Pedersen, B., & Gomo, Z. A. (2016). Inflammation-modulating cytokine profile and lipid interaction in HIV-related risk factors for Cardiovascular Diseases. *Therapeutics and Clinical risk Management*, *12*, 1659–1666. https://doi.org/10.2147/TCRM.S117980
72. Williams, P., Wu, J., Cohn, S., Koletar, S., McCutchan, J., Murphy, R., Currier, J., & AIDS Clinical Trials Group 362 Study Team. (2009). Improvement in lipid profiles over 6 years of follow-up in adults with AIDS and immune reconstitution. *HIV Medicine*, *10*(5), 290–301. https://doi.org/10.1111/j.1468-1293.2008.00685.x
73. Xu, Y., Chen, X., & Wang, K. (2017). Global prevalence of Hypertension among people living with HIV: A systematic review and meta-analysis. *Journal of the American Society of Hypertension*, *11*(8), 530–540. https://doi.org/10.1016/j.jash.2017.06.004
74. Jackson, I. L., Lawrence, S. M., Igwe, C. N., et al. (2022). Prevalence and control of Hypertension among people living with HIV receiving care at a Nigerian hospital. *The Pan African Medical Journal*, *41*, 153. https://doi.org/10.11604/pamj.2022.41.153.21809
75. Chepchirchir, A., Nyagol, J., & Jaoko, W. (2018). Cytokine expression and Hypertension Comorbidity in HIV/AIDS patients at Kenyatta National Hospital HIV Care Centre, Nairobi, Kenya. *International Journal of Cardiovascular Research*, *07*, 2. https://doi.org/10.4172/2324-8602.1000343
76. Siedner, M. J., Kim, J. H., Nakku, R. S., Bibangambah, P., Hemphill, L., Triant, V. A., Haberer, J. E., Martin, J. N., Mocello, A. R., Boum, Y. 2nd, Kwon, D. S., Tracy, R. P., Burdo, T., Huang, Y., Cao, H., Okello, S., Bangsberg, D. R., & Hunt, P. W. (2016). Persistent Immune activation and carotid Atherosclerosis in HIV-Infected ugandans receiving antiretroviral therapy. *The Journal of Infectious Diseases*, *213*(3), 370–378. https://doi.org/10.1093/infdis/jiv450
77. Shen, G., Thomas, T. S., Walpert, A. R., McClure, C. M., Fitch, K. V., deFilippi, C., Torriani, M., Buckless, C. G., Adler, G. K., Grinspoon, S. K., & Srinivasa, S. (2022). Role of renin-angiotensin-aldosterone system activation and other metabolic variables in relation to arterial inflammation in HIV. *Clinical Endocrinology*, *97*(5), 581–587. https://doi.org/10.1111/cen.14784
78. Peck, R. N., Shedafa, R., Kalluvya, S., Downs, J. A., Todd, J., Suthanthiran, M., Fitzgerald, D. W., & Kataraihya, J. B. (2014). Hypertension, Kidney Disease, HIV and antiretroviral therapy among Tanzanian adults: A cross-sectional study. *BMC Medicine*, *12*, 125. https://doi.org/10.1186/s12916-014-0125-2
79. Xu, Y., Chen, X., & Wang, K. (2017). Global prevalence of Hypertension among people living with HIV: A systematic review and meta-analysis. *Journal of the American Society of Hypertension: JASH*, *11*(8), 530–540. https://doi.org/10.1016/j.jash.2017.06.004
80. Byonanebye, D. M., Polizzotto, M. N., Neesgaard, B., Sarcletti, M., Matulionyte, R., Braun, D. L., Castagna, A., de Wit, S., Wit, F., Fontas, E., Vehreschild, J. J., Vesterbacka, J., Greenberg, L., Hatleberg, C., Garges, H., Gallant, J., Volny Anne, A., Öllinger, A., Mozer-Lisewska, I., Surial, B., et al. (2022). Incidence of Hypertension in people with HIV who are treated with integrase inhibitors versus other antiretroviral regimens in the RESPOND cohort consortium. *HIV Medicine*, *23*(8), 895–910. https://doi.org/10.1111/hiv.13273
81. González-Cordón, A., Doménech, M., Camafort, M., Martínez-Rebollar, M., Torres, B., Laguno, M., Rojas, J., Loncà, M., Blanco, J. L., Mallolas, J., Gatell, J. M., de Lazzari, E., & Martínez, E. (2018). Subclinical Cardiovascular Disease in patients starting contemporary protease inhibitors. *HIV Medicine*. https://doi.org/10.1111/hiv.12619

