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WLISSES HENRIQUE VELOSO DE CARVALHO DA SILVA

FATORES IMUNOLÓGICOS E GENÉTICOS ENVOLVIDOS NA RECONSTITUIÇÃO DE LINFÓCITOS T CD4+ DE PACIENTES HIV-POSITIVOS EM TERAPIA ANTIRRETROVIRAL

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Orientador: Prof. Dr. Rafael Lima Guimarães
Coorientador: Prof. Dr. Fabrício Oliveira Souto

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Dedico esta tese a todos pacientes HIV-positivos, que direta ou indiretamente contribuíram para realização desse trabalho. Vocês não estão sozinhos nessa batalha contra o HIV.

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“É a ciência que conduz o cientista e não o contrário; o que um cientista não faz hoje, outro certamente o fará amanhã.”

Autor Desconhecido

RESUMO

A atual terapia antirretroviral (ART) tem revolucionado o tratamento contra o vírus da imunodeficiência humana (HIV) por suprimir ao máximo a replicação viral, reduzir as taxas de transmissões e de progressão da doença, e melhorar consideravelmente a qualidade de vida dos pacientes. Todavia, apesar da eficácia da ART, 15-30% dos pacientes HIV-positivos apresentam deficiência na reconstituição de linfócitos T CD4+, mesmo em supressão viral, sendo definidos como não-respondedores imunológicos (INR). Essa deficiência na recuperação imunológica tem sido descrita como uma condição multifatorial, mas ainda não está claro quais mecanismos determinam precisamente essa condição. Sendo assim, o presente estudo objetivou avaliar o perfil imunológico fenotípico dos pacientes HIV-positivos submetidos à ART e investigar alterações genéticas (por genotipagem e/ou revisão da literatura) em genes do sistema imune desses indivíduos. Foi realizada uma revisão da literatura em editorial acerca de fatores genéticos envolvidos na produção, ativação, proliferação e nos mecanismos de morte celular dos linfócitos T CD4+ durante ART. Análises de imunofenotipagem e de genotipagem (para os polimorfismos *SDF1*-3'A e *CCR5*Δ32) foram realizadas em amostras de pacientes HIV-positivos sob ART com prolongada supressão da carga viral. Eles foram classificados em dois grupos (respondedores imunológicos – IR e não-respondedores imunológicos – INR) de acordo com as mudanças na contagem de células T CD4+. Dados sociodemográficos e clínicos também foram avaliados a partir dos prontuários médicos. Heterozigose para o alelo *CCR5*Δ32, sexo masculino, contagem baixa de células T CD4+ pré-tratamento que se manteve reduzida mesmo após início da ART, baixa razão CD4/CD8, níveis reduzidos de células T CD4+ recentes emigradas do timo (RTE) (CD45RA+CD31+) e naïve (CD45RA+CD62L+), altos níveis de células T CD4+ de memória efetora (CD45RA-CD62L-) e níveis elevados de morte celular por piroptose em linfócitos T CD4+ RTE (CD31+FLICA-Caspase1+) foram mais frequentes nos INR que nos IR, sendo estatisticamente associados com a deficiência na recuperação imunológica. Esses resultados evidenciam alguns dos fatores genéticos e imunológicos determinantes envolvidos na reconstituição imune dos pacientes HIV-positivos em ART.

Palavras-chave: ART; *CCR5*Δ32; INR; Polimorfismo; Recuperação imunológica.

ABSTRACT

The current antiretroviral therapy (ART) has revolutionized the treatment against human immunodeficiency virus (HIV) by maximally suppressing viral replication, preventing further transmission and disease progression, and considerably improving the quality of life of HIV-positive patients. However, despite the efficiency of ART, 15-30% of HIV-positive patients exhibit impaired CD4+ T-cell reconstitution even though achieving viral suppression, being defined as immunological non-responders (INR). This incomplete immunological recovery has been described as a multifactorial condition, but it is still unclear what mechanisms precisely determine this condition. Therefore, the present study aimed to evaluate the immunophenotypic profile of HIV-positive patients receiving ART and to investigate genetic alterations (by genotyping and/or literature review) in immune system genes of these individuals. We performed a literature review in editorial concerning genetics factors involved in CD4 T-lymphocytes production, activation, proliferation, and mechanisms of cell death under ART. Immunophenotypic and genetic analyses were performed in samples from ART-treated HIV-positive patients with prolonged viral suppression. They were classified into two groups (immunological responders – IR and immunological non-responders – INR) according to their CD4+ T-cell count changes. Sociodemographic and clinical data were also evaluated from medical records. Heterozygosity for CCR5Δ32 allele, male sex, lower pre-treatment CD4+ T-cell count that remained low even after ART start, lower CD4/CD8 ratio, lower levels of recent thymic emigrant (RTE) CD4+ T-cell (CD45RA+CD31+) and naïve CD4+ T-cell (CD45RA+CD62L+), high levels of effector memory CD4+ T cells (CD45RA-CD62L-) and high pyroptosis levels of RTE CD4+Tcells (CD31+FLICA-Caspase1+) were more frequent in INR than IR group, being statistically associated with immunological recovery failure. These results evidence some determinant immunological and genetic factors involved in immune reconstitution of HIV-positive patients under ART.

Key-words: ART; *CCR5*Δ32; INR; Polymorphism; Immunological recovery.

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Revisão de Literatura

Artigo II

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LISTA DE ABREVIATURAS

ATV	- Atazanavir
AZT	- Azidotimidina (Zidovudina)
CMV	- *Citomegalovírus*
céls	- Células
DRV	- Darunavir
DTG	- Dolutegravir
EFZ	- Efavirenz
et al.	- E outros
gp	- Glicoproteína
LPV	- Lopinavir
mL	- Mililitro
NVP	- Nevirapina
r	- Ritonavir
RAL	- Raltegravir
TDF	- Tenofovir
3TC	- Lamivudina
µL	- Microlitro

LISTA DE SIGLAS

ART	- *Antiretroviral Therapy*
AIDS	- *Acquired Immunodeficiency Syndrome*
APC	- *Antigen-presenting cell*
CCR5	- *Chemokine (C-C Motif) Receptor 5*
CDC	- *Center for Disease Control*
CVP	- Carga viral plasmática
CXCR4	- *Chemokine (C-X-C Motif) Receptor 4*
EDTA	*-Ethylenediamine tetraacetic acid*
FSC	*-Forward Scatter*
HAART	- *Highly Active Antiretroviral Therapy*
HBV	- *Hepatitis B Virus*
HCV	- *Hepatitis C Virus*
HIV-1	- *Human Immunodeficiency Virus type 1*
HIV-2	- *Human Immunodeficiency Virus type 2*
HTLV	- *Human T lymphotropic virus*
IIQ	- Intervalo Interquartis
INI	- Inibidor de Integrase
INNTR	- Inibidor Não-Nucleosídeo da Transcriptase Reversa
INR	- *Immunological non-responders*
INTR	- Inibidor Nucleosídeo da Transcriptase Reversa
IP	- Inibidor de Protease (do inglês PI, *protease inhibitor*)
IR	- *Immunological responders*
PBMC	- *Peripheral blood mononuclear cells*
PBS	- *Phosphate Buffered Saline*
PVHA	- Pessoas vivendo com HIV/AIDS
RTE	- *Recent thymic emigrantes*
SD	- *Standard Deviation*
SIV	- *Simian Immunodeficiency Virus*
SSC	- *Side Scatter*
UNAIDS	- *Joint United Nations Program on HIV/AIDS*
VDLR	- *Venereal Disease Research Laboratory*
WHO	- *World Health Organization*

SUMÁRIO

1 INTRODUÇÃO

Desde a descoberta do vírus da imunodeficiência humana (HIV) em 1983, como agente causador da síndrome da imunodeficiência adquirida (AIDS), o número de pessoas vivendo com HIV/AIDS ao redor do mundo cresceu consideravelmente, tornando-se um grave problema mundial de saúde. Estima-se que existam cerca de 38 milhões de indivíduos infectados pelo HIV. Por essa razão, nas últimas quatro décadas houve intensos esforços da comunidade científica para o desenvolvimento de medicamentos anti-HIV e erradicação do vírus dos indivíduos infectados.

O tratamento contra o HIV consiste em combater a replicação viral por meio de drogas antirretrovirais usadas em combinações, que constitui a terapia antirretroviral (ART, na sigla em inglês). Apesar da eficácia da ART, cerca de um terço dos indivíduos que iniciam a terapia apresentam deficiência na reconstituição de células T CD4+, mesmo com a supressão da carga viral plasmática, que atinge níveis indetectáveis por longo período de tratamento. Isso é o que se caracteriza como deficiência na recuperação imunológica, e esses pacientes em ART são definidos como não-respondedores imunológicos (INR, sigla em inglês).

Os mecanismos que precisamente determinam essa deficiência na recuperação imunológica ainda não foram completamente elucidados, e caracteriza-se como uma condição multifatorial, destacando-se principalmente os fatores genéticos e imunológicos. Todavia, essa baixa reconstituição de linfócitos T CD4+ tem sido visto na literatura como consequência de dois principais processos: produção insuficiente e destruição excessiva das células T CD4+. Acredita-se que a desregulação na homeostase das células T desempenha um papel fundamental na recuperação imunológica dos pacientes tratados com ART. Essa homeostase é regulada por um balanço dinâmico entre produção, ativação, proliferação, migração e destruição dos linfócitos T nos órgãos linfoides e na circulação periférica. Além disso, variações genéticas, tais como polimorfismos, podem afetar a regulação gênica e o funcionamento das proteínas, aumentando ou diminuindo sua produção no indivíduo, e assim influenciando na homeostase das células T dos pacientes em tratamento.

1.1 OBJETVIOS

1.1.1 Objetivo Geral

Avaliar o perfil imunológico fenotípico dos pacientes HIV-positivos submetidos à ART e investigar alterações genéticas em genes do sistema imune desses indivíduos.

1.1.2 Objetivos Específicos

- Verificar a influência das variáveis sociodemográficas e clínicas com a recuperação imunológica dos pacientes durante a ART;
- Investigar, a partir de uma revisão na forma de editorial, fatores genéticos envolvidos na produção, ativação, proliferação e nos mecanismos de destruição das células T CD4+ durante ART;
- Avaliar a associação dos polimorfismos *SDF1*-3'A e *CCR5*Δ32 com a reconstituição imune dos indivíduos HIV-positivos em tratamento;
- Analisar fenotipicamente as populações dos linfócitos T de pacientes HIV-positivos em terapia;
- Avaliar a influência dos fatores genéticos e imunológicos na reconstituição de linfócitos T CD4+ dos indivíduos HIV-positivos durante a ART;

2 REVISÃO DE LITERATURA

2.1 SÍNDROME DA IMUNODEFICIÊNCIA ADQUIRIDA (AIDS)

2.1.1 Histórico

Embora casos esporádicos de Síndrome da Imunodeficiência Adquirida (AIDS) tenham ocorrido nas décadas 50 e 60, sugere-se que a epidemia tenha iniciado no final dos anos 70 de forma silenciosa e despercebida, onde Estados Unidos, Haiti e África Central apresentavam os primeiros casos da doença. Até o começo da década de 80, não se sabia como as pessoas teriam adquirido HIV ou desenvolvido AIDS. O vírus ainda era desconhecido e a transmissão não era acompanhada por sinais ou sintomas perceptíveis (AIDS.GOV, 2020; AVERT, 2022; FIOCRUZ, 2007; MANN; CHIN, 1988).

Em 1981, surgiram alguns casos de uma rara infecção pulmonar causada pelo fungo *Pneumocystis carinii (*atual *P. jirovecii)*, que acometeu cinco jovens homens que faziam sexo com outros homens, previamente saudáveis, em Los Angeles. Estes casos marcam oficialmente o que viria a ser conhecido como epidemia da AIDS. No mesmo ano, foi reportada a mesma infecção pulmonar em usuários de drogas injetáveis, mas que não apresentavam histórico de sexo com outros homens. Nesse período, Nova York e Califórnia registravam casos de homens gays apresentando pneumonia por *P. carinii* e um agressivo câncer de pele chamado de Sarcoma de Kaposi. Essas patologias indicavam uma deficiência do sistema imune desses pacientes, que até então afetava principalmente homens que faziam sexo com outros homens. Devido a isso a doença foi definida inicialmente como deficiência imune relacionada a gays (GRID, do inglês *gay-related immune deficiency*) (CDC, 1981a, 1981b; HYMES, 1981; MASUR, 1981).

Contudo, em meados de 1982, a doença foi reportada em pacientes hemofílicos e haitianos, que inclusive induziu muitos a acreditarem que ela teria sido originada no Haiti. Desde então, foram surgindo casos de infecções oportunistas em indivíduos homens que faziam seco com outros homens (anteriormente denominados de “homossexuais”), heroinômanos (usuários de drogas injetáveis, principalmente heroína), hemofílicos, haitianos e *hookers* (do inglês, profissionais do sexo), e assim, adota-se temporariamente o termo “Doença dos 5 H”. Com esses acontecimentos, foi sugerida a hipótese que o agente causador, até então desconhecido, era transmitido

por contato sexual, sangue ou materiais contaminados por fluidos corporais (CDC, 1982a, 1982b; FIOCRUZ, 2007).