82. Brennan, A. T., Nattey, C., Kileel, E. M., Rosen, S., Maskew, M., Stokes, A. C., Fox, M. P., & Venter, W. D. F. (2023). Change in body weight and risk of hypertension after switching from efavirenz to dolutegravir in adults living with HIV: evidence from routine care in Johannesburg, South Africa. *eClinicalMedicine* 57, 101836. https://doi.org/10.1016/j.eclinm.2023.101836
83. Santiprabhob, J., Chokephaibulkit, K., Khantee, P., Maleesatharn, A., Phonrat, B., Phongsamart, W., Lapphra, K., Wittawatmongkol, O., Rungmaitree, S., Tanchaweng, S., Maturapat, S., Lermankul, W., & Tungtrongchitr, R. (2020). Adipocytokine dysregulation, abnormal glucose metabolism, and lipodystrophy in HIV-infected adolescents receiving protease inhibitors. *Cytokine*, *136*, 155145. https://doi.org/10.1016/j.cyto.2020.155145
84. Ketlogetswe, K. S., Post, W. S., Li, X., Palella, F. J., Jr, Jacobson, L. P., Margolick, J. B., Kingsley, L. A., Witt, M. D., Dobs, A. S., Budoff, M. J., & Brown, T. T. (2014). Lower adiponectin is associated with subclinical Cardiovascular Disease among HIV-infected men. *AIDS (London England)*, *28*(6), 901–909. https://doi.org/10.1097/QAD.0000000000000186
85. Blázquez, D., Ramos-Amador, J. T., Saínz, T., Mellado, M. J., García-Ascaso, M., De José, M. I., Rojo, P., Navarro, M. L., Muñoz-Fernández, M., Saavedra, J., Roa, M. A., Jiménez, S., Becciro, J., Prieto, L., Hortelano, M. G., & González-Tomé, M. I. (2015). Lipid and glucose alterations in perinatally-acquired HIV-infected adolescents and young adults. *BMC infectious diseases* 15, 119. https://doi.org/10.1186/s12879-015-0853-8
86. Putcharoen, O., Wattanachanya, L., Sophonphan, J., Siwamogsatham, S., Sapsirisavat, V., Gatechompol, S., Phonphithak, S., Kerr, S. J., Chattranukulchai, P., Avihingsanon, Y., Ruxrungtham, K., Avihingsanon, A., & HIV-NAT 006 team. (2017). New-onset Diabetes in HIV-treated adults: Predictors, long-term renal and cardiovascular outcomes. *AIDS (London England)*, *31*(11), 1535–1543. https://doi.org/10.1097/QAD.0000000000001496
87. Birabaharan, M., Strunk, A., Kaelber, D. C., Smith, D. M., & Martin, T. C. S. (2022). Sex differences in type 2 Diabetes Mellitus prevalence among persons with HIV. *AIDS (London England)*, *36*(3), 383–389. https://doi.org/10.1097/QAD.0000000000003127
88. Poznyak, A., Grechko, A. V., Poggio, P., Myasoedova, V. A., Alfieri, V., & Orekhov, A. N. (2020). The Diabetes Mellitus-Atherosclerosis Connection: The Role of Lipid and Glucose Metabolism and Chronic Inflammation. *International journal of molecular sciences 21*(5), 1835. https://doi.org/10.3390/ijms21051835
89. Dikaiou, P., Björck, L., Adiels, M., Lundberg, C. E., Mandalenakis, Z., Manhem, K., & Rosengren, A. (2021). Obesity, overweight and risk for Cardiovascular Disease and mortality in young women. *European Journal of Preventive Cardiology*, *28*(12), 1351–1359. https://doi.org/10.1177/2047487320908983
90. Thompson-Paul, A. M., Wei, S. C., Mattson, C. L. (2015). Obesity Among HIV-Infected Adults Receiving Medical Care in the United States: Data From the Cross-Sectional Medical Monitoring Project and National Health and Nutrition Examination Survey. *Medicine 94*(27), e1081. https://doi.org/10.1097/MD.0000000000001081
91. Silverberg, M., Leyden, W., Alexeeff, S. (2020). Changes in body mass index over time in persons with and without HIV. AIDS 2020: 23rd International AIDS Conference Virtual. July 6–10, 2020.
92. Kumar, S., & Samaras, K. (2018). The Impact of Weight Gain During HIV Treatment on Risk of Pre-diabetes, Diabetes Mellitus, Cardiovascular Disease, and Mortality. *Frontiers in Endocrinology* 9, 705. Published 2018 Nov 27. https://doi.org/10.3389/fendo.2018.00705
93. Sax, P. E., Erlandson, K. M., Lake, J. E., Mccomsey, G. A., Orkin, C., Esser, S., Brown, T. T., Rockstroh, J. K., Wei, X., Carter, C. C., Zhong, L., Brainard, D. M., Melbourne, K., Das, M., Stellbrink, H. J., Post, F. A., Waters, L., & Koethe, J. R. (2020). Weight Gain Following Initiation of Antiretroviral Therapy: Risk Factors in Randomized Comparative Clinical Trials. *Clinical infectious diseases*: an official publication of the Infectious Diseases Society of America, *71*(6), 1379–1389. https://doi.org/10.1093/cid/ciz999
94. Erlandson, K. M., Wu, K., Lake, J. E., Samuels, D. C., Bares, S. H., Tassiopoulos, K., Koethe, J. R., Brown, T. T., Leonard, M., Benson, C. A., Haas, D. W., & Hulgan, T., A5001, A5322 protocol teams (2021) mitochondrial DNA haplogroups and weight gain following switch to integrase strand transfer inhibitor-based antiretroviral therapy. *AIDS (London England) 35*(3), 439–445. https://doi.org/10.1097/QAD.0000000000002771
95. McMahon, C., Trevaskis, J. L., Carter, C., Holsapple, K., White, K., Das, M., Collins, S., Martin, H., & Burns-Naas, L. A. (2020). Lack of an association between clinical INSTI-related body weight gain and direct interference with MC4 receptor (MC4R), a key central regulator of body weight. *PloS One*, *15*(2), e0229617. https://doi.org/10.1371/journal.pone.0229617
96. Jeong, S. W., Kim, S. H., Kang, S. H., Kim, H. J., Yoon, C. H., Youn, T. J., & Chae, I. H. (2019). Mortality reduction with physical activity in patients with and without Cardiovascular Disease. *European Heart Journal*, *40*(43), 3547–3555. https://doi.org/10.1093/eurheartj/chz564
97. Sahel-Gozin, N., Loutfy, M., & O'Brien, K. K. (2023). Exploring experiences engaging in exercise from the perspectives of women living with HIV: A qualitative study. *PloS One*, *18*(6), e0286542. https://doi.org/10.1371/journal.pone.0286542
98. Martin, K., Naclerio, F., Karsten, B., et al. (2019). Physical activity and quality of life in people living with HIV. *Aids Care*, *31*(5), 589–598. https://doi.org/10.1080/09540121.2019.1576848
99. García-Lucio, J., Peinado, V. I., de Jover, L., Del Pozo, R., Blanco, I., Bonjoch, C., Coll-Bonfill, N., Paul, T., Tura-Ceide, O., & Barberà, J. A. (2018). Imbalance between endothelial damage and repair capacity in Chronic Obstructive Pulmonary Disease. *PloS One*, *13*(4), e0195724. https://doi.org/10.1371/journal.pone.0195724
100. Lanza, G. A., Spera, F. R., Villano, A., Russo, G., Di Franco, A., Lamendola, P., & Crea, F. (2015). Effect of smoking on endothelium-independent vasodilatation. *Atherosclerosis 240*(2), 330–332. https://doi.org/10.1016/j.atherosclerosis.2015.03.041
101. Wu, M., Lai, T., Jing, D., Yang, S., Wu, Y., Li, Z., Wu, Y., Zhao, Y., Zhou, L., Chen, H., Shen, J., Li, W., Ying, S., Chen, Z., Wu, X., & Shen, H. (2021). Epithelium-derived IL17A Promotes Cigarette Smoke-induced Inflammation and Mucus Hyperproduction. *American journal of respiratory cell and molecular biology 65*(6), 581–592. https://doi.org/10.1165/rcmb.2020-0424OC
102. Giebe, S., Hofmann, A., Brux, M., et al. (2021). Comparative study of the effects of cigarette smoke versus next generation Tobacco and nicotine product extracts on endothelial function. *Redox Biology*, *47*, 102150. https://doi.org/10.1016/j.redox.2021.102150
103. Bui, T. C., Hoogland, C. E., Chhea, C., Sopheab, H., Ouk, V., Samreth, S., Hor, B., Vidrine, J. I., Businelle, M. S., Shih, Y. C. T., Sutton, S. K., Jones, S. R., Shorey Fennell, B., Cottrell-Daniels, C., Frank-Pearce, S. G., Ngor, C., Kulkarni, S., & Vidrine, D. J. (2023). Ending Tobacco Use Through Interactive Tailored Messaging for Cambodian People With HIV (Project EndIT): Protocol for a Randomized Controlled Trial. *JMIR research protocols 12*, e48923. https://doi.org/10.2196/48923
104. Bui, T. C., Hoogland, C. E., Chhea, C., Sopheab, H., Ouk, V., Samreth, S., Hor, B., Vidrine, J. I., Businelle, M. S., Shih, Y. C. T., Sutton, S. K., Jones, S. R., Shorey Fennell, B., Cottrell-Daniels, C., Frank-Pearce, S. G., Ngor, C., Kulkarni, S., & Vidrine, D. J. (2023). Ending Tobacco Use through interactive tailored messaging for Cambodian people with HIV (Project EndIT): Protocol for a Randomized Controlled Trial. *JMIR Research Protocols*, *12*, e48923.