No final de 1982, o *Center for Disease Control* (CDC) dos Estados Unidos nomeou essa nova doença de Síndrome da Imunodeficiência Adquirida (AIDS, sigla em inglês), e a partir de então a comunidade científica voltou suas pesquisas para elucidar as muitas dúvidas sobre a doença. Nesse período, o CDC publicava também que a AIDS era de fato transmissível por um agente infeccioso (ainda desconhecido) que debilitava o sistema imune lentamente, e, portanto, só após algum tempo no organismo, o indivíduo desenvolvia a doença. Enquanto isso, o vírus já tinha se espalhado por vários países, infectando milhares de pessoas. Os Estados Unidos registravam cerca de três mil casos e mais de 1200 óbitos, e Brasil, Canadá, Austrália e Países europeus reportavam seus primeiros casos de AIDS. No ano seguinte, surge a primeira notificação em criança e os primeiros casos registrados em mulheres e profissionais de saúde, sugerindo que a doença podia ser transmitida também por via heterossexual e de mãe para filho (transmissão vertical) (AIDS.GOV, 2020; AVERT, 2022; CDC, 1982c).

O agente causador da AIDS só veio ser descrito em meados de 1983, como um novo retrovírus, quando foi primeiramente isolado a partir de células linfocitárias de pacientes com quadro de deficiência na resposta imune e apresentando doenças oportunistas severas, principais sintomas da AIDS. O isolamento do vírus foi realizado de forma independente por dois grupos de pesquisa. Um grupo liderado por Luc Montagnier e sua colega Françoise Barré-Sinoussi, do Instituto Pasteur na França, que nomearam ele de Vírus Associado a Linfadenopatia (LAV, sigla em inglês). E o outro grupo coordenado por Robert Gallo, do Instituto Nacional do Câncer nos Estados Unidos, que nomeou o novo retrovírus de Vírus T-Linfotrófico Humano Tipo III (HTLV-III, sigla em inglês). Apenas em meados de 1986, o vírus causador da AIDS foi oficialmente nomeado de Vírus da Imunodeficiência Humana (HIV, sigla em inglês) pelo Comitê Internacional de Taxonomia de Vírus (BARRÉ-SINOUSSI et al., 1983; CASE, 1986; GALLO et al., 1983).

2.1.2 Origem do HIV e da Epidemia da AIDS

Em 1999, um grupo de pesquisadores encontrou uma cepa viral, chamada de vírus da imunodeficiência símia (SIV, sigla em inglês), que infectava os chimpanzés e apresentava-se geneticamente quase idêntica ao HIV. Essa evidência levou os

pesquisadores a sugerir que os chimpanzés fossem a fonte da infecção do HIV em humanos. A teoria mais aceita atualmente é que essa transmissão zoonótica (*spillover*) tenha ocorrido durante os processos de caça dos chimpanzés no final do século XIX para o início do século XX, na África Central. Portanto, o SIV foi provavelmente transmitido para humanos por meio de contato de fluidos de chimpanzés com sangue humano durante a caça, captura e tratamento da carne desses animais. Além disso, acredita-se que as cepas do SIV de chimpanzés tenham se originado de outros primatas, conhecidos como “macacos do velho mundo”, que são naturalmente infectados por diversas variantes do SIV. Dessa forma, ao longo do tempo, o SIV sofreu mutações, gerando novas linhagens do vírus por meio de seleção natural, passando a infectar chimpanzés e posteriormente os humanos, com mais eficiência, tornando-se assim HIV (GAO et al., 1999; PEETERS; JUNG; AYOUBA, 2013; SHARP; HAHN, 2011).

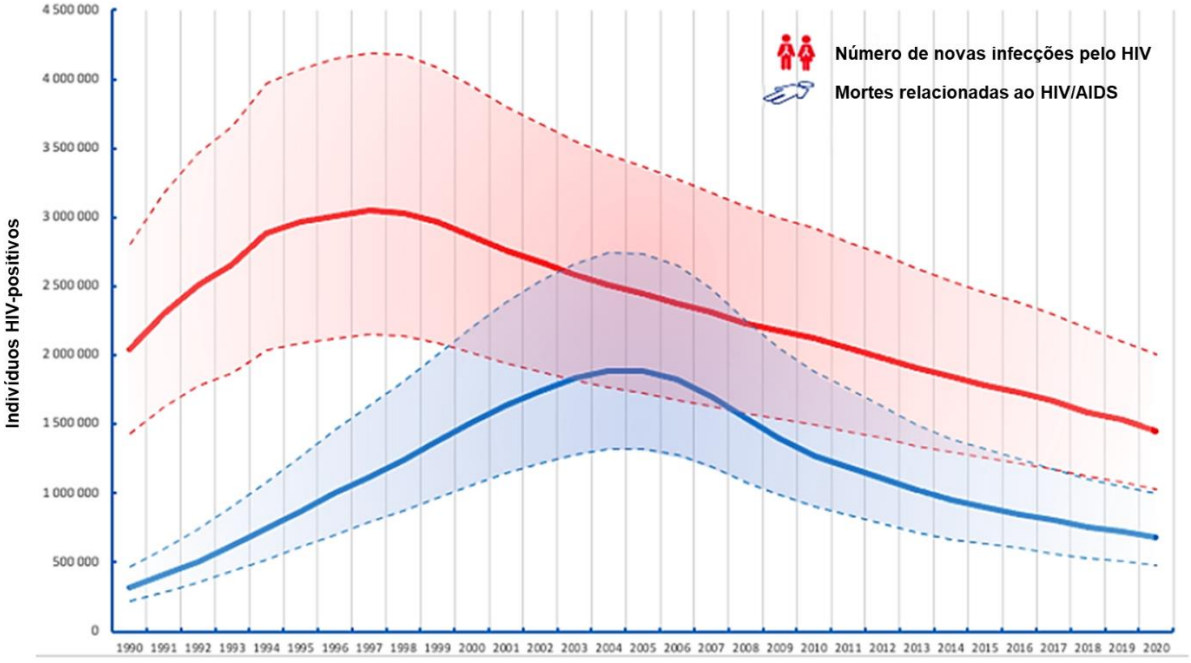
Alguns estudos evidenciaram que a primeira transmissão do SIV para humanos tenha ocorrido por volta de 1920 em Kinshasa, atual capital da República Democrática do Congo (DRC, sigla em inglês) e provável origem da epidemia global da AIDS. Pesquisadores conseguiram demonstrar esses resultados com base em análises filogenéticas de duas sequências tidas até o momento como dos primeiros casos de HIV em humanos. Essas análises foram realizadas em amostras recuperadas retrospectivamente de sangue e tecido coletadas em 1959 (ZR59) e 1960 (DRC60), ambas oriundas de Kinshasa. Isso possibilitou aos pesquisadores inferir quando o vírus apareceu pela primeira vez em humanos e como ele evoluiu (FARIA et al., 2014; WOROBEY et al., 2008; ZHU et al., 1998).

A região de Kinshasa é rica em áreas comerciais e vias de transporte, tais como estradas, ferrovias e rios. Além disso, na época que o HIV começou a se espalhar a cidade apresentava elevada população de migrantes e crescente comércio sexual. Esses fatores podem explicar como o vírus facilmente se propagou para outras regiões, e com o passar das décadas ele lentamente se espalhou por toda a África. Não é surpresa que hoje a região da África Central e outros países da África Subsaariana serem o epicentro da epidemia e apresentar a maior diversidade genética de cepas do HIV. Isso reflete o número de vezes que o SIV possa ter sido transmitido para humanos e dessa região ser a origem da epidemia global da AIDS (FARIA et al., 2014; HEMELAAR, 2012; LIHANA et al., 2012; UNAIDS, 2021a).

2.1.3 Epidemiologia

Com menos de uma década do início da epidemia, todo território mundial já apresentava casos de pessoas infectadas e vivendo com HIV/AIDS. O maior crescimento da epidemia ocorreu nos anos 90, onde a incidência global do HIV atingiu o pico em 1997, com 2,9 milhões de novas infecções. Enquanto isso, o número de mortes relacionados a AIDS só aumentava, chegando a 1,9 milhões de óbitos em 2004 (Figura 1). Contudo, desde a introdução e distribuição dos medicamentos antirretrovirais, esses números vêm reduzindo significativamente (UNAIDS, 2021a; WANG et al., 2016; WHO, 2022).

Figura 1 – Evolução da epidemia do HIV desde 1990. Estimativa global das novas infecções pelo HIV e do número de mortes relacionados a AIDS.

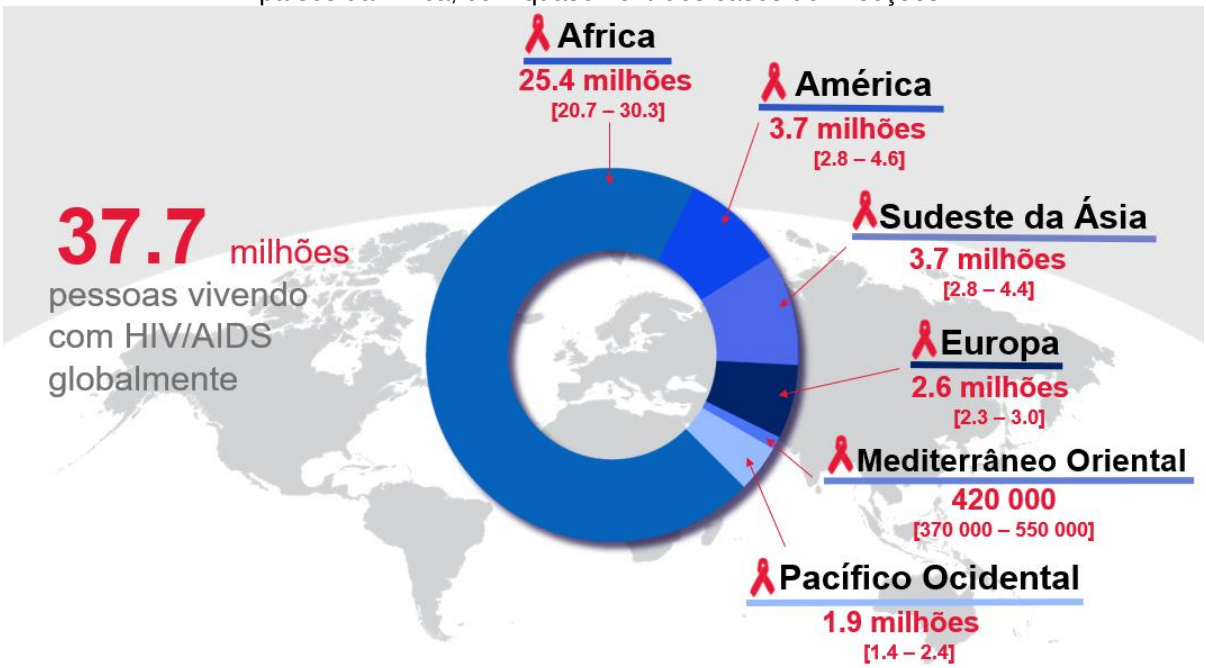


Fonte: Adaptado de WHO (2022).

A infecção pelo HIV, ao longo dos anos, rapidamente tornou-se uma pandemia, e continua sendo um grave problema de saúde pública mundial. Estima-se que existam aproximadamente 38 milhões de pessoas vivendo com o HIV/AIDS (PVHA) ao redor do mundo (Figura 2), e mais de 32 milhões já morreram por causas relacionadas à AIDS desde que a epidemia começou. De acordo com o relatório anual do Programa das Nações Unidas para HIV/AIDS (UNAIDS), 1,5 milhões de novas infecções pelo HIV e 680 mil óbitos foram registrados em 2020 no mundo todo. Isso

representa uma redução de 64% no número de mortes relacionadas a AIDS desde seu pico em 2004 (UNAIDS, 2021a; WHO, 2022).

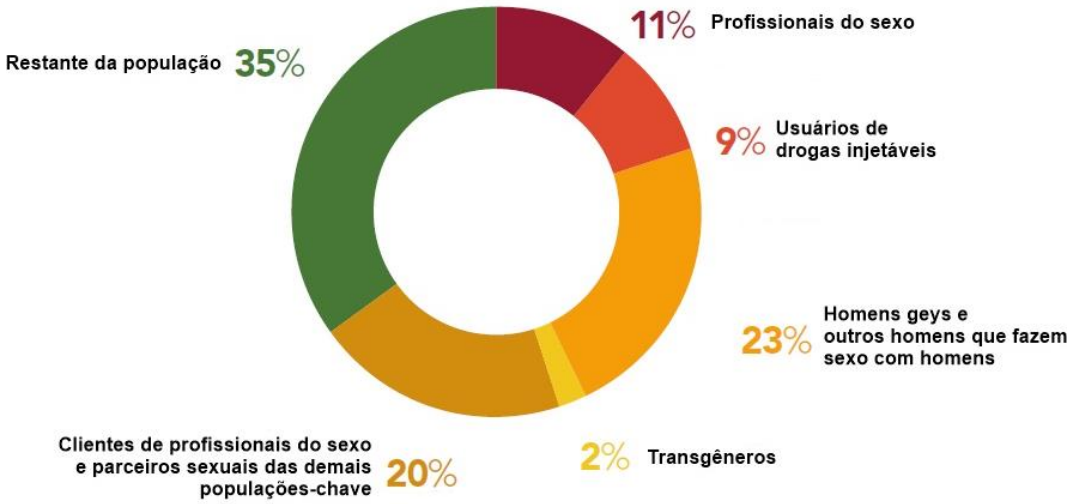
Figura 2 – Estimativa mundial de pessoas vivendo com HIV/AIDS. Atualmente 37.7 milhões de adultos e crianças estão infectadas com o vírus. A prevalência do HIV continua sendo mais alta nos países da África, com quase 70% dos casos de infecções.