105. Ashare, R. L., Thompson, M., Leone, F. (2019). Differences in the rate of nicotine metabolism among smokers with and without HIV. *AIDS 33*(6), 1083–1088. https://doi.org/10.1097/QAD.0000000000002127
106. Gillis, J., Smieja, M., Cescon, A., Rourke, S. B., Burchell, A. N., Cooper, C., Raboud, J. M., & OHTN Cohort Study Group. (2014). Risk of Cardiovascular Disease associated with HCV and HBV coinfection among antiretroviral-treated HIV-infected individuals. *Antiviral Therapy*, *19*(3), 309–317. https://doi.org/10.3851/IMP2724
107. National Center for HIV/AIDS, Hepatitis, V., STD, and, Prevention, T. B., & Mortality, H. I. V. (2021). https://www.cdc.gov/hiv/pdf/library/slidesets/cdc-hiv-surveillance-slideset-mortality-2021.pdf (accessed on 05 October 2023).
108. Zicari, S., Sessa, L., Cotugno, N. (2019). Immune Activation, Inflammation, and Non-AIDS Co-Morbidities in HIV-Infected Patients under Long-Term ART. *Viruses 11*(3), 200. https://doi.org/10.3390/v11030200
109. Juno, J. A., Phetsouphanh, C., Klenerman, P., et al. (2019). Perturbation of mucosal-associated invariant T cells and iNKT cells in HIV Infection. *Current Opinion in HIV and AIDS*, *14*(2), 77–84. https://doi.org/10.1097/COH.0000000000000526
110. Chen, X., Liu, X., Duan, S., et al. (2020). Plasma inflammatory biomarkers Associated with Advanced Liver Fibrosis in HIV-HCV-Coinfected individuals. *International Journal of Environmental Research and Public Health*, *17*(24), 9474. https://doi.org/10.3390/ijerph17249474
111. Shata, M. T. M., Abdel-Hameed, E. A., Rouster, S. D., et al. (2019). HBV and HIV/HBV infected patients have distinct Immune exhaustion and apoptotic serum biomarker profiles. *Pathogens and Immunity*, *4*(1), 39–65. https://doi.org/10.20411/pai.v4i1.267
112. Maidji, E., Somsouk, M., Rivera, J. M., Hunt, P. W., & Stoddart, C. A. (2017). Replication of CMV in the gut of HIV-infected individuals and epithelial barrier dysfunction. *PLoS Pathogens*, *13*(2), e1006202. https://doi.org/10.1371/journal.ppat.1006202
113. Li, H., Weng, P., Najarro, K., et al. (2014). Chronic CMV Infection in older women: Longitudinal comparisons of CMV DNA in peripheral monocytes, anti-CMV IgG titers, serum IL-6 levels, and CMV pp65 (NLV)-specific CD8(+) T-cell frequencies with twelve year follow-up. *Experimental Gerontology*, *54*, 84–89. https://doi.org/10.1016/j.exger.2014.01.010
114. Nazim, F., Kayani, H. A., Nathwani, A. (2022). A.,. CMV and EBV Co-Infection in HIV-Infected Children: Infection Rates and Analysis of Differential Expression of Cytokines in HIV Mono- and HIV-CMV-EBV Co-Infected Groups. *Viruses 14*(8), 1823. https://doi.org/10.3390/v14081823
115. EACS European AIDS Clinical Society (2022). Guidelines Version 11.1 October 2022. Available online: https://www.eacsociety.org/media/guidelines-11.1_final_09-10.pdf (accessed on 01 August 2023).
116. Menéndez-Arias, L., & Delgado, R. (2022). Update and latest advances in antiretroviral therapy. *Trends in Pharmacological Sciences*, *43*(1), 16–29. https://doi.org/10.1016/j.tips.2021.10.004
117. ang, L. L., Li, Q., Zhou, L. B., & Chen, S. Q. (2019). Meta-analysis and systematic review of the efficacy and resistance for human immunodeficiency virus type 1 integrase strand transfer inhibitors. *International journal of antimicrobial agents 54*(5), 547–555. https://doi.org/10.1016/j.ijantimicag.2019.08.008
118. Neesgaard, B., Greenberg, L., Miró, J. M., et al. (2022). Associations between integrase strand-transfer inhibitors and Cardiovascular Disease in people living with HIV: A multicentre prospective study from the RESPOND cohort consortium. *The Lancet HIV*, *9*(7), e474–e485. https://doi.org/10.1016/S2352-3018(22)00094-7
119. O'Halloran, J. A., Sahrmann, J., Butler, A. M., Olsen, M. A., & Powderly, W. G. (2020). Brief Report: Integrase Strand Transfer Inhibitors Are Associated With Lower Risk of Incident Cardiovascular Disease in People Living With HIV. *Journal of acquired immune deficiency syndromes (1999) 84*(4), 396–399. https://doi.org/10.1097/QAI.0000000000002357
120. Chen, Y. F., Stampley, J. E., Irving, B. A., & Dugas, T. R. (2019). Chronic Nucleoside Reverse transcriptase inhibitors disrupt mitochondrial homeostasis and promote premature endothelial senescence. *Toxicological Sciences: An Official Journal of the Society of Toxicology*, *172*(2), 445–456. https://doi.org/10.1093/toxsci/kfz203
121. Chen, Y. F., Hebert, V. Y., Stadler, K., Xue, S. Y., Slaybaugh, K., Luttrell-Williams, E., et al. (2019). Coenzyme Q10 alleviates chronic nucleoside reverse transcriptase inhibitor-induced premature endothelial senescence. *Cardiovascular Toxicology*, *19*(6), 500–509. https://doi.org/10.1007/s12012-019-09520-1
122. Lacey, A., Savinelli, S., Barco, E. A. (2020). Investigating the effect of antiretroviral switch to tenofovir alafenamide on lipid profiles in people living with HIV. *AIDS 34*(8), 1161–1170. https://doi.org/10.1097/QAD.0000000000002541
123. Iannone, V., Farinacci, D., D'Angelillo, A., et al. (2022). Cardiovascular Disease Risk in a cohort of Virologically Suppressed people living with HIV switching to Doravirine: Preliminary Data from the Real Life. *AIDS Research and Human Retroviruses*, *38*(11), 878–880. https://doi.org/10.1089/AID.2022.0050
124. Martini, S., Pisaturo, M., Russo, A., Palamone, M. G., Russo, M. T., Zollo, V., Maggi, P., & Coppola, N. (2023). Evaluation of lipid Profile and Intima Media Thickness in antiretroviral-experienced HIV-Infected patients treated with protease inhibitor-based regimens versus protease inhibitor-sparing regimens. *Pathogens (Basel Switzerland)*, *12*(7), 925. https://doi.org/10.3390/pathogens12070925
125. Sharif, S., Van der Graaf, Y., Cramer, M. J., Kapelle, L. J., de Borst, G. J., Visseren, F. L. J., Westerink, J., & SMART study group. (2021). Low-grade inflammation as a risk factor for cardiovascular events and all-cause mortality in patients with type 2 Diabetes. *Cardiovascular Diabetology*, *20*(1), 220. https://doi.org/10.1186/s12933-021-01409-0
126. Martínez-Ayala, P., Alanis-Sánchez, G. A., Álvarez-Zavala, M., Sánchez-Reyes, K., Ruiz-Herrera, V. V., Cabrera-Silva, R. I., González-Hernández, L. A., Ramos-Becerra, C., Cardona-Muñoz, E., & Andrade-Villanueva, J. F. (2023). Effect of antiretroviral therapy on decreasing arterial stiffness, metabolic profile, vascular and systemic inflammatory cytokines in treatment-naïve HIV: A one-year prospective study. *PloS One*, *18*(3), e0282728. https://doi.org/10.1371/journal.pone.0282728
127. Unver, N., & McAllister, F. (2018). IL-6 family cytokines: Key inflammatory mediators as biomarkers and potential therapeutic targets. *Cytokine & Growth Factor Reviews*, *41*, 10–17. https://doi.org/10.1016/j.cytogfr.2018.04.004
128. Borges, Á. H., O'Connor, J. L., Phillips, A. N., Rönsholt, F. F., Pett, S., Vjecha, M. J., French, M. A., Lundgren, J. D., & INSIGHT SMART and ESPRIT Study Groups and the SILCAAT Scientific Committee. (2015). Factors Associated with plasma IL-6 levels during HIV Infection. *The Journal of Infectious Diseases*, *212*(4), 585–595. https://doi.org/10.1093/infdis/jiv123
129. Guo, H., Gao, J., Taxman, D. J., et al. (2014). HIV-1 Infection induces interleukin-1β production via TLR8 protein-dependent and NLRP3 inflammasome mechanisms in human monocytes. *Journal of Biological Chemistry*, *289*(31), 21716–21726. https://doi.org/10.1074/jbc.M114.566620
130. Collora, J. A., Liu, R., Pinto-Santini, D., Ravindra, N., Ganoza, C., Lama, J. R., Alfaro, R., Chiarella, J., Spudich, S., Mounzer, K., Tebas, P., Montaner, L. J., van Dijk, D., Duerr, A., & Ho, Y. C. (2022). Single-cell multiomics reveals persistence of HIV-1 in