Fonte: Adaptado de WHO (2022).

Embora o HIV possa afetar a população no geral, existem grupos em que a prevalência do vírus é consideravelmente maior, por serem particularmente vulneráveis (alto risco) ao HIV e frequentemente não tem acesso adequado aos serviços de saúde. São populações marginalizadas e criminalizadas por suas identidades e expressão de gênero, orientação sexual, estilo de vida e meios de subsistência. Esses grupos são definidos como “populações-chave”, e são atualmente cinco: homens gays e outros homens que fazem sexo com homens, profissionais do sexo, transgêneros, usuários de drogas injetáveis e indivíduos privados de liberdade. A maioria das infecções globais pelo HIV (65%) ainda se encontra nesses grupos e em seus parceiros sexuais (Figura 3). Em 2020, fora da África Subsaariana, as populações-chave e seus parceiros sexuais representaram mais de 93% das novas infecções pelo HIV (UNAIDS, 2021b).

Figura 3 – Distribuição das infecções pelo HIV por população.



Fonte: Adaptado de UNAIDS (2021b).

Do total de pessoas infectadas pelo HIV ao redor do mundo, 2,1 milhões vivem na América Latina. Com mais de um milhão casos, o Brasil abriga quase metade dos indivíduos latino-americanos HIV-positivos, sendo registrados desde o início da epidemia cerca de 688 mil (66%) casos em homens e 357 mil (34%) em mulheres. Além disso, já foram identificados no Brasil mais de 360 mil óbitos tendo como causa básica o HIV/AIDS. Em relação às regiões do país, a maior concentração dos casos se encontra nas regiões Sudeste (50,6%) e Sul (19,8%) seguidas pelas regiões Nordeste, Norte e Centro-Oeste com 16,5%, 6,9% e 6,2%, respectivamente. A Tabela 1 sumariza a distribuição proporcional dos casos de HIV/AIDS no Brasil (MINISTÉRIO DA SAÚDE, 2021; UNAIDS, 2021a).

Tabela 1 – Variáveis epidemiológicas de HIV/AIDS no Brasil em suas respectivas regiões desde 1980 a julho de 2021.

Indicadores	Norte	Nordeste	Sudeste	Sul	Centro-Oeste	Brasil
Casos Notificados	72.223	172.129	529.034	206.759	65.210	1.045.355
Proporção dos casos (%)	6,9	16,5	50,6	19,8	6,2	100
Óbitos	19.625	51.157	206.139	64.146	19.256	360.323

Fonte: Ministério da Saúde (2021).

Entre os estados do Nordeste, Pernambuco possui o segundo maior número de casos de HIV notificados (39.277 casos), representando 22,8% do total, equivalente a Bahia (23% dos casos). Com 23 indivíduos infectados pelo HIV a cada 100 mil habitantes em 2020, a cidade do Recife após 13 anos consecutivos sai da lista

das dez capitais brasileiras com a maior taxa de detecção de casos de HIV/AIDS no Brasil, ficando com a 12ª posição (MINISTÉRIO DA SAÚDE, 2021).

2.2 VÍRUS DA IMUNODEFICIÊNCIA HUMANA TIPO 1 (HIV-1)

2.2.1 Classificação e Diversidade do HIV

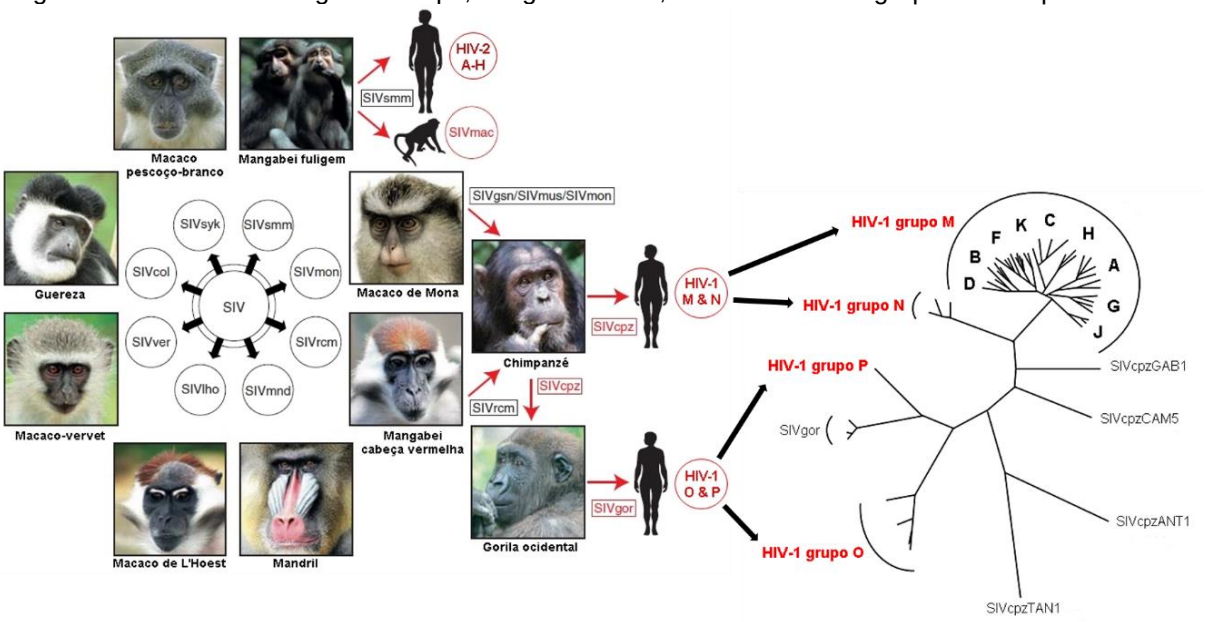
O vírus da imunodeficiência humana (HIV) é um lentivírus pertencente à família Retroviridae, responsável por causar uma infecção crônica no hospedeiro que gradualmente prejudica o sistema imune do mesmo e, se não tratada, pode ser fatal (HUTCHINSON, 2001; MELHUISH; LEWTHWAITE, 2018).

Existem atualmente dois tipos desse vírus responsáveis pela infecção em humanos, mas que apresentam diferentes especificidades genéticas e virológicas: HIV-1 e HIV-2. O HIV-1 está amplamente distribuído no território mundial, possui maior diversidade de cepas virais e é o responsável pela pandemia global (representando aproximadamente 95% das infecções mundiais). Já o HIV-2, pouco disseminado e menos agressivo, causa uma forma da doença de progressão mais lenta, onde os indivíduos apresentam cargas virais mais baixas, menor declínio das células T CD4, e apenas 20-30% desenvolvem AIDS. Além disso, ele é encontrado quase que exclusivamente na região da África Ocidental. Ambos evoluíram de cepas do vírus da imunodeficiência símia (SIV) presente em diversas espécies de primatas da África Ocidental e Central. Estudos evidenciam que o HIV-1 tenha sido originado primariamente de chimpanzés, enquanto o HIV-2 de uma espécie de macaco mangabei (*Cercocebus atys*), a única espécie de primata naturalmente infectada por cepas virais que são relacionadas com o HIV-2 (Figura 4) (JAFFAR et al., 2004; PEETERS; JUNG; AYOUBA, 2013; VIJAYAN et al., 2017).

Com base em análises filogenéticas, o HIV-1 apresenta diversas linhagens que são classificadas em quatro grupos (M, N, O e P) e foram originadas das cepas virais SIVcpz e SIVgor, presentes em chimpanzés (*Pantroglo dytestroglodytes*) e gorilas (*Gorilla gorilla gorilla*), respectivamente. Essas linhagens apresentam diferentes distribuições geográficas de acordo com suas origens. O grupo M (de *Main/Major*) é responsável por aproximadamente 35 milhões de indivíduos infectados mundialmente, o grupo O (de *Outlier*) causa alguns milhares de infecções na África Centro-Ocidental, e os grupos N (de *New* ou *non-M/non-O*) e P têm sido encontrados em alguns indivíduos provenientes de Camarões (país da África Central). O grupo M

tem sido evidenciado ser a linhagem mais antiga do HIV-1 em humanos, e atualmente apresenta nove subtipos: A-D, F-H, J e K (Figura 4), que ainda possui subdivisões tais como A1-A4 para o subtipo A e F1-F2 para o F. O HIV-1 pode também apresentar recombinações entre esses diferentes grupos e subtipos devido as múltiplas infecções que alguns pacientes possuem: formas recombinantes circulantes (CRFs) e únicas (URFs), e as recombinantes de segunda geração (SGRs) (HEMELAAR, 2012; MOUREZ; SIMON; PLANTIER, 2013; SHARP; HAHN, 2011; VUILLEUMIER; BONHOEFFER, 2015).

Figura 4 – Origem e Diversidade do HIV. Os primatas conhecidos como “macacos do velho mundo” são naturalmente infectados por diversos SIVs, que passaram a infectar chimpanzés (pelo SIVcpz), gorilas (pelo SIVgor) e posteriormente os humanos (pelos HIV-1 e 2). À esquerda estão as relações filogenéticas entre as linhagens SIVcpz, SIVgor e HIV-1, evidenciando os grupos e subtipos do HIV-1.



Fonte: Adaptado de Sharp e Hahn (2011).

Estima-se que as cepas virais do subtipo C (grupo M) representam mais de 50% das infecções globais pelo HIV-1, seguido dos subtipos B e A. No Brasil, assim como em diversos outros países e regiões do mundo, são encontradas apenas cepas virais do grupo M. Contudo, apenas os subtipos B e C predominam no país, sendo o B mais amplamente distribuído enquanto as cepas do subtipo C se concentram na região Sul do Brasil. Algumas formas recombinantes e outros subtipos virais também são encontrados, mas em menor frequência (ALVES et al., 2019; BBOSA; KALEEBU; SSEMWANGA, 2019; TAYLOR et al., 2008).

Além das suas relações filogenéticas, o HIV-1 também pode ser classificado com relação ao seu tropismo, que depende do correceptor celular no qual a partícula

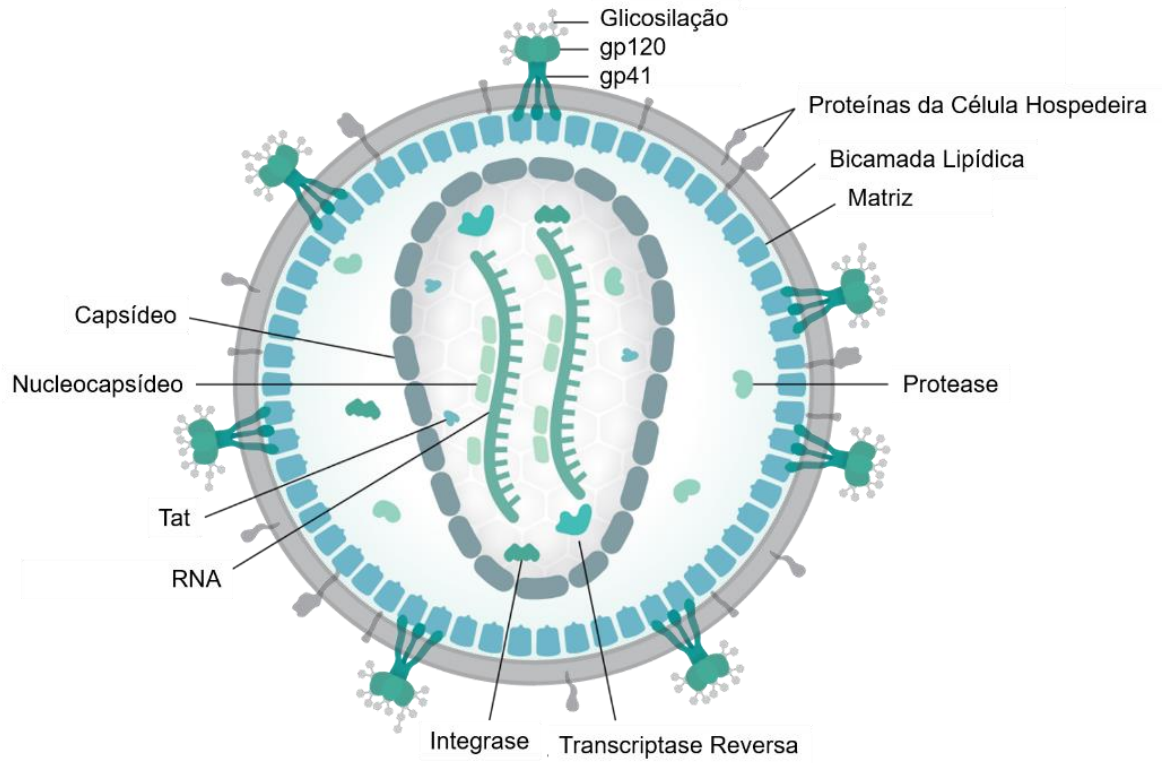
viral se liga, temos então: HIV-1 de tropismo R5, pois infectam células do sistema imune que apresentam CCR5 na superfície celular, principalmente macrófagos; HIV-1 de tropismo X4, infectam células que apresentam CXCR4, principalmente linfócitos T CD4+; e o de tropismo duplo (R5/X4), que podem interagir com ambos os correceptores (CCR5 ou CXCR4) no processo de fusão celular durante a infecção pelo HIV-1. As cepas virais de tropismo R5 aparecem mais no início da infecção e são responsáveis pela transmissão do HIV-1, ademais, eles têm um impacto relativamente menor no sistema imune do hospedeiro e predominam nos indivíduos infectados. Enquanto as cepas de tropismo X4 aparecem tardiamente e têm sido associados com o declínio mais rápido das células T CD4+ e progressão à AIDS. Contudo, estudos têm evidenciado que existem vírus de tropismo X4 que podem aparecer ainda no início da infecção, sendo responsáveis pela transmissão inicial do vírus. Essas variantes estão mais presentes no HIV-2, são menos infecciosas, e assim, menos frequentes (ALKHATIB, 2009; GRANDE et al., 2019; NAIF, 2013).