expanded cytotoxic T cell clones. *Immunity*, *55*(6), 1013–1031e7. https://doi.org/10.1016/j.immuni.2022.03.004
131. Li, J., Das, J. R., Tang, P., Han, Z., Jaiswal, J. K., & Ray, P. E. (2017). Transmembrane TNF-α facilitates HIV-1 Infection of Podocytes cultured from children with HIV-Associated Nephropathy. *Journal of the American Society of Nephrology: JASN*, *28*(3), 862–875. https://doi.org/10.1681/ASN.2016050564
132. Chinnapaiyan, S., Dutta, R. K., Nair, M., Chand, H. S., Rahman, I., & Unwalla, H. J. (2019). TGF-β1 increases viral burden and promotes HIV-1 latency in primary differentiated human bronchial epithelial cells. *Scientific Reports*, *9*(1), 12552. https://doi.org/10.1038/s41598-019-49056-6
133. Mahmud, F. J., Boucher, T., Liang, S., et al. (2020). Osteopontin and integrin mediated modulation of Post-synapses in HIV Envelope Glycoprotein exposed hippocampal neurons. *Brain Science*, *10*(6), 346. https://doi.org/10.3390/brainsci10060346
134. Affi, R., Gabillard, D., Dunyach-Remy, C., et al. (2021). Association of plasma Soluble Vascular Cell Adhesion Molecule-1 and sCD14 with mortality in HIV-1-Infected west African adults with high CD4 counts. *Journal of Acquired Immune Deficiency Syndromes*, *86*(1), 138–145. https://doi.org/10.1097/QAI.0000000000002533
135. Pu, H., Tian, J., Flora, G., Lee, Y. W., Nath, A., Hennig, B., & Toborek, M. (2003). HIV-1 Tat protein upregulates inflammatory mediators and induces monocyte invasion into the brain. *Molecular and cellular neurosciences 24*(1), 224–237. https://doi.org/10.1016/s1044-7431(03)00171-4
136. Saud, A., Ali, N. A., Gali, F., & Hadi, N. (2022). The role of cytokines, adhesion molecules, and toll-like receptors in atherosclerosis progression: the effect of Atorvastatin. *Journal of medicine and life 15*(6), 751–756. https://doi.org/10.25122/jml-2021-0187
137. Steiner, O., Coisne, C., Cecchelli, R., Boscacci, R., Deutsch, U., Engelhardt, B., & Lyck, R. (2010). Differential roles for endothelial ICAM-1, ICAM-2, and VCAM-1 in shear-resistant T cell arrest, polarization, and directed crawling on blood-brain barrier endothelium. *Journal of immunology (Baltimore, Md.: 1950) 185*(8), 4846–4855. https://doi.org/10.4049/jimmunol.0903732
138. Guha, D., Misra, V., Yin, J., Horiguchi, M., Uno, H., & Gabuzda, D. (2023). Vascular injury markers associated with cognitive impairment in people with HIV on suppressive antiretroviral therapy. *AIDS (London, England)* Advance online publication. https://doi.org/10.1097/QAD.0000000000003675
139. Affi, R., Gabillard, D., Kouame, G. M., et al. (2022). Plasma sVCAM-1, antiretroviral therapy and mortality in HIV-1-infected west African adults. *HIV Medicine*, *23*(7), 717–726. https://doi.org/10.1111/hiv.13230
140. Okay, G., Koc, M. M., Guler, E. M., et al. (2020). The effect of antiretroviral therapy on IL-6, IL-1β, TNF-α, IFN-γ levels and their relationship with HIV-RNA and CD4 + T cells in HIV patients. *Current HIV Research*, *18*, 354–361. https://doi.org/10.2174/1570162X18666200712174642
141. Teasdale, C. A., Hernandez, C., Zerbe, A., et al. (2020). Changes in D-dimer after initiation of antiretroviral therapy in adults living with HIV in Kenya. *BMC Infectious Diseases*, *20*(1), 508. https://doi.org/10.1186/s12879-020-05213-1
142. Mercurio, V., Fitzgerald, W., Vanpouille, C., Molodtsov, I., & Margolis, L. (2021). Mechanisms of residual immune activation in HIV-1-infected human lymphoid tissue ex vivo. *AIDS (London England)*, *35*(8), 1179–1190. https://doi.org/10.1097/QAD.0000000000000288

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Podsumowanie i wnioski

1. Leczenie antyretrowirusowe jest kluczowym elementem terapii zakażenia HIV, umożliwia rekonstrukcję immunologiczną, a tym samym prowadzi do wydłużenia życia pacjentów zakażonych HIV.
2. W związku z obserwowanym wzrostem długości życia pacjentów zakażonych HIV, również w tej grupie pacjentów częściej występują choroby cywilizacyjne, w tym schorzenia układu sercowo naczyniowego oraz polipragmazja.
3. Po włączeniu leczenia antyretrowirusowego obserwuje się odbudowę immunologiczną, jednak u większości pacjentów nie udaje się osiągnąć prawidłowej wartości parametrów układu immunologicznego.
4. Rozpoczęcie leczenia na wczesnym etapie zakażenia HIV zwiększa szansę na pełną odbudowę układu immunologicznego.
5. Rodzaj zastosowanego schematu leczenia antyretrowirusowego nie wpływa na tempo odbudowy immunologicznej.
6. Leczenie antyretrowirusowe może zwiększać ryzyko sercowo-naczyniowe, poprzez wzrost stężenia cholesterolu całkowitego oraz cholesterolu LDL.
7. Zaburzenie funkcji śródbłonka (wyrażone za pomocą stężenia VCAM-1) u pacjentów zakażonych HIV, jest bardziej nasilone u osób nieotrzymujących leczenia antyretrowirusowego.
8. Pacjenci zakażeni HIV otrzymujący leczenie antyretrowirusowe powinni być regularnie oceniani pod kątem wystąpienia chorób układu sercowo-naczyniowego.

Piśmiennictwo

1. Marcus JL, Leyden WA, Alexeeff SE, Anderson AN, Hechter RC, Hu H, Lam JO, Towner WJ, Yuan Q, Horberg MA, Silverberg MJ. Comparison of Overall and Comorbidity-Free Life Expectancy Between Insured Adults With and Without HIV Infection, 2000-2016. JAMA Netw Open. 2020 Jun 1;3(6):e207954. doi: 10.1001/jamanetworkopen.2020.7954. PMID: 32539152; PMCID: PMC7296391.

2. Freiberg MS, Chang CH, Skanderson M, Patterson OV, DuVall SL, Brandt CA, So-Armah KA, Vasan RS, Oursler KA, Gottdiener J, Gottlieb S, Leaf D, Rodriguez-Barradas M, Tracy RP, Gibert CL, Rimland D, Bedimo RJ, Brown ST, Goetz MB, Warner A, Crothers K, Tindle HA, Alcorn C, Bachmann JM, Justice AC, Butt AA. Association Between HIV Infection and the Risk of Heart Failure With Reduced Ejection Fraction and Preserved Ejection Fraction in the Antiretroviral Therapy Era: Results From the Veterans Aging Cohort Study. JAMA Cardiol. 2017 May 1;2(5):536-546. doi: 10.1001/jamacardio.2017.0264. PMID: 28384660; PMCID: PMC5541383.

3. Perkins MV, Joseph SB, Dittmer DP, Mackman N. Cardiovascular Disease and Thrombosis in HIV Infection. Arterioscler Thromb Vasc Biol. 2023 Feb;43(2):175-191. doi: 10.1161/ATVBAHA.122.318232. Epub 2022 Dec 1. PMID: 36453273; PMCID: PMC10165851.

4. Yu X, Westra JR, Giordano TP, Berenson AB, Baillargeon JG, Kuo YF. Assessing comorbidities and survival in HIV-infected and uninfected matched Medicare enrollees. AIDS. 2021 Aug 1;35(10):1667-1675. doi: 10.1097/QAD.0000000000002963. PMID: 34049353; PMCID: PMC8286326.

5. Rosoff DB, Davey Smith G, Mehta N, Clarke TK, Lohoff FW. Evaluating the relationship between alcohol consumption, tobacco use, and cardiovascular disease: A multivariable Mendelian randomization study. PLoS Med. 2020 Dec 4;17(12):e1003410. doi: 10.1371/journal.pmed.1003410. PMID: 33275596; PMCID: PMC7717538.

6. Gillis J, Smieja M, Cescon A, Rourke SB, Burchell AN, Cooper C, Raboud JM; OHTN Cohort Study Group. Risk of cardiovascular disease associated with HCV and HBV coinfection among antiretroviral-treated HIV-infected individuals. Antivir Ther. 2014;19(3):309-17. doi: 10.3851/IMP2724. Epub 2014 Jan 16. PMID: 24429380.

7. Bagheri Amiri F, Mostafavi E, Mirzazadeh A. HIV, HBV and HCV Coinfection Prevalence in Iran--A Systematic Review and Meta-Analysis. PLoS One. 2016 Mar 31;11(3):e0151946. doi: 10.1371/journal.pone.0151946. PMID: 27031352; PMCID: PMC4816272.

8. Centers for Disease Control and Prevention. Hepatitis C. CDC Yellow Book 2024. Available at: https://wwwnc.cdc.gov/travel/yellowbook/2024/infections-diseases/hepatitis-c

9. Shahmanesh M, Schultze A, Burns F, Kirk O, Lundgren J, Mussini C, Pedersen C, De Wit S, Kutsyna G, Mocroft A; EuroSIDA in EuroCOORD. The cardiovascular risk management for people living with HIV in Europe: how well are we doing? AIDS. 2016 Oct 23;30(16):2505-2518. doi: 10.1097/QAD.0000000000001207. PMID: 27456984.

10. Menéndez-Arias L, Delgado R. Update and latest advances in antiretroviral therapy. Trends Pharmacol Sci. 2022 Jan;43(1):16-29. doi: 10.1016/j.tips.2021.10.004. Epub 2021 Nov 3. PMID: 34742581.

11. Neesgaard B, Greenberg L, Miró JM, Grabmeier-Pfistershammer K, Wandeler G, Smith C, De Wit S, Wit F, Pelchen-Matthews A, Mussini C, Castagna A, Pradier C, d'Arminio Monforte A, Vehreschild JJ, Sönnerborg A, Anne AV, Carr A, Bansi-Matharu L, Lundgren JD, Garges H, Rogatto F, Zangerle R, Günthard HF, Rasmussen LD, Necsoi C, van der Valk M, Menozzi M, Muccini C, Peters L, Mocroft A, Ryom L. Associations between integrase strand-transfer inhibitors and cardiovascular disease in people living with HIV: a multicentre prospective study from the RESPOND cohort consortium. Lancet HIV. 2022 Jul;9(7):e474-e485. doi: 10.1016/S2352-3018(22)00094-7. Epub 2022 Jun 7. PMID: 35688166.