2.2.2 Estrutura Biológica do HIV-1

A partícula viral do HIV-1 apresenta uma forma esférica com aproximadamente 100ηm de diâmetro, que é externamente envelopada por uma bicamada lipídica de origem da célula hospedeira contendo diversas glicoproteínas virais. Essas proteínas do envelope se organizam em estruturas triméricas fortemente glicosiladas, chamadas de *spikes.* Cada estrutura dessa é composta por três moléculas de proteínas transmembrana (gp41), envolvidas na fusão de membranas; e três proteínas de superfície (gp120), que atuam no reconhecimento e ligação aos receptores celulares (Figura 5) (ARAÚJO; ALMEIDA, 2013; ENGELMAN; CHEREPANOV, 2012; HUTCHINSON, 2001).

Internamente à membrana bilipídica encontra-se o capsídeo viral estruturalmente cônico (o núcleo do vírus), que é formado pelas proteínas p24 e envolvido por uma matriz estrutural constituída pelas proteínas associadas, p17. Nesse núcleo em forma de cone localiza-se o genoma (duas fitas simples de RNA) e as enzimas virais: integrases, proteases e a transcriptase reversa (BRIGGS; KRÄUSSLICH, 2011; FANALES-BELASIO et al., 2010; LI; DE CLERCQ, 2016).

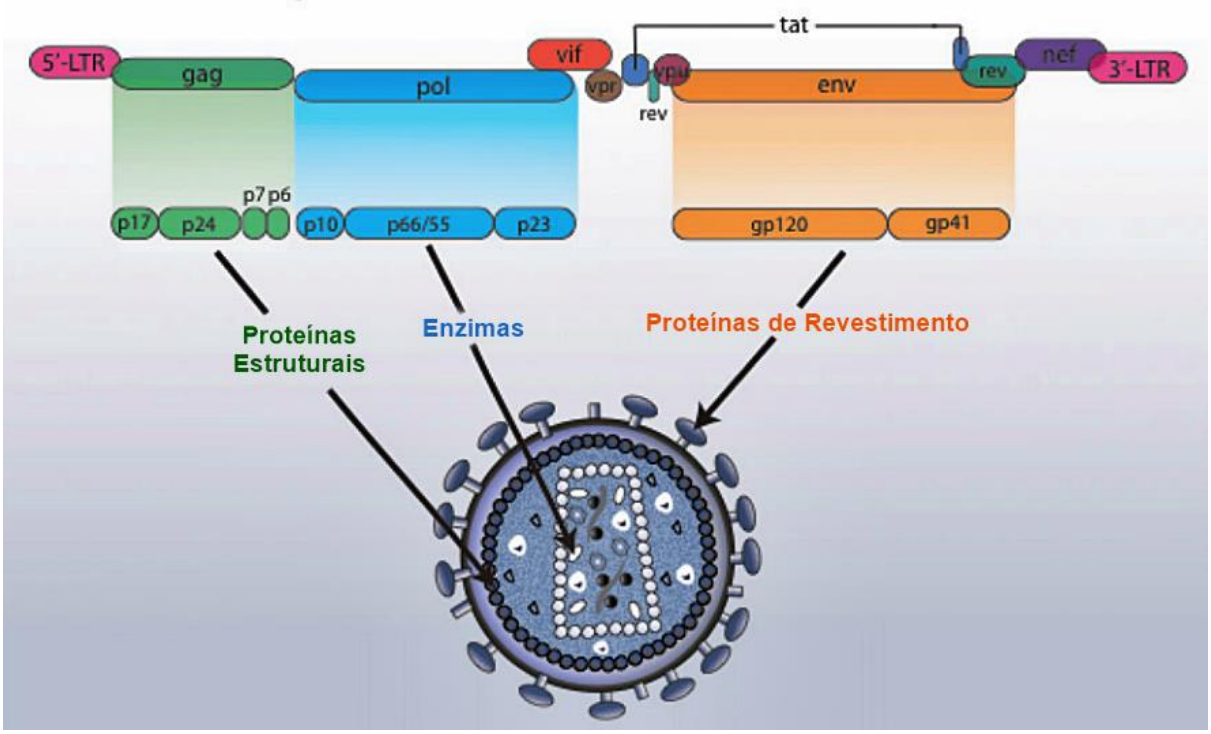
Figura 5 – Diagrama do HIV-1. Uma partícula viral evidenciando seus respectivos componentes estruturais: o genoma viral representado por duas fitas de RNA, as enzimas (protease, integrase, transcriptase reversa e Tat), o capsídeo, a matriz proteica e os complexos de glicoproteínas do envelope (*spikes*).



Fonte: Adaptado de Splettstoesser (2014). Disponível em: <https://commons.wikimedia.org/wiki/File:HI-virion-structure_en.svg> Acesso: 05 de abril de 2020.

O genoma do HIV-1 contém aproximadamente 9200 ácidos nucleicos (somando ambas as fitas de RNA), organizados em nove regiões gênicas responsáveis por codificar as diferentes proteínas virais. Esses genes são delimitados e regulados por duas regiões terminais longas (5'LTR e 3'LTR) e podem ser categorizados em dois grupos principais de acordo com sua funcionalidade: os genes estruturais encarregados pela codificação das proteínas estruturais (*gag*), enzimas (*pol*) e proteínas do envelope (*env*); e os genes regulatórios (*vif*, *vpr*, *vpu*, *nef, rev* e *tat*) responsáveis pela codificação das proteínas auxiliares e regulatórias, que atuam na replicação, transcrição, tradução, maturação e infectividade das partículas virais (Figura 6) (ABBAS; LICHTMAN; PILLAI, 2018; DONNELLY; CIBOROWSKI, 2016; FANALES-BELASIO et al., 2010; WATTS et al., 2009).

Figura 6 – Estrutura genômica do HIV-1. Os genes ao longo do genoma linear estão indicados por blocos de cores, agrupados de acordo com a categoria da proteína que será sintetizada. *LTR*, repetição terminal longa; *gag,* antígeno específico de grupo; *pol,* polimerase; *vif*, fator de infectividade viral; *vpr*, proteína viral R; *rev*, regulador de expressão de genes virais; *vpu*, proteína viral U; *tat,* ativador transcricional; *env*, envelope; *nef*, efetor negativo.



Fonte: Adaptado de Donnelly e Ciborowski (2016).

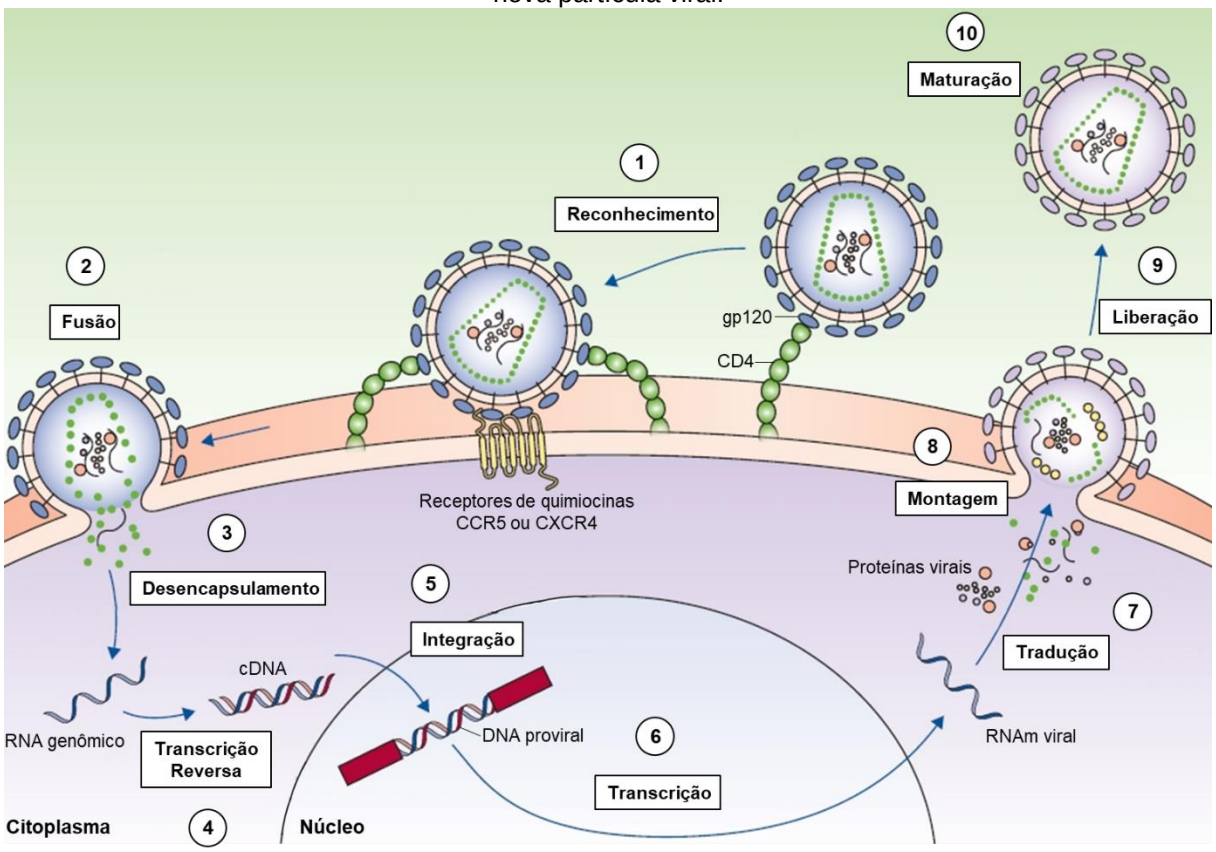
2.2.3 Ciclo de Replicação Viral

A infecção pelo HIV-1 ocorre em células que apresentam em sua superfície o receptor CD4 e os correceptores CCR5 e CXCR4, sendo os linfócitos T CD4+ as principais células-alvo do vírus no organismo. Ademais, o HIV-1 é capaz de infectar outros tipos celulares, tais como monócitos, macrófagos e células dendríticas, que expressam também esses receptores (ABBAS; LICHTMAN; PILLAI, 2018; DEEKS et al., 2015; WILEN; TILTON; DOMS, 2012).

A infecção viral tem início quando as glicoproteínas do envelope viral gp120 reconhecem a células-alvo por meio de sua ligação aos receptores de superfície CD4, e em seguida aos receptores de quimiocinas CCR5 ou CXCR4, que atuam como correceptores para fusão do HIV-1 à célula. Após o reconhecimento e ligação celular, a glicoproteína viral gp41 sofre uma mudança conformacional, que permite a fusão da membrana viral com a membrana da célula-hospedeira. Assim, o capsídeo é liberado no citoplasma celular para posteriores etapas do ciclo viral (Figura 7). Vários estudos sugerem que a entrada do HIV-1 na célula-alvo também pode ocorrer por via de

endocitose (BLUMENTHAL; DURELL; VIARD, 2012; BRANDENBERG et al., 2015; DUMAS; PREIRA; SALOME, 2014; LINDEMANN; STEFFEN; PÖHLMANN, 2013).

Figura 7 – Ciclo de replicação do HIV-1. As etapas sequenciais do ciclo viral, evidenciando os processos que ocorrem durante a infecção pelo HIV-1 nas células hospedeira até a liberação de uma nova partícula viral.



Fonte: Adaptado de Maartens et al. (2014).