12. Sax PE, Erlandson KM, Lake JE, Mccomsey GA, Orkin C, Esser S, Brown TT, Rockstroh JK, Wei X, Carter CC, Zhong L, Brainard DM, Melbourne K, Das M, Stellbrink HJ, Post FA, Waters L, Koethe JR. Weight Gain Following Initiation of Antiretroviral Therapy: Risk Factors in Randomized Comparative Clinical Trials. Clin Infect Dis. 2020 Sep 12;71(6):1379-1389. doi: 10.1093/cid/ciz999. PMID: 31606734; PMCID: PMC7486849.

13. O'Halloran JA, Sahrmann J, Butler AM, Olsen MA, Powderly WG. Brief Report: Integrase Strand Transfer Inhibitors Are Associated With Lower Risk of Incident

Cardiovascular Disease in People Living With HIV. J Acquir Immune Defic Syndr. 2020 Aug 1;84(4):396-399. doi: 10.1097/QAI.0000000000002357. PMID: 32243280; PMCID: PMC7401319.

14. Chen YF, Stampley JE, Irving BA, Dugas TR. Chronic Nucleoside Reverse Transcriptase Inhibitors Disrupt Mitochondrial Homeostasis and Promote Premature Endothelial Senescence. Toxicol Sci. 2019 Dec 1;172(2):445-456. doi: 10.1093/toxsci/kfz203. Erratum in: Toxicol Sci. 2021 Sep 28;183(2):415. PMID: 31545371.

15. Chen YF, Hebert VY, Stadler K, Xue SY, Slaybaugh K, Luttrell-Williams E, Glover MC, Krzywanski DM, Dugas TR. Coenzyme Q10 Alleviates Chronic Nucleoside Reverse Transcriptase Inhibitor-Induced Premature Endothelial Senescence. Cardiovasc Toxicol. 2019 Dec;19(6):500-509. doi: 10.1007/s12012-019-09520-1. PMID: 31020509.

16. Lacey A, Savinelli S, Barco EA, Macken A, Cotter AG, Sheehan G, Lambert JS, Muldoon E, Feeney E, Mallon PW, Tinago W; UCD ID Cohort Study. Investigating the effect of antiretroviral switch to tenofovir alafenamide on lipid profiles in people living with HIV. AIDS. 2020 Jul 1;34(8):1161-1170. doi: 10.1097/QAD.0000000000002541. PMID: 32310899.

17. Iannone V, Farinacci D, D'Angelillo A, Dusina A, Lamanna F, Passerotto R, Baldin G, Visconti E, Tamburrini E, Borghetti A, Di Giambenedetto S, Ciccullo A. Cardiovascular Disease Risk in a Cohort of Virologically Suppressed People Living with HIV Switching to Doravirine: Preliminary Data from the Real Life. AIDS Res Hum Retroviruses. 2022 Nov;38(11):878-880. doi: 10.1089/AID.2022.0050. Epub 2022 Oct 19. PMID: 36166220.

18. Martini S, Pisaturo M, Russo A, Palamone MG, Russo MT, Zollo V, Maggi P, Coppola N. Evaluation of Lipid Profile and Intima Media Thickness in Antiretroviral-Experienced HIV-Infected Patients Treated with Protease Inhibitor-Based Regimens versus Protease Inhibitor-Sparing Regimens. Pathogens. 2023 Jul 10;12(7):925. doi: 10.3390/pathogens12070925. PMID: 37513772; PMCID: PMC10383365.

19. Zicari S, Sessa L, Cotugno N, Ruggiero A, Morrocchi E, Concato C, Rocca S, Zangari P, Manno EC, Palma P. Immune Activation, Inflammation, and Non-AIDS Co-

Morbidities in HIV-Infected Patients under Long-Term ART. Viruses. 2019 Feb 27;11(3):200. doi: 10.3390/v11030200. PMID: 30818749; PMCID: PMC6466530.

20. Liu CH, Abrams ND, Carrick DM, Chander P, Dwyer J, Hamlet MRJ, Macchiarini F, PrabhuDas M, Shen GL, Tandon P, Vedamony MM. Biomarkers of chronic inflammation in disease development and prevention: challenges and opportunities. Nat Immunol. 2017 Oct 18;18(11):1175-1180. doi: 10.1038/ni.3828. PMID: 29044245.

21. Affi R, Gabillard D, Kouame GM, Ntakpe JB, Moh R, Badje A, Danel C, Inwoley A, Eholié SP, Anglaret X, Weiss L. Plasma sVCAM-1, antiretroviral therapy and mortality in HIV-1-infected West African adults. HIV Med. 2022 Aug;23(7):717-726. doi: 10.1111/hiv.13230. Epub 2022 Jan 12. PMID: 35023284.

22. Okay G, Koc MM, Guler EM, Yabaci A, Kocyigit A, Akkoyunlu Y. The Effect of Antiretroviral Therapy on IL-6, IL-1β, TNF-α, IFN-γ Levels and their Relationship with HIV-RNA and CD4+ T Cells in HIV Patients. Curr HIV Res. 2020;18(5):354-361. doi: 10.2174/1570162X18666200712174642. PMID: 32652911.

23. Teasdale CA, Hernandez C, Zerbe A, Chege D, Hawken M, El-Sadr WM. Changes in D-dimer after initiation of antiretroviral therapy in adults living with HIV in Kenya. BMC Infect Dis. 2020 Jul 14;20(1):508. doi: 10.1186/s12879-020-05213-1. PMID: 32664854; PMCID: PMC7362533.

24. Mercurio V, Fitzgerald W, Vanpouille C, Molodtsov I, Margolis L. Mechanisms of residual immune activation in HIV-1-infected human lymphoid tissue ex vivo. AIDS. 2021 Jul 1;35(8):1179-1190. doi: 10.1097/QAD.0000000000002881. PMID: 33710022; PMCID: PMC8183484.

25. Pu H, Tian J, Flora G, Lee YW, Nath A, Hennig B, Toborek M. HIV-1 Tat protein upregulates inflammatory mediators and induces monocyte invasion into the brain. Mol Cell Neurosci. 2003 Sep;24(1):224-37. doi: 10.1016/s1044-7431(03)00171-4. PMID: 14550782.

26. Saud A, Ali NA, Gali F, Hadi N. The role of cytokines, adhesion molecules, and toll-like receptors in atherosclerosis progression: the effect of Atorvastatin. J Med Life. 2022 Jun;15(6):751-756. doi: 10.25122/jml-2021-0187. PMID: 35928361; PMCID: PMC9321484.

27. Steiner O, Coisne C, Cecchelli R, Boscacci R, Deutsch U, Engelhardt B, Lyck R. Differential roles for endothelial ICAM-1, ICAM-2, and VCAM-1 in shear-resistant T cell arrest, polarization, and directed crawling on blood-brain barrier endothelium. J Immunol. 2010 Oct 15;185(8):4846-55. doi: 10.4049/jimmunol.0903732. Epub 2010 Sep 22. PMID: 20861356.

28. Guha D, Misra V, Yin J, Horiguchi M, Uno H, Gabuzda D. Vascular injury markers associated with cognitive impairment in people with HIV on suppressive antiretroviral therapy. AIDS. 2023 Nov 15;37(14):2137-2147. doi: 10.1097/QAD.0000000000003675. Epub 2023 Jul 27. PMID: 37503603; PMCID: PMC10615701.

29. Shenoy N, Ramapuram JT, Shenoy A, Ahmed J, Srikant N. Incidence of Opportunistic Infections among HIV-Positive Adults on Highly Active Antiretroviral Therapy in a Teaching Hospital, India: Prospective Study. J Int Assoc Provid AIDS Care. 2017 May/Jun;16(3):309-311. doi: 10.1177/2325957416686192. Epub 2017 Jan 4. PMID: 28050923.

30. Sobrino-Vegas P, Moreno S, Rubio R, Viciana P, Bernardino JI, Blanco JR, Bernal E, Asensi V, Pulido F, del Amo J, Hernando V; Cohorte de la Red de Investigación en Sida, Spain. Impact of late presentation of HIV infection on short-, mid- and long-term mortality and causes of death in a multicenter national cohort: 2004-2013. J Infect. 2016 May;72(5):587-96. doi: 10.1016/j.jinf.2016.01.017. Epub 2016 Feb 24. PMID: 26920789.