Dentro da célula-alvo, o capsídeo é desencapsulado, liberando o material genético do HIV-1 e tornando as enzimas do complexo núcleocapsídeo ativas. O RNA genômico viral é então convertido, por meio da transcriptase reversa, em uma fita dupla de DNA (chamado de DNA complementar, cDNA). A grande variabilidade do HIV-1, sua notável e mais vantajosa característica, é proveniente principalmente da alta taxa de mutações durante a replicação viral, consequência dos erros introduzidos pela transcriptase reversa, que não tem atividade revisora durante a síntese do cDNA. E assim, esse cDNA é transportado para o núcleo celular onde será inserido ao genoma do hospedeiro por meio da enzima integrase. O DNA viral integrado é agora chamado de provírus (ou DNA proviral) podendo permanecer inativo por meses ou anos no hospedeiro, e assim, a infecção pelo HIV no organismo torna-se latente (ARAÚJO; ALMEIDA, 2013; DEEKS et al., 2015; ENGELMAN; CHEREPANOV, 2012; FANALES-BELASIO et al., 2010; SLEASMAN; GOODENOW, 2003).

No núcleo, o genoma proviral, integrado ao material genético da célula infectada, inicia a etapa de transcrição e processamento do RNAm viral. Os transcritos, por sua vez, irão originar o RNA genômico e as proteínas virais. A transcrição dos genes do HIV-1 nas células infectadas inicia-se por meio da ativação dessas células por citocinas. Dessa maneira, após a transcrição do genoma proviral no núcleo, os transcritos virais (processados por *splicing* alternativo e os não processados) são transportados para o citoplasma onde formarão o genoma do HIV-1 e fornecerão as informações genéticas para a tradução das proteínas regulatórias, estruturais e das enzimas (ABBAS; LICHTMAN; PILLAI, 2018; ENGELMAN; CHEREPANOV, 2012; LI; DE CLERCQ, 2016).

Após a síntese de todo material viral, ocorrem as etapas de montagem e liberação das novas partículas do HIV-1. Durante a montagem, o capsídeo é reestabelecido em torno do genoma e das enzimas virais, e direcionado para a periferia celular. Por fim, o capsídeo adquire o envelope a partir da membrana celular do hospedeiro (processo chamado de brotamento), e as novas partículas virais são liberadas para o meio extracelular, onde passarão por maturação (promovida pela protease viral) e modificação morfológica, tornando-se um vírion (a forma infecciosa do vírus) (BRIGGS; KRÄUSSLICH, 2011; FREED, 2015; MAARTENS; CELUM; LEWIN, 2014).

2.2.4 Transmissão e Patogênese

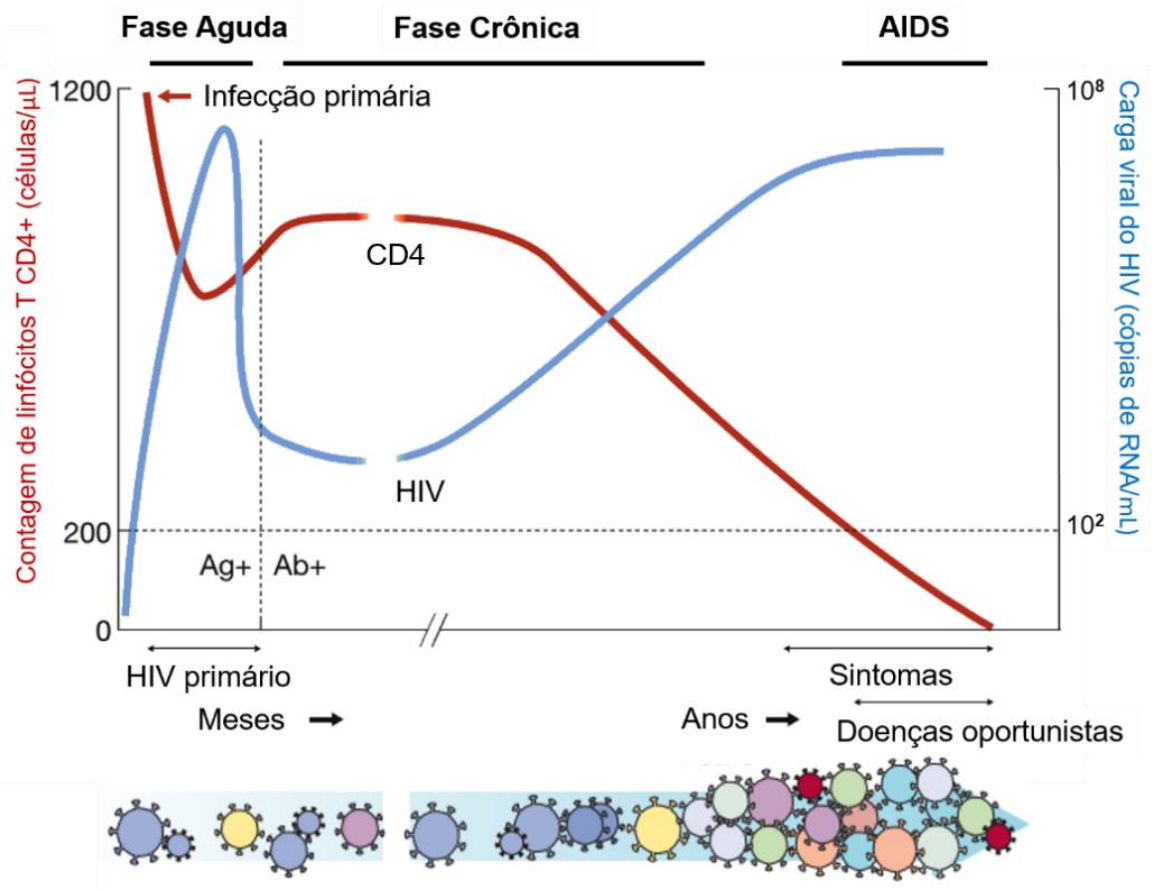
A transmissão do HIV-1 ocorre apenas por meio de certos fluidos corporais de uma pessoa infectada, tais como sangue, sêmen, fluídos vaginal e retal, e leite materno. Para que o vírus possa ser transmitido, o indivíduo deve entrar em contato direto com esses fluidos por meio de regiões de mucosas, tecido lesado ou inoculações (intravenosa e cutânea). Sendo assim, a transmissão do HIV pode ocorrer em relações sexuais sem proteção, transfusões sanguíneas, compartilhamentos de objetos perfurocortantes e transmissão vertical (da mãe para o filho). Mais de 80% dos adultos vivendo com HIV-1 tornaram-se infectados devido à exposição das regiões de mucosa ao vírus, principalmente via relações sexuais desprotegidas (COHEN et al., 2013; HLADIK; MCELRATH, 2008; SHAW; HUNTER, 2012).

Uma vez o organismo exposto ao vírus (geralmente via mucosa epitelial genital ou retal), ocorrem os primeiros sinais da reposta imune à infecção pelo HIV-1, e assim, tem início a fase aguda. Imediatamente após a exposição e transmissão do vírus, as

células são infectadas (principalmente macrófagos presentes nas mucosas) e imunologicamente ativadas. Durante os primeiros dias (7-21 dias) de contato com os vírions, há um período denominado de fase eclipse, onde o HIV-1 mantém sua replicação nas regiões de mucosas, não sendo detectado no plasma e impossibilitando geralmente o diagnóstico por ensaios clínicos (mesmo os ultrassensíveis). Em seguida, as células infectadas migram para os linfonodos locais e depois para os demais tecidos linfoides, onde por meio do contato direto intercelular o vírus é disseminado. Dessa forma, por volta de 28 dias após a exposição ao HIV-1, a replicação torna-se mais ativa e a circulação livre das partículas virais na corrente sanguínea resulta em uma alta viremia, caracterizando assim a fase aguda. Esse pico de viremia vem associado a um declínio acentuado na contagem de células T CD4+, uma vez que a resposta imune adaptativa ainda não foi desenvolvida, aumentando o potencial infeccioso do indivíduo (Figura 8). Nas primeiras semanas após a exposição ao HIV-1, alguns indivíduos podem apresentar febre e mal-estar, sintomas usualmente inespecíficos (CARTER; EHRLICH, 2008; COHEN et al., 2013; FRASER et al., 2014; MINISTÉRIO DA SAÚDE, 2018a).

À medida que o HIV-1 infecta e destrói os linfócitos T CD4+ (os quais coordenam a resposta imune), o sistema imunológico do hospedeiro é debilitado uma vez que o vírus induz vias de mortes celular tanto nas células infectadas quanto nas saudáveis. Isso ocorre devido à liberação de sinais inflamatórios pelas células mortas, que recrutam mais células para o local de inflamação, e consequentemente gerando mais morte celular. Esse mecanismo da infecção pelo HIV-1 cria um ciclo vicioso, que por fim causa no indivíduo a depleção das células T CD4+ e uma inflamação crônica nos órgãos linfoides. As manifestações clínicas da infecção variam desde um estado assintomático até o desenvolvimento de doenças oportunistas graves e neoplasias, que são potencialmente letais devido à condição imunológica do indivíduo (DOITSH; GREENE, 2016; MELHUISH; LEWTHWAITE, 2018; STEVENSON, 2003).

Figura 8 – Curso natural da infecção pelo HIV-1. Em destaque os níveis de CD4 (linha vermelha) e carga viral (linha azul), evidenciando a progressão da infecção pelo HIV-1 e a depleção de células T CD4+ ao longo de cada fase.



Fonte: Adaptado de Melhuish e Lewthwaite (2018).

Quando o sistema imune do hospedeiro começa a gerar a resposta adaptativa contra HIV-1 inicia-se a fase crônica da infecção. Esta fase é marcada pela forte interação entre as células de defesa e as constantes e rápidas mutações do vírus, que não enfraquece o organismo o suficiente para permitir novas doenças. Embora a resposta imune específica seja tardia e insuficiente para erradicar a infecção, ela consegue elevar os níveis de células T CD4+ (mesmo que abaixo do normal) e estabilizar a viremia. Porém, o HIV-1 apresenta mecanismos de escape da resposta imune, além de aumentar sua diversidade genética a cada ciclo de replicação dificultando uma resposta imune eficiente, o vírus consegue entrar em latência nas células infectadas em diversos tecidos e órgãos, formando os reservatórios virais. Esse período de latência pode durar anos, resultando em uma doença crônica e assintomática (Figura 8) (MAARTENS; CELUM; LEWIN, 2014; SILICIANO; GREENE, 2011; STEVENSON, 2003; SWANSTROM; COFFIN, 2012).

Na maioria dos casos, a contagem de linfócitos T CD4+ diminui ao longo dos anos, à medida que a disseminação do vírus persiste e ocasionando a exaustão do sistema imunológico. Dessa forma, em média dez anos após a exposição pelo HIV-1, os indivíduos infectados e não tratados apresentam elevada carga viral plasmática (CVP) e níveis de linfócitos T CD4+ abaixo de 200 céls/mm^3, caracterizando o estágio clínico de AIDS. AIDS é a fase final de uma doença progressiva e patogênica que causa uma profunda deficiência do sistema imune, tornando os indivíduos susceptíveis a infecções oportunistas e neoplasias. Nesse momento, na ausência de tratamento antirretroviral, o indivíduo tem grande risco de morrer (DEEKS et al., 2015; MELHUISH; LEWTHWAITE, 2018; NAIF, 2013; SWANSTROM; COFFIN, 2012).

2.3 TERAPIA ANTIRRETROVIRAL (ART)

2.3.1 Antirretrovirais e a ART

Desde a descoberta do HIV-1 e o crescente número de pessoas vivendo com HIV/AIDS mundialmente, houve um aumento acentuado em pesquisas pela comunidade científica mundial para a descoberta de drogas antirretrovirais. O desenvolvimento e aprovação desses medicamentos anti-HIV surgiu ainda na década de 1980, com o objetivo de impedir a multiplicação do vírus no organismo e sua disseminação. A azidotimidina (AZT) ou zidovudina (ZVD) foi o primeiro antirretroviral aprovado para o tratamento da infecção pelo HIV-1, e foi lançado em 1987. Desde então, o número de drogas anti-HIV produzidas e implementadas tem aumentado consideravelmente, aprimorando cada vez mais o tratamento para PVHA (ARTS; HAZUDA, 2012; MAEDA et al., 2019; TSENG; SEET; PHILLIPS, 2014).