31. CDC. Revised Guidelines for Performing CD4+ T-Cell Determinations in Persons Infected with Human Immunodeficiency Virus (HIV). 1997. Available online: https://www.cdc.gov/mmwr/preview/mmwrhtml/00045580.htm

32. Mussini C, Lorenzini P, Cozzi-Lepri A, Lapadula G, Marchetti G, Nicastri E, Cingolani A, Lichtner M, Antinori A, Gori A, d'Arminio Monforte A; Icona Foundation Study Group. CD4/CD8 ratio normalisation and non-AIDS-related events in individuals with HIV who achieve viral load suppression with antiretroviral therapy: an observational cohort study. Lancet HIV. 2015 Mar;2(3):e98-106. doi: 10.1016/S2352-3018(15)00006-5. Epub 2015 Feb 6. PMID: 26424550.

33. Zhang Y, Jiang T, Li A, Li Z, Hou J, Gao M, Huang X, Su B, Wu H, Zhang T, Jiang W. Adjunct Therapy for CD4+ T-Cell Recovery, Inflammation and Immune Activation in

People Living With HIV: A Systematic Review and Meta-Analysis. Front Immunol. 2021 Feb 17;12:632119. doi: 10.3389/fimmu.2021.632119. PMID: 33679779; PMCID: PMC7925844.

34. Panel on Antiretroviral Guidelines for Adults and Adolescents. Guidelines for the use of antiretroviral agents in HIV-1 infected adults and adolescents. Washington, DC: Department of Health and Human Services, 2008. Available online: https://www.ncbi.nlm.nih.gov/books/NBK586306/

35. Yang X, Su B, Zhang X, Liu Y, Wu H, Zhang T. Incomplete immune reconstitution in HIV/AIDS patients on antiretroviral therapy: Challenges of immunological non-responders. J Leukoc Biol. 2020 Apr;107(4):597-612. doi: 10.1002/JLB.4MR1019-189R. Epub 2020 Jan 22. PMID: 31965635; PMCID: PMC7187275.

36. Stiksrud B, Aass HCD, Lorvik KB, Ueland T, Trøseid M, Dyrhol-Riise AM. Activated dendritic cells and monocytes in HIV immunological nonresponders: HIV-induced interferon-inducible protein-10 correlates with low future CD4+ recovery. AIDS. 2019 Jun 1;33(7):1117-1129. doi: 10.1097/QAD.0000000000002173. PMID: 30789356; PMCID: PMC6511429.

37. Wei T, Zhu Z, Liu L, Liu B, Wu M, Zhang W, Cui Q, Liu F, Zhang R. Circulating levels of cytokines and risk of cardiovascular disease: a Mendelian randomization study. Front Immunol. 2023 May 25;14:1175421. doi: 10.3389/fimmu.2023.1175421. PMID: 37304261; PMCID: PMC10247976.

38. Hsue PY, Lo JC, Franklin A, Bolger AF, Martin JN, Deeks SG, Waters DD. Progression of atherosclerosis as assessed by carotid intima-media thickness in patients with HIV infection. Circulation. 2004 Apr 6;109(13):1603-8. doi: 10.1161/01.CIR.0000124480.32233.8A. Epub 2004 Mar 15. PMID: 15023877.

39. Alonso A, Barnes AE, Guest JL, Shah A, Shao IY, Marconi V. HIV Infection and Incidence of Cardiovascular Diseases: An Analysis of a Large Healthcare Database. J Am Heart Assoc. 2019 Jul 16;8(14):e012241. doi: 10.1161/JAHA.119.012241. Epub 2019 Jul 2. PMID: 31266386; PMCID: PMC6662120.

40. Obare LM, Priest S, Ismael A, Mashayekhi M, Zhang X, Stolze LK, Sheng Q, Vue Z, Neikirk K, Beasley H, Gabriel C, Temu T, Gianella S, Mallal S, Koethe JR, Hinton A, Bailin S, Wanjalla CN. Cytokine and Chemokine Receptor Profiles in Adipose Tissue

Vasculature Unravel Endothelial Cell Responses in HIV. bioRxiv [Preprint]. 2024 Mar 12:2024.03.10.584280. doi: 10.1101/2024.03.10.584280. PMID: 38559150; PMCID: PMC10979923.

41. Yu F, Ma C, Jin X, Zhao H, Xiao J, Li L, Song S, Xie X, Yang S, Tang Y, Wang L, Zhang F. Mitochondrial disturbance related to increased caspase-1 of CD4+T cells in HIV-1 infection. BMC Infect Dis. 2024 Jan 24;24(1):129. doi: 10.1186/s12879-023-08485-5. PMID: 38267841; PMCID: PMC10809604.

Opinia Komisji Bioetycznej

Komisja Bioetyczna
przy Warszawskim Uniwersytecie Medycznym

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

ul. Żwirki i Wigury nr 61
02-091 Warszawa

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

Warszawa, dnia 30 lipca 2021r.

AKBE/ 128/ 2021

Lek. Agnieszka Lembas
Klinika Chorób Zakaźnych, Tropikalnych i Hepatologii
ul. Wolska 37
01 – 201 Warszawa

OŚWIADCZENIE

Niniejszym oświadczam, że Komisja Bioetyczna przy Warszawskim Uniwersytecie Medycznym w dniu 30 lipca 2021 r. przyjęła do wiadomości informację na temat badania pt.: „ VCAM-1 jako biomarker funkcji śródbłonka u pacjentów zakażonych HIV leczonych i nieleczonych antyretrowirusowo." Przedstawione badanie nie stanowi eksperymentu medycznego w rozumieniu art. 21 ust. 1 ustawy z dnia 5 grudnia 1996 r. o zawodach lekarza i lekarza dentysty(Dz.U. z 2018 r. poz. 617) i nie wymaga uzyskania opinii Komisji Bioetycznej przy Warszawskim Uniwersytecie Medycznym, o której mowa w art. 29 ust.1 ww. ustawy.

Przewodnicząca Komisji Bioetycznej

Prof. dr hab. n. med. Magdalena Kuźma –Kozakiewicz

Komisja Bioetyczna
przy Warszawskim Uniwersytecie Medycznym

Tel.: 022/ 57 - 20 -303
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ul. Żwirki i Wigury nr 61
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e-mail: komisja.bioetyczna@wum.edu.pl
www.komisja-bioetyczna.wum.edu.pl

Warszawa, dnia 12.06 2023

AKBE/ 188 / 2023

Lek. Agnieszka Lembas
Klinika Chorób Zakaźnych ,Tropikalnych i Hepatologii
Warszawskiego Uniwersytetu Medycznego
ul. Wolska 37
01 – 201 Warszawa

OŚWIADCZENIE

Niniejszym oświadczam, że Komisja Bioetyczna przy Warszawskim Uniwersytecie Medycznym w dniu 12 czerwca 2023 r. przyjęła do wiadomości informację na temat badania pt. "Czynniki wpływające na skuteczność immunologiczną terapii antyretrowirusowej wśród pacjentów zakażonych HIV" Przedstawione badanie nie stanowi eksperymentu medycznego w rozumieniu art. 21ust.1 ustawy z dnia 5 grudnia 1996 r. o zawodach lekarza i lekarza dentysty (Dz.U. z 2018 r poz. 617) i nie wymaga uzyskania opinii Komisji Bioetycznej przy Warszawskim Uniwersytecie Medycznym, o której mowa w art. 29 ust.1 ww. ustawy.

Przewodnicząca Komisji Bioetycznej

Prof. dr hab. n. med. Magdalena Kuźma –Kozakiewicz

Oświadczenia współautorów publikacji

WARSZAWA, 14.04.2024
(miejscowość, data)

AGNIESZKA LEMBAS
(imię i nazwisko)

OŚWIADCZENIE

Jako współautor pracy pt. „VCAM-1 as a Biomarker of Endothelial Function among HIV-Infected Patients Receiving and Not Receiving Antiretroviral Therapy" oświadczam, iż mój własny wkład merytoryczny w przygotowanie, przeprowadzenie i opracowanie badań oraz przedstawienie pracy w formie publikacji stanowi: przygotowanie koncepcji, metodykę, przygotowanie danych do analizy, analizę statystyczną, przygotowanie wykresów, pozyskanie materiałów źródłowych, pisanie manuskryptu oraz zarządzanie projektem.

Mój udział procentowy w przygotowaniu publikacji określam jako 70%.

Agnieszka Lembas
(podpis oświadczającego)

Warszawa 14.04.2014
(miejscowość, data)

KATARZYNA ZAWARTKO
(imię i nazwisko)

OŚWIADCZENIE

Jako współautor pracy pt. „VCAM-1 as a Biomarker of Endothelial Function among HIV-Infected Patients Receiving and Not Receiving Antiretroviral Therapy" oświadczam, iż mój własny wkład merytoryczny w przygotowanie, przeprowadzenie i opracowanie badań oraz przedstawienie pracy w formie publikacji stanowi: pozyskanie materiałów źródłowych oraz pisanie manuskryptu.

Mój udział procentowy w przygotowaniu publikacji określam jako 10%.
Wkład Agnieszki Lembas w powstawanie publikacji określam jako 70%,
obejmował on: przygotowanie koncepcji, metodykę, przygotowanie danych do analizy, analizę statystyczną, przygotowanie wykresów, pozyskanie materiałów źródłowych, pisanie manuskryptu oraz zarządzanie projektem.

Jednocześnie wyrażam zgodę na wykorzystanie w/w pracy jako część rozprawy doktorskiej lek. Agnieszki Lembas.

(podpis oświadczającego)

WARSZAWA, 15.04.24
(miejscowość, data)

MARIUSZ SAPUŁA
(imię i nazwisko)

OŚWIADCZENIE

Jako współautor pracy pt. „VCAM-1 as a Biomarker of Endothelial Function among HIV-Infected Patients Receiving and Not Receiving Antiretroviral Therapy" oświadczam, iż mój własny wkład merytoryczny w przygotowanie, przeprowadzenie i opracowanie badań oraz przedstawienie pracy w formie publikacji stanowi: współuczestnictwo w opracowaniu analizy statystycznej.

Mój udział procentowy w przygotowaniu publikacji określam jako 5%.
Wkład Agnieszki Lembas w powstawanie publikacji określam jako 70%,
obejmował on: przygotowanie koncepcji, metodykę, przygotowanie danych do analizy, analizę statystyczną, przygotowanie wykresów, pozyskanie materiałów źródłowych, pisanie manuskryptu oraz zarządzanie projektem.

Jednocześnie wyrażam zgodę na wykorzystanie w/w pracy jako część rozprawy doktorskiej lek. Agnieszki Lembas.

Mariusz Sapuła
(podpis oświadczającego)

Warsaw, 15.04.2024
(miejscowość, data)

Tomasz Milune
(imię i nazwisko)

OŚWIADCZENIE

Jako współautor pracy pt. „VCAM-1 as a Biomarker of Endothelial Function among HIV-Infected Patients Receiving and Not Receiving Antiretroviral Therapy" oświadczam, iż mój własny wkład merytoryczny w przygotowanie, przeprowadzenie i opracowanie badań oraz przedstawienie pracy w formie publikacji stanowi: pozyskanie materiałów źródłowych oraz wsparcie merytoryczne.

Mój udział procentowy w przygotowaniu publikacji określam jako 5%.
Wkład Agnieszki Lembas w powstawanie publikacji określam jako 70%,
obejmował on: przygotowanie koncepcji, metodykę, przygotowanie danych do analizy, analizę statystyczną, przygotowanie wykresów, pozyskanie materiałów źródłowych, pisanie manuskryptu oraz zarządzanie projektem.

Jednocześnie wyrażam zgodę na wykorzystanie w/w pracy jako część rozprawy doktorskiej lek. Agnieszki Lembas.

(podpis oświadczającego)

Warmae, 17.04.2024
.....
(miejscowość, data)

Joanna Korbowska
.....
(imię i nazwisko)

OŚWIADCZENIE

Jako współautor pracy pt. „VCAM-1 as a Biomarker of Endothelial Function among HIV-Infected Patients Receiving and Not Receiving Antiretroviral Therapy" oświadczam, iż mój własny wkład merytoryczny w przygotowanie, przeprowadzenie i opracowanie badań oraz przedstawienie pracy w formie publikacji stanowi: pozyskanie materiałów źródłowych.

Mój udział procentowy w przygotowaniu publikacji określam jako 5%.
Wkład Agnieszki Lembas w powstawanie publikacji określam jako 70%,
obejmował on: przygotowanie koncepcji, metodykę, przygotowanie danych do analizy, analizę statystyczną, przygotowanie wykresów, pozyskanie materiałów źródłowych, pisanie manuskryptu oraz zarządzanie projektem.

Jednocześnie wyrażam zgodę na wykorzystanie w/w pracy jako część rozprawy doktorskiej lek. Agnieszki Lembas.

J. Korbowska
.....
(podpis oświadczającego)

WARSZAWA, 16.04.2024
(miejscowość, data)

ALICJA WIERCIŃSKA-DRAPAŁO
(imię i nazwisko)

OŚWIADCZENIE

Jako współautor pracy pt. „VCAM-1 as a Biomarker of Endothelial Function among HIV-Infected Patients Receiving and Not Receiving Antiretroviral Therapy" oświadczam, iż mój własny wkład merytoryczny w przygotowanie, przeprowadzenie i opracowanie badań oraz przedstawienie pracy w formie publikacji stanowi: wsparcie merytoryczne oraz nadzorowanie projektu.

Mój udział procentowy w przygotowaniu publikacji określam jako 5%.
Wkład Agnieszki Lembas w powstawanie publikacji określam jako 70%,
obejmował on: przygotowanie koncepcji, metodykę, przygotowanie danych do analizy, analizę statystyczną, przygotowanie wykresów, pozyskanie materiałów źródłowych, pisanie manuskryptu oraz zarządzanie projektem.

Jednocześnie wyrażam zgodę na wykorzystanie w/w pracy jako część rozprawy doktorskiej lek. Agnieszki Lembas.

KIEROWNIK
Kliniki Chorób Zakaźnych, Tropikalnych i Hepatologii
prof. dr hab. n. med. Alicja Wiercińska-Drapało

(podpis oświadczającego)

WARSZAWA, 14.04.2024
(miejscowość, data)

AGNIESZKA LEMBAS
(imię i nazwisko)

OŚWIADCZENIE

Jako współautor pracy pt. „Evaluation of Clinical Biomarkers Related to CD4 Recovery in HIV-Infected Patients—5-Year Observation" oświadczam, iż mój własny wkład merytoryczny w przygotowanie, przeprowadzenie i opracowanie badań oraz przedstawienie pracy w formie publikacji stanowi: przygotowanie koncepcji, metodykę, przygotowanie danych do analizy, analizę statystyczną, przygotowanie wykresów, pozyskanie materiałów źródłowych, pisanie manuskryptu oraz zarządzanie projektem.

Mój udział procentowy w przygotowaniu publikacji określam jako 75%.

Agnieszka Lembas
(podpis oświadczającego)

Warszawa 16/04/2024
(miejscowość, data)

Andrzej Zajkowski
(imię i nazwisko)

OŚWIADCZENIE

Jako współautor pracy pt. „Evaluation of Clinical Biomarkers Related to CD4 Recovery in HIV-Infected Patients—5-Year Observation" oświadczam, iż mój własny wkład merytoryczny w przygotowanie, przeprowadzenie i opracowanie badań oraz przedstawienie pracy w formie publikacji stanowi: wsparcie merytoryczne.

Mój udział procentowy w przygotowaniu publikacji określam jako 5%.
Wkład Agnieszki Lembas w powstawanie publikacji określam jako 75%,
obejmował on: przygotowanie koncepcji, metodykę, przygotowanie danych do analizy, analizę statystyczną, przygotowanie wykresów, pozyskanie materiałów źródłowych, pisanie manuskryptu oraz zarządzanie projektem.

Jednocześnie wyrażam zgodę na wykorzystanie w/w pracy jako część rozprawy doktorskiej lek. Agnieszki Lembas.

Andrzej Zajkowski
(podpis oświadczającego)

Warsaw, 15.04.2024
(miejscowość, data)

Tomasz Milewski
(imię i nazwisko)

OŚWIADCZENIE

Jako współautor pracy pt. „Evaluation of Clinical Biomarkers Related to CD4 Recovery in HIV-Infected Patients—5-Year Observation" oświadczam, iż mój własny wkład merytoryczny w przygotowanie, przeprowadzenie i opracowanie badań oraz przedstawienie pracy w formie publikacji stanowi: wsparcie merytoryczne.