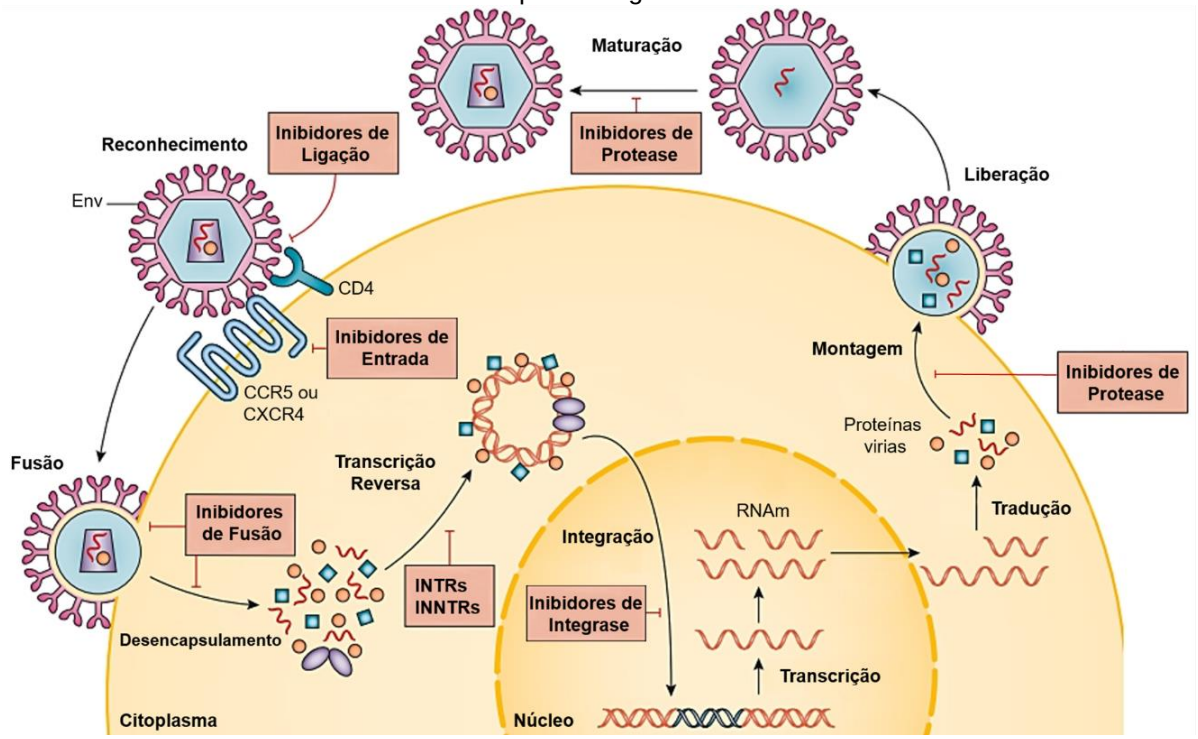
Os antirretrovirais são fármacos usados no tratamento de infecções causadas por retrovírus, principalmente o HIV-1. Esses medicamentos não eliminam completamente o vírus, que permanece em latência proviral nas células infectadas, mas suprime a replicação viral, evitando o enfraquecimento do sistema imunológico e aumentando o tempo e a qualidade de vida dos indivíduos vivendo com HIV/AIDS (ARTS; HAZUDA, 2012; DE CLERCQ; LI, 2016; PAU; GEORGE, 2014).

Atualmente, estão disponíveis no mercado diversos fármacos anti-HIV, usados usualmente em combinações de três drogas, constituindo a terapia antirretroviral (TARV, ou do inglês ART), anteriormente chamada de HAART (do inglês, *Highly Active Antiretroviral Therapy*). Essa terapia é responsável pela melhoria do

prognóstico dos pacientes, queda da carga viral e recuperação do sistema imune. Tais drogas podem ser divididas em sete classes de acordo com o seu modo de ação (Figura 9) (CAMBOU; LANDOVITZ, 2020; DE CLERCQ; LI, 2016; MAEDA et al., 2019; PAU; GEORGE, 2014; TSENG; SEET; PHILLIPS, 2014):

- (1) Inibidores de Ligação (IL): Tipo I – são anticorpos que se ligam aos receptores CD4 e atuam como inibidor pós-ligação, pois impedem que a proteína gp120 do HIV-1 mude sua conformação dificultando a ligação do vírus aos correceptores; Tipo II – ligam-se a gp120 do envelope viral impossibilitando a interação do vírus com a célula hospedeira, e consequentemente, prevenindo a entrada do HIV-1;
- (2) Inibidores de Entrada (IE): ligam-se ao correceptor CCR5 na célula, atuando como antagonista do CCR5 e bloqueando a entrada do HIV-1 de cepa R5 nas células-alvo;
- (3) Inibidores de Fusão (IF): se ligam a proteína gp41 do vírus, impedindo o rearranjo estrutural da gp41, e assim, bloqueando a fusão do vírus na membrana celular hospedeira;
- (4) Inibidores Nucleosídeos da Transcriptase Reversa (INTR): são análogos de nucleosídeos ou nucleotídeos que atuam na enzima transcriptase reversa, interrompendo a síntese do material genético do vírus;
- (5) Inibidores Não-Nucleosídeos da Transcriptase Reversa (INNTR): impedem a síntese do material genético do vírus por se ligarem a sítios alostéricos da transcriptase reversa, inibindo sua atividade;
- (6) Inibidores de Integrase (INI): agem no bloqueio da atividade da enzima integrase responsável pela inserção do DNA viral ao DNA humano. Assim, inibe a replicação do vírus e sua capacidade de infectar novas células;
- (7) Inibidores de Protease (IP): interagem com a protease viral, impedindo o processamento correto das proteínas e suprimindo a infectividade das partículas virais.

Figura 9 – As principais classes de antirretrovirais e as etapas do ciclo viral que são bloqueadas pelas drogas.



Fonte: Adaptado de Deeks et al. (2015).

O Brasil foi o primeiro país a distribuir gratuitamente os antirretrovirais para os indivíduos portadores do HIV-1. Desde 1996, logo após a implementação da terapia anti-HIV combinada, foi estabelecida a oferta universal ao tratamento antirretroviral, que permitiu reduzir a morbimortalidade relacionada à infecção pelo HIV-1, e consequentemente, aumentou a sobrevida e a qualidade de vida das PVHA no país (MINISTÉRIO DA SAÚDE, 2018b; WOLFF et al., 2017).

Atualmente, recomenda-se o início da terapia antirretroviral para todas as PVHA assim que são diagnosticas com a infecção, independentemente do seu estágio clínico e/ou imunológico (contagem de células T CD4+). Além disso, algumas situações exigem maior urgência no início da ART, tais como: gestantes, indivíduos HIV-positivos com contagem de células T CD4+ <350 células/µL e quadro clínico avançado da doença. A recomendação de início precoce da ART tem proporcionado melhores respostas terapêuticas aos pacientes. No entanto, é importante destacar que em países onde os recursos são limitados e, consequentemente, apresentam menor disponibilidade de fármacos, a prioridade no início da ART é para indivíduos em estágios mais avançados da infecção (MINISTÉRIO DA SAÚDE, 2018b; WHO, 2016).

Até 2016, a Organização Mundial de Saúde (WHO) estabelecia como primeira linha de antirretrovirais para terapia anti-HIV uma combinação de dois INTR e um INNTR: Tenofovir (TDF) ou Zidovudina (AZT) + Lamivudina (3TC) + Efavirenz (EFZ) ou Nevirapina (NVP). Contudo, com os avanços nos estudos com esses esquemas antirretrovirais e uma forma de minimizar a resistência viral que vinha sendo reportada, o atual esquema recomendado pela WHO consiste em dois INTR e um INI: TDF + 3TC + DTG (Dolutegravir); ou dois INTR e um INNTR, sendo: TDF + 3TC + EFZ400 (Efavirenz 400 mg). Esses novos esquemas terapêuticos têm sido associados com uma melhor tolerabilidade dos pacientes, maior eficácia antirretroviral, taxas mais baixas de descontinuação do tratamento, uma maior barreira genética à resistência e menos interações medicamentosas. Já na segunda linha de esquemas, geralmente utilizada em caso de falha na primeira, o INNTR podia ser substituído por um Inibidor de Protease (IP) com ritonavir (r). Neste caso os antirretrovirais recomendados eram: Lopinavir (LPV) e o Atazanavir (ATV). Entretanto, após as novas recomendações em 2016, esses antirretrovirais passaram a ser substituídos pelos: Darunavir/r (DRV/r, um IP/r) e o Raltegravir (RAL, um INI). Essas são algumas das diretrizes estabelecidas pela WHO que os países mundiais vêm adotando no tratamento anti-HIV e atualizando ao longo do tempo (WHO, 2016, 2017).

2.3.2 Respostas à ART

Na última década, com os avanços da terapia antirretroviral foi possível observar um aumento progressivo de sucesso na resposta terapêutica. Cerca de 80% dos pacientes atingem CVP indetectável (variando de <20 a <75 cópias/mL, dependendo do método laboratorial) em até um ano após início do tratamento e conseguem manter a supressão viral ao longo do tempo. Um dos mais notáveis benefícios dessa supressão do HIV por meio da ART, que vem sendo observado nos pacientes, é a intransmissibilidade do vírus devido a indetectabilidade, do inglês *Undetectable* = *Untransmittable* (U = U). Este conceito, baseado em fortes evidências científicas, assume que indivíduos HIV-positivos em ART e com CVP suprimida pode não transmitir sexualmente o vírus para outras pessoas, visto que o HIV se encontra em concentrações desprezíveis no sêmen, demostrando assim a eficácia e importância da terapia anti-HIV também para prevenção. Essas evidências têm possibilitado ao UNAIDS desenvolver companhas que visam acabar com a epidemia mundial da AIDS até 2030, reduzindo o número de novas infecções e de mortes

relacionadas a AIDS, e eliminando o estigma e a discriminação associado ao HIV (EISINGER; DIEFFENBACH; FAUCI, 2019; MINISTÉRIO DA SAÚDE, 2018b; UNAIDS, 2021b; WHO, 2016; WOLFF et al., 2017).

Desde sua criação em 1996 pelas Nações Unidas (ONU), o UNAIDS tem realizado muitas ações no combate ao HIV/AIDS, que objetivam prevenir o avanço do vírus, promover tratamento e assistência aos infectados pelo HIV e reduzir o impacto socioeconômico da epidemia nos países afetados. E usualmente tem alcançado sucesso em suas propostas. Em 2014, o UNAIDS estabeleceu o objetivo dos “90-90-90”, que propõe até o final 2020 90% das pessoas vivendo com HIV serem diagnosticas, 90% desses diagnosticados estarem em tratamento e 90% desses em terapia alcançarem o sucesso terapêutico, supressão da CVP. Com essa estratégia, até 2030 o número de novas infecções pelo HIV-1 e mortes relacionadas à AIDS reduzirá em até 80%. Para isso, é necessária ampliação nos métodos de diagnóstico do HIV, expandir o acesso à ART, e melhorar a adesão ao tratamento e qualidade na assistência às PVHA. Até o final de 2020, alguns países já tinham conseguido atingir os 90-90-90 e passaram a aderir novas metas (95-95-95), enquanto vários outros estavam bem próximos de alcançar os três 90. No Brasil, cerca de 94% dos pacientes HIV-positivos em ART alcançam esse sucesso terapêutico. Atualmente, dos 37.7 milhões de pessoas vivendo com HIV/AIDS ao redor do mundo, 84% foram diagnosticadas e sabem seus *status* clínico, 87% delas estão em ART e 90% dessas em tratamento apresentam supressão da carga viral. Contudo, o número total de indivíduos infectados pelo HIV que estão em tratamento antirretroviral ainda não é satisfatório para os objetivos – 75% (~28 milhões) até junho de 2021. Ou seja, mais de nove milhões de PVHA não tem acesso aos antirretrovirais, ainda sendo necessário melhorar a ampliação no acesso aos medicamentos da ART (HILL; POZNIAK, 2015; UNAIDS, 2017, 2021a).

Apesar das taxas de sucesso terapêutico serem elevadas, muitos pacientes ainda apresentam falha da terapia anti-HIV e precisam de alterações em seus esquemas antirretrovirais, algumas vezes necessitando do “esquema de resgate” (esquema utilizado em casos de falha a primeira linha e segunda linha de antirretroviral). A falha terapêutica é consequência de inúmeros fatores, tais como baixa adesão ao tratamento, esquema antirretrovirais inadequados, resistência viral e fatores farmacológicos (interações medicamentosas, erros de prescrição, má absorção e/ou eliminação acelerada dos antirretrovirais). Além disso, para

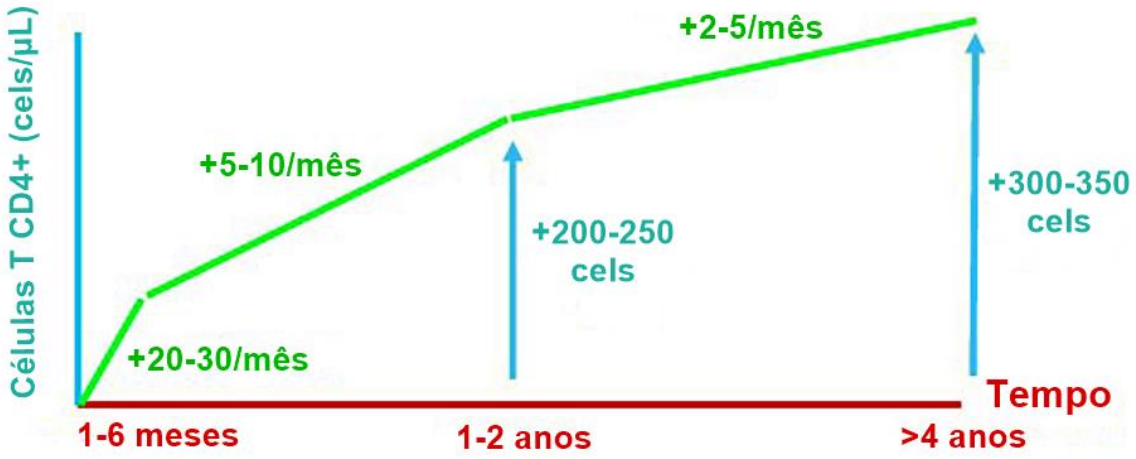
caracterização da falha terapêutica, a falha virológica é o principal parâmetro, que é definida quando o indivíduo após seis meses de tratamento não suprime a CVP a níveis indetectáveis (<40 cópias/mL) ou rebote da carga viral nos pacientes que mantinham supressão viral durante a ART (ALDOUS; HAUBRICH, 2009; LEDERGERBER et al., 2004; MINISTÉRIO DA SAÚDE, 2018b; TSENG; SEET; PHILLIPS, 2014; WHO, 2016).