Mój udział procentowy w przygotowaniu publikacji określam jako 5%.
Wkład Agnieszki Lembas w powstawanie publikacji określam jako 75%,
obejmował on: przygotowanie koncepcji, metodykę, przygotowanie danych do analizy, analizę statystyczną, przygotowanie wykresów, pozyskanie materiałów źródłowych, pisanie manuskryptu oraz zarządzanie projektem.

Jednocześnie wyrażam zgodę na wykorzystanie w/w pracy jako część rozprawy doktorskiej lek. Agnieszki Lembas.

(podpis oświadczającego)

Warszawa, 15.04.2024 r
(miejscowość, data)

Tomasz Dyda
(imię i nazwisko)

OŚWIADCZENIE

Jako współautor pracy pt. „Evaluation of Clinical Biomarkers Related to CD4 Recovery in HIV-Infected Patients—5-Year Observation" oświadczam, iż mój własny wkład merytoryczny w przygotowanie, przeprowadzenie i opracowanie badań oraz przedstawienie pracy w formie publikacji stanowi: opisanie metodologii.

Mój udział procentowy w przygotowaniu publikacji określam jako 5%.
Wkład Agnieszki Lembas w powstawanie publikacji określam jako 75%,
obejmował on: przygotowanie koncepcji, metodykę, przygotowanie danych do analizy, analizę statystyczną, przygotowanie wykresów, pozyskanie materiałów źródłowych, pisanie manuskryptu oraz zarządzanie projektem.

Jednocześnie wyrażam zgodę na wykorzystanie w/w pracy jako część rozprawy doktorskiej lek. Agnieszki Lembas.

Tomasz Dyda
(podpis oświadczającego)

Warszawa 15.04.2024
(miejscowość, data)

Wojciech Staniczek
(imię i nazwisko)

OŚWIADCZENIE

Jako współautor pracy pt. „Evaluation of Clinical Biomarkers Related to CD4 Recovery in HIV-Infected Patients—5-Year Observation" oświadczam, iż mój własny wkład merytoryczny w przygotowanie, przeprowadzenie i opracowanie badań oraz przedstawienie pracy w formie publikacji stanowi: wsparcie merytoryczne.

Mój udział procentowy w przygotowaniu publikacji określam jako 5%.
Wkład Agnieszki Lembas w powstawanie publikacji określam jako 75%,
obejmował on: przygotowanie koncepcji, metodykę, przygotowanie danych do analizy, analizę statystyczną, przygotowanie wykresów, pozyskanie materiałów źródłowych, pisanie manuskryptu oraz zarządzanie projektem.

Jednocześnie wyrażam zgodę na wykorzystanie w/w pracy jako część rozprawy doktorskiej lek. Agnieszki Lembas.

.....
(podpis oświadczającego)

WARSZAWA, 16.04.2024
(miejscowość, data)

ALICJA WIERCIŃSKA - DRAPAŁO
(imię i nazwisko)

OŚWIADCZENIE

Jako współautor pracy pt. „Evaluation of Clinical Biomarkers Related to CD4 Recovery in HIV-Infected Patients—5-Year Observation" oświadczam, iż mój własny wkład merytoryczny w przygotowanie, przeprowadzenie i opracowanie badań oraz przedstawienie pracy w formie publikacji stanowi: wsparcie merytoryczne oraz nadzorowanie projektu.

Mój udział procentowy w przygotowaniu publikacji określam jako 5%.
Wkład Agnieszki Lembas w powstawanie publikacji określam jako 75%,
obejmował on: przygotowanie koncepcji, metodykę, przygotowanie danych do analizy, analizę statystyczną, przygotowanie wykresów, pozyskanie materiałów źródłowych, pisanie manuskryptu oraz zarządzanie projektem.

Jednocześnie wyrażam zgodę na wykorzystanie w/w pracy jako część rozprawy doktorskiej lek. Agnieszki Lembas.

KIEROWNIK
Kliniki Chorób Zakaźnych, Tropikalnych i Hepatologii
prof. dr hab. n. med. Alicja Wiercińska-Drapało

.....
(podpis oświadczającego)

WARSZAWA, 14.04.2024
(miejscowość, data)

AGNIESZKA LEMBAS
(imię i nazwisko)

OŚWIADCZENIE

Jako współautor pracy pt. „Human Immunodeficiency Virus as a Risk Factor for Cardiovascular Disease" oświadczam, iż mój własny wkład merytoryczny w przygotowanie, przeprowadzenie i opracowanie badań oraz przedstawienie pracy w formie publikacji stanowi: przygotowanie koncepcji, metodykę, przygotowanie ryciny, pozyskanie materiałów źródłowych, pisanie manuskryptu oraz zarządzanie projektem.

Mój udział procentowy w przygotowaniu publikacji określam jako 80%.

Agnieszka Lembas
(podpis oświadczającego)

Warszawa 16/04/2024
(miejscowość, data)

Andrzej Zajączkowski
(imię i nazwisko)

OŚWIADCZENIE

Jako współautor pracy pt. „Human Immunodeficiency Virus as a Risk Factor for Cardiovascular Disease" oświadczam, iż mój własny wkład merytoryczny w przygotowanie, przeprowadzenie i opracowanie badań oraz przedstawienie pracy w formie publikacji stanowi: wsparcie merytoryczne.

Mój udział procentowy w przygotowaniu publikacji określam jako 5%.
Wkład Agnieszki Lembas w powstawanie publikacji określam jako 80%,
obejmował on: przygotowanie koncepcji, metodykę, przygotowanie ryciny, pozyskanie materiałów źródłowych, pisanie manuskryptu oraz zarządzanie projektem.

Jednocześnie wyrażam zgodę na wykorzystanie w/w pracy jako część rozprawy doktorskiej lek. Agnieszki Lembas.

Andrzej Zajączkowski
(podpis oświadczającego)

Warszawa 13/4/2024
(miejscowość, data)

Dr n. med. Michał Peller
LEKARZ
specjalista kardiolog
2982751

.....
(imię i nazwisko)

OŚWIADCZENIE

Jako współautor pracy pt. „Human Immunodeficiency Virus as a Risk Factor for Cardiovascular Disease" oświadczam, iż mój własny wkład merytoryczny w przygotowanie, przeprowadzenie i opracowanie badań oraz przedstawienie pracy w formie publikacji stanowi: pisanie trzech podrozdziałów manuskryptu.

Mój udział procentowy w przygotowaniu publikacji określam jako 5%.
Wkład Agnieszki Lembas w powstawanie publikacji określam jako 80%,
obejmował on: przygotowanie koncepcji, metodykę, przygotowanie ryciny, pozyskanie materiałów źródłowych, pisanie manuskryptu oraz zarządzanie projektem.

Jednocześnie wyrażam zgodę na wykorzystanie w/w pracy jako część rozprawy doktorskiej lek. Agnieszki Lembas.

Dr n. med. Michał Peller
LEKARZ
specjalista kardiolog
2982751

.....
(podpis oświadczającego)

Warsaw 15.04.2025
(miejscowość, data)

Tomasz Milewski
(imię i nazwisko)

OŚWIADCZENIE

Jako współautor pracy pt. „Human Immunodeficiency Virus as a Risk Factor for Cardiovascular Disease" oświadczam, iż mój własny wkład merytoryczny w przygotowanie, przeprowadzenie i opracowanie badań oraz przedstawienie pracy w formie publikacji stanowi: wsparcie merytoryczne.

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Jednocześnie wyrażam zgodę na wykorzystanie w/w pracy jako część rozprawy doktorskiej lek. Agnieszki Lembas.

(podpis oświadczającego)

WARSZAWA, 16.04.2024
(miejscowość, data)

ALICJA WIERCIŃSKA-DRAPAŁO
(imię i nazwisko)

OŚWIADCZENIE

Jako współautor pracy pt. „Human Immunodeficiency Virus as a Risk Factor for Cardiovascular Disease" oświadczam, iż mój własny wkład merytoryczny w przygotowanie, przeprowadzenie i opracowanie badań oraz przedstawienie pracy w formie publikacji stanowi: wsparcie merytoryczne oraz nadzorowanie projektu.

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Jednocześnie wyrażam zgodę na wykorzystanie w/w pracy jako część rozprawy doktorskiej lek. Agnieszki Lembas.

KIEROWNIK
Kliniki Chorób Zakaźnych, Tropikalnych i Hepatologii
prof. dr hab. n. med. Alicja Wiercińska-Drapało
(podpis oświadczającego)