Dessa forma, a ART visa suprimir a CVP-HIV no organismo a níveis indetectáveis, diminuir as morbimortalidades associadas ao HIV-1 e a taxa de progressão da doença, diminuir as taxas de transmissão viral, e como uma consequência, espera-se que ocorra a reconstituição imunológica (recuperação dos níveis de linfócitos T CD4+). Existem casos em que apesar da supressão da CVP-HIV durante o tratamento, o indivíduo apresenta deficiência na reconstituição do número de linfócitos T CD4+, caracterizando assim, a falha na recuperação imunológica. No entanto, mesmo na ausência de benefício imunológico, a supressão viral completa ainda é o que define o sucesso terapêutico (BACK; MARZOLINI, 2020; CORBEAU; REYNES, 2011; MINISTÉRIO DA SAÚDE, 2018b; PAU; GEORGE, 2014; YANG et al., 2020).

2.3.3 Recuperação Imunológica

Durante a ART, o ganho de células T CD4+ é constante, mas com etapas graduais, apresentando um rápido ganho nos primeiros meses e tornando-se menos progressivo ao longo do tempo de tratamento. Nos primeiros seis meses de ART a média no ganho de linfócitos T CD4+ nos pacientes é de 20-30 células/µL ao mês. Em seguida, até o segundo ano de ART, esse aumento de células diminui para cerca de 5-10 céls/µL/mês. Nos anos seguintes o ganho de linfócitos a cada mês é de apenas 2-5 céls/µL (Figura 10), tornando a reconstituição imune dos pacientes HIV-positivos cada vez mais lenta, principalmente se o início da ART for tardio. Entretanto, alguns pacientes não conseguem manifestar esse perfil de aumento no número de linfócitos T CD4+, podendo apresentar um ganho ainda mais reduzido, e assim, uma deficiência na recuperação imunológica durante o tratamento (CORBEAU; REYNES, 2011; GUIHOT; TUBIANA; BRETON, 2010; PANTAZIS et al., 2019; SMITH et al., 2003).

Figura 10 – Ganho médio ao mês de células T CD4+ durante ART. O aumento na contagem de linfócitos T CD4+ está representando em vários pontos ao longo do tempo de terapia.



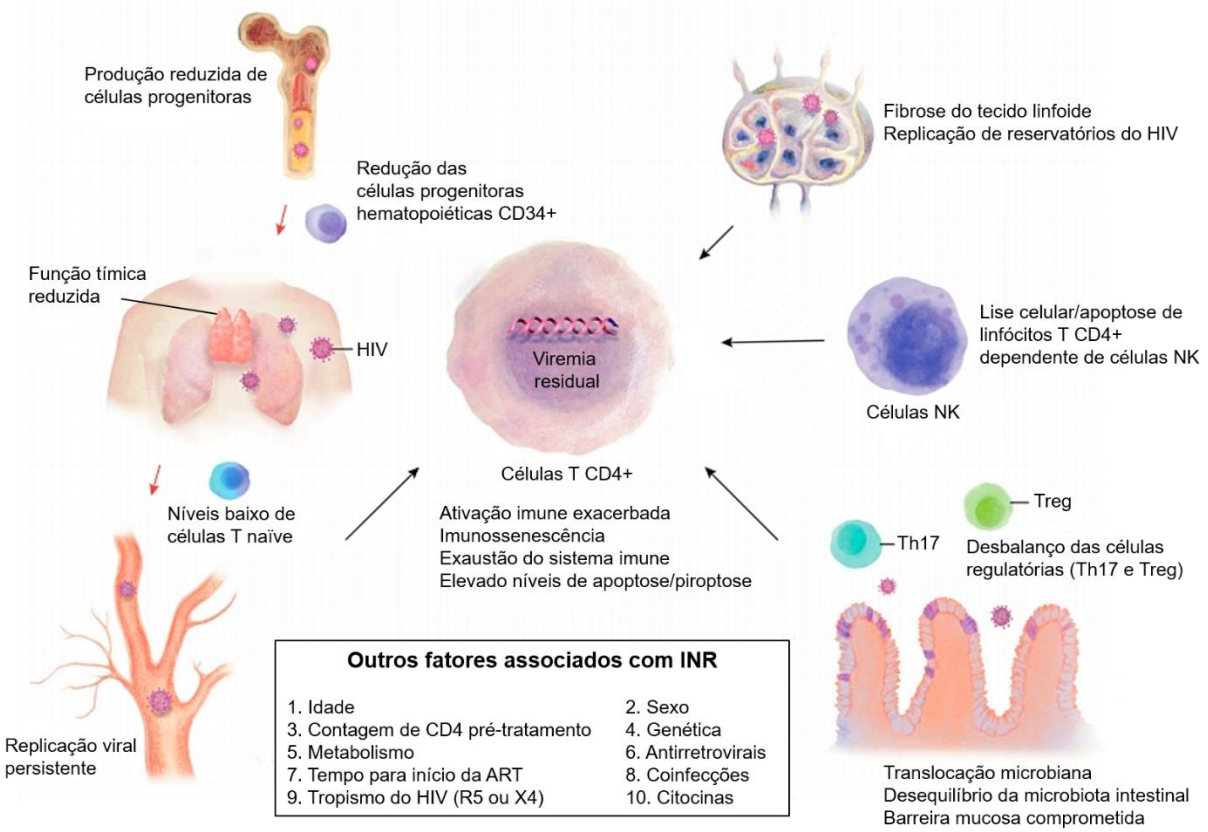
Fonte: Adaptado de Corbeau e Reynes (2011).

Embora a supressão viral sempre tenha sido a prioridade da ART, o aumento de linfócitos T CD4 durante o tratamento tornou-se também um objetivo importante, uma vez que melhora a qualidade de vida dos pacientes e aumenta a sobrevida deles, obtendo resultados terapêuticos mais satisfatórios. De 15% a 30% dos indivíduos que iniciam ART apresentam recuperação imunológica insuficiente, mesmo atingindo supressão completa da CVP. Esses pacientes são conhecidos como respondedores virológicos, mas não-respondedores imunológicos (INR, do inglês *immunological non-responders)*. Essa deficiência na reconstituição imune tem sido descrita na literatura como uma condição multifatorial, tendo sexo masculino, idade avançada no início da ART, carga viral alta e baixa contagem de células T CD4+ no início do tratamento, coinfecções durante a terapia, replicação viral residual e perfil genético, como alguns dos principais fatores de risco associados à esse ganho reduzido de linfócitos T CD4+ durante a ART (Figura 11) (CASTILHO; MELEKHIN; STERLING, 2014; MINISTÉRIO DA SAÚDE, 2018b; PACHECO et al., 2014; PINZONE et al., 2012; YANG et al., 2020).

Além desses diversos fatores envolvidos na reconstituição imune dos pacientes em ART, ainda não se tem um consenso sobre a definição ou classificação dos INR, devido também a alta heterogeneidade imunológica desses indivíduos. Sabemos que a contagem de células T CD4+ é o principal parâmetro, mas o ganho dessas células ao longo da terapia pode variar consideravelmente entre os pacientes, particularmente quando se leva em consideração as muitas variáveis que influenciam essa recuperação imunológica. A maioria dos estudos têm baseado a classificação dos INR comparando o perfil de linfócitos T CD4+ pré-tratamento com o ganho dessas células

nos primeiros anos de terapia, e nesse período mantendo a supressão da carga viral a níveis indetectáveis. Ou seja, quando os pacientes após um ou dois anos de ART, em supressão viral, não apresentam um ganho satisfatório de linfócitos CD4+ (geralmente <200 céls/µL), eles são classificados como INR (CENDERELLO; DE MARIA, 2016; CORBEAU; REYNES, 2011; KONG et al., 2019; LI et al., 2011; YANG et al., 2020).

Figura 11 – Fatores e mecanismos envolvidos na recuperação imunológica dos pacientes HIV-positivos durante ART. Linhas em vermelho indicam a rota de maturação das células T, enquanto as linhas em preto evidenciam os fatores associados com a deficiência na reconstituição do sistema imune. ART: terapia antirretroviral; INR: não-respondedores imunológicos; Th: célula T *helper*; Treg: célula T regulatória; NK: *natural killer*.



Fonte: Adaptado de Yang et al. (2020).

Apesar de já terem se passados mais de três décadas de estudos e avanços em tratamentos anti-HIV, os mecanismos que precisamente determinam essa deficiência na recuperação imunológica ainda não foram totalmente esclarecidos. Mesmo assim, diversos estudos têm demonstrado que a reconstituição de células T CD4+ reduzida durante ART possa ser consequência de dois principais processos: produção insuficiente e destruição excessiva dos linfócitos T CD4+. Este último, consequência de patogênese da infecção pelo HIV-1, ativação imunológica e/ou aumento, geneticamente determinado, da morte celular programada dos linfócitos. A

desregulação na homeostase das células T desempenha um papel fundamental na recuperação imunológica dos pacientes HIV-positivos em ART. Essa homeostase é regulada por um balanço dinâmico entre produção, proliferação, migração e destruição dos linfócitos T nos órgãos linfoides e na circulação periférica (Figura 11). Em outras palavras, se a destruição celular excede a produção ou houver redução na biossíntese celular ou proliferação, a contagem de células T CD4+ diminui mesmo em tratamento e com CVP suprimida (CENDERELLO; DE MARIA, 2016; CORBEAU; REYNES, 2011; GAARDBO et al., 2012; OKOYE; PICKER, 2013; YANG et al., 2020).

Apesar do grande interesse em tentar diminuir a probabilidade de deficiência na recuperação imunológica durante o tratamento, pouco ainda se sabe a respeito dos fatores do hospedeiro que estão envolvidos nesse fenômeno. Os estudos da literatura destacam essencialmente os aspectos imunológicos, maioria mediados pelo background genético, como principais fatores que influenciam na reconstituição de linfócitos T CD4+ durante a ART. Diante desse cenário, torna-se necessário investigar o perfil imunológico bem como alterações genéticas em genes do sistema imune dos pacientes em terapia antirretroviral que possam interferir na reconstituição imune desses indivíduos. Estudos assim possibilitarão entender melhor os mecanismos envolvidos nas respostas imunológicas desses pacientes em tratamento, e poderá fornecer subsídios para futuras pesquisas que visem melhorar os esquemas da ART e estabelecer marcadores de prognósticos para a condição dos não respondedores imunológicos.

3 RESULTADOS

3.1 ARTIGO 1 – GENETICS AND IMMUNOLOGICAL RECOVERY WITH ANTIRETROVIRAL TREATMENT FOR HIV

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Pharmacogenomics

Genetics and immunological recovery with antiretroviral treatment for HIV

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"Several studies have demonstrated the heterogeneity of patient's immunological recovery during ART, mostly focusing on immune factors that could be regulated by genetic variations."

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Keywords: apoptosis • ART • CD4$^+$ T-cells • immune reconstitution • INR • polymorphisms • pyroptosis • thymic function

Since the introduction of antiretroviral therapy (ART) HIV infection, treatment of the HIV-positive patient has improved considerably. ART consists of a combination of different antiretrovirals that can suppress plasma viral load to undetectable levels (<40 RNA copies/ml), preventing further transmission, disease progression and AIDS related deaths [1].

More than three decades of studies and advances in anti-HIV therapy have passed and several antiretrovirals have been developed since the first drug, zidovudine (ZDV or AZT), was introduced against HIV infection [2]. HIV viral load suppression always has been a priority for ART, and, consequently, the CD4$^+$ T-lymphocyte counts can increase for a better treatment outcome and quality of life of HIV-infected individuals. However, even though achieving undetectable plasma viral concentration, 15–30% of ART-treated patients do not significantly restore their CD4$^+$ T-cell count. This condition is defined as immunological recovery failure (IRF), and these patients are known as virological responders but immunological nonresponders (INR) [3,4].

The mechanisms that precisely determine this poor immune reconstitution have not been completely elucidated yet, and it is one of the main areas of research on HIV treatment. Nevertheless, several studies have demonstrated that IRF during ART may be the result of two main processes: insufficient production and excessive destruction of CD4$^+$ T cells [4,5]. It is believed that dysregulation of CD4$^+$ T-lymphocytes homeostasis plays a major role for immune restoration in HIV-positive patients on ART, and it is regulated by a dynamic balance between CD4$^+$ T-cells destruction, production and trafficking to and from lymphoid organs and peripheral circulation [6,7]. Furthermore, to date, IRF has been described as a multifactorial condition, including male sex, older age at ART start, advanced HIV infection with low pretreatment CD4$^+$ T-cell count, coinfections during therapy, reduced thymic output, residual viral production and genetic background as some of the main factors involved in the poor CD4$^+$ T-cell restoration under ART [4,8].

CD4$^+$ T-lymphocytes production

T-lymphocytes originate from CD34$^+$ hematopoietic progenitor cells (HPCs) in bone marrow, but their development occurs in the thymus, where T-cells migrate for maturation, selection and subsequent export of naive T-lymphocytes to the peripheral circulation [9]. It has been demonstrated that a proportion of CD34$^+$ HPCs can express CD4, CCR5 and CXCR4 receptors being potentially susceptible to HIV infection. Consequently, HIV can infect CD34$^+$ HPCs causing active infection and cell death, establishing latent reservoirs and influencing the inflow of progenitors for thymic function [4,6]. HIV can also infect and kill the developing CD4 thymocytes

and inhibit its maturation [10], affecting thymopoiesis and thus immune reconstitution of ART-treated patients even though achieving viral suppression since residual HIV replication by reservoir cells can persist.

The thymus is responsible for establishing the peripheral repertoire of T-lymphocytes during infancy and childhood, which would be sufficient for homeostatic proliferation and to maintain a wide compartment of $CD4^+$ T cells in adulthood. It is known that thymus begins to atrophy and is replaced by fatty tissue during aging [9]. Thymopoiesis is again required when a massive exhaustion of the T-lymphocytes pool occurs, and accelerated T-cell regeneration is then necessary, as observed in HIV infection [10]. However, a long HIV infection without treatment can cause thymic exhaustion and result in incomplete immune reconstitution even after ART initiation, indicating that early treatment initiation is essential for immunological recovery in HIV-positive patients. Diverse studies, including from our group, have shown that reduced thymic function plays an important role in the IRF of ART-treated patients although it alone does not explain the poor peripheral $CD4^+$ T-cell restoration during treatment [11–13]. Genetic factors that may influence hematopoiesis and thymic production are still unclear and have been less studied since they are regulated mainly by immune elements.

Inasmuch as thymic output is dramatically reduced with age, $CD4^+$ T-lymphocytes are increasingly generated from proliferation of already existing $CD4^+$ cells [9]. Peripheral proliferation of $CD4^+$ T-lymphocytes is an essential process for immune recovery of individuals living with HIV and under ART. This mechanism is mediated, among other factors, by the activity of interleukins, such as IL-2, IL-7 and IL-15 acting in activation, peripheral proliferation, differentiation and survival of $CD4^+$ T cells [14,15]. The presence of genetic variants, such as polymorphisms and expression variations, in the genes that encode these interleukins or their receptors can lead to alterations in their functions. Consequently, it has been shown to affect the peripheral $CD4^+$ T-lymphocytes homeostasis, contributing to IRF status. Additionally, genetic factors may interfere in the activity of these three interleukins [16].

It has been shown, through gene expression analysis, that although the IL-7 plasma levels are increased in the INR, its alpha-receptor expression (IL-7Rα or CD127) is decreased, causing a destabilization in the interaction between interleukin and receptor. On the other hand, HIV infection is characterized by reduced plasma IL-2 concentration, which tends to increase because of therapeutic success [6,15]. However, in INR, these low IL-2 levels can persist. In fact, a reduction in the *IL2* expression by mRNA quantification has been observed, leading to lower $CD4^+$ T-cell proliferation. Previous investigations have demonstrated that peripheral blood mononuclear cells from untreated HIV-positive individuals show decreased IL-15 production, and this is maintained in ART-treated individuals with IRF status. Moreover, some polymorphisms have also been reported in the genes encoding IL-2, IL-2Rα, IL-2Rβ, IL-2Rγ, IL-7, IL-7Rα, IL-15 and IL-15Rα that they could influence on $CD4^+$ T-lymphocytes homeostasis, isolated or in haplotypes [16,17].

In addition, alterations in genes that encode other interleukins, such as IL-4, IL-10, IL-12 and IL-19, among others [16], have also been reported to influence the immune recovery of HIV-infected individuals. However, the mechanism by which these genetic factors affect the $CD4^+$ T-cell restoration has not been completely elucidated yet.

$CD4^+$ T-lymphocytes destruction

The various mechanisms described in association with T-cell destruction during HIV infection occur substantially as a result of excessive immune activation since it contributes to increased cell death, rapid T-lymphocyte turnover and immune system exhaustion [18]. Studies have shown that even though decreasing HIV-induced immune activation and inflammatory process during ART, they can continue for a long time because of residual viral production by reservoirs, one of the main causes of immune activation [19,20].

Residual viral replication promotes a constant inflammatory process in lymphoid organs. Intrinsic HIV characteristics, such as its tropism, can influence this process. Studies have shown that X4 viral strains are more frequent in INR, inducing faster disease progression and persistent T-cell activation [6,19]. Thus, the inflammation advances progressively, leading to exhaustion of activated and naive $CD4^+$ T cells by cell death pathways. Additionally, microbial translocation has also been associated with immune activation. This contributes to lymphocytes destruction in the course of HIV infection, mainly in the initial stages where R5 viral strains have a preference for lymphoid tissues in the GI tract, destroying the defense barrier and leaving a way for microbia coming from the intestinal lumen to invade the systemic circulation [19,21].

Infections by cytomegalovirus, tuberculosis and hepatitis type B and C have also been found in ART-treated patients with poor immune recovery, leading to increased $CD4^+$ and $CD8^+$ T-cell activation, which results in

thymic exhaustion and lymphopenia mediated by high cell death levels [22]. These suggest that coinfections also contribute to exacerbated immune activation and thus the IRF condition.

Untreated HIV-infected individuals commonly have elevated levels of some inflammatory markers, such as TNF-α, IFN, IL-6, IL-8, IL-1β, TGF-β, RANTES and others, in addition to elevated levels of activated $CD8^+$ T cells since higher expression of $CD38^+$ and HLA-DR is observed [4,23]. Furthermore, variants in genes that produce these molecules have demonstrated influence in the $CD4^+$ T-cell restoration. For instance, the TT genotype of promoter region polymorphism (-179G/T) in *IFNγ* was associated with rapid decrease in $CD4^+$ T cells. Additionally, the +3954 G/A polymorphism (at exon 5) in *IL1B* has been associated with increased protein secretion, which may enhance the cell death by pyroptosis leading to $CD4^+$ T-cell depletion [23].

Exacerbated cell death levels, by apoptosis or, highlighted by more recent evidence, by pyroptosis of nonpermissive $CD4^+$ T-lymphocytes, have been described as a direct consequence of viral infection, contributing to a hyperinflammatory condition [24]. Therefore, functional alterations in proteins or genes of these cell death pathways could exacerbate $CD4^+$ T-cell destruction.

Mechanisms of apoptosis during HIV infection can be triggered in several ways. Studies have shown that persistent viral interaction with T-cell receptor as well as gp120–CXCR4 interaction is able to cause apoptosis since it has direct regulation. Moreover, it has been observed that INRs have shorter telomeres and dysregulated telomere activity, leading to higher activation of cell cycle and apoptosis [6]. Increased expression of Fas (receptor) and FasL (ligand), essential to apoptosis pathways, has been found in HIV-infected patients, being regulated by viral proteins such as Nef, Tat and gp120 [25]. In addition, polymorphisms in *Fas* and *FasL* genes have been associated to $CD4^+$ T-cells count. The GG genotype (-124A/G variation at promoter region) of *FasL*, for example, has been demonstrated to be associated with lower $CD4^+$ T-cell counts. Similar outcomes were observed with genetic variations in *TRAIL*, *BIM*, mtDNA and other genes of proteins involved in apoptosis mechanisms [5,16].

Regarding the pyroptosis, inflammasome pathways genes, such as *NLRP3*, *IFI16*, *CASPASE-1*, *IL1B* and *IL18*, have emerged as the main candidates to influence the immunological recovery of ART-treated patients. Transcriptional analyses have demonstrated higher *NLRP3*, *CASP1*, *IL1* and *IL18* expression in INRs [4]. In fact, our research group recently found an association of the rs187238 C>G polymorphism in the promoter region of *IL18* with higher pyroptosis levels of recent thymic emigrant $CD4^+$ cells in INR [26]. Although other types of cell death have already been observed in the HIV infection process, recent studies still point to these as the two main cell death pathways that induce $CD4^+$ T-cell depletion.

Research with lymphoid tissue have demonstrated that cell–cell interactions are also responsible for promoting constant immune activation. Thus, adhesion molecules such as ICAM-1 and LFA-1 that are responsible for T-cell trapping in the lymphoid organs can induce cell death activation in neighboring lymphocytes – a mechanism called virological synapse [8,19]. Furthermore, the IL-1β and IL-18, released by inflammasome activation, also induce the expression of these adhesion molecules on endothelium, promoting the migration of $CD4^+$ T cells to die and produce more inflammation [24].

Therapeutic possibilities for INR

Although suitable therapeutic strategies to satisfactorily improve immune reconstitution in INR have not been achieved, researchers have suggested several possibilities to restore $CD4^+$ T-cell numbers in INRs during ART [3– 6,8]. Some of these already have been implemented, such as ART optimizing, adopting new antiretroviral drugs in first-line ART regimens (dolutegravir and efavirenz in low dose – 400 mg), and early treatment starting regardless of clinical stage and $CD4^+$ cell count as recommended by WHO since 2015 [27]. This has considerably improved treatment outcomes, including immunological recovery in these ART-treated individuals with complete control of plasma viral load.

Most of the therapeutic strategies to restore $CD4^+$ cell count in the HIV-infected INR aim to increase thymus function and peripheral proliferation. Of all suggested possibilities, the administration of recombinant human (rh)IL-2, IL-7 or IL-15 in combination with ART, which could boost the immune system and improve CD4 recovery, have been intensively evaluated [6,14,15].

Persistent immune activation, as mentioned above, is a crucial factor in causing poor $CD4^+$ reconstitution in INR. Although it is not possible to eliminate HIV from reservoir yet [28], several strategies aiming at reducing immune activation have been suggested, such as the use of immunomodulatory agents and first-line ART regimens intensified with raltegravir and/or maraviroc (CCR5–antagonist) [4–6]. Moreover, gene therapy

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To reduce *CCR5* expression has been demonstrated as not only one strategy to decrease immune activation during ART but also a possibility to eradicate reservoirs and prevent new HIV infection [8,28].

Conclusion

Several studies have demonstrated the heterogeneity of patient's immunological recovery during ART, mostly focusing on immune factors that could be regulated by genetic variations. In this context, more studies, mainly with genome-wide approaches and in populations with different genetic backgrounds, should be performed in order to improve the knowledge of the genetic variations impact on immune reconstitution of ART-treated patients.

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References

1. Arts EJ, Hazuda DJ. HIV-1 antiretroviral drug therapy. *Cold Spring Harb. Perspect. Med.* 2(4), a007161 (2012).
2. Tseng A, Seet J, Phillips EJ. The evolution of three decades of antiretroviral therapy: challenges, triumphs and the promise of the future. *Br. J. Clin. Pharmacol.* 79(2), 182–194 (2014).
3. Cenderello G, De Maria A. Discordant responses to cART in HIV-1 patients in the era of high potency antiretroviral drugs: clinical evaluation, classification, management prospects. *Expert Rev. Anti. Infect. Ther.* 14(1), 29–40 (2016).
4. 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 nonresponders. *J. Leukoc. Biol.* 107(4), 597–612 (2020).
5. Corbeau P, Reynes J. Immune reconstitution under antiretroviral therapy: the new challenge in HIV-1 infection. *Blood* 117(21), 5582–5590 (2011).
6. Gaardbo JC, Hartling HJ, Gerstoft J, Nielsen SD. Incomplete immune recovery in HIV infection: mechanisms, relevance for clinical care, and possible solutions. *Clin. Dev. Immunol.* 67(670957), 1–17 (2012).
7. Okoye AA, Picker LJ. $CD4^+$ T-cell depletion in HIV infection: mechanisms of immunological failure. *Immunol. Rev.* 254(1), 54–64 (2013).
8. Pinzone MR, Di Rosa M, Cacopardo B, Nunnari G. HIV RNA suppression and immune restoration: can we do better? *Clin. Dev. Immunol.* 51(515962), 1–12 (2012).
9. Kumar BV, Connors TJ, Farber DL. Human T cell development, localization, and function throughout life. *Immunity* 48(2), 202–213 (2018).
10. Kolte L. Thymic function in HIV-infection. *Dan. Med. J.* 60(4), 44–52 (2013).
11. Carvalho-Silva WHV, Andrade-Santos JL, Souto FO, Coelho VAC, Crovella S, Guimaraes˜ RL. Immunological recovery failure in cART-treated HIV-positive patients is associated with reduced thymic output and RTE $CD4^+$ T cell death by pyroptosis. *J. Leukoc. Biol.* 107(1),85–94 (2020).
12. Li T, Wu N, Dai Y *et al.* Reduced thymic output is a major mechanism of immune reconstitution failure in HIV-infected patients after long-term antiretroviral therapy. *Clin. Infect. Dis.* 53(9), 944–951 (2011).
13. Rb-Silva R, Nobrega C, Azevedo C *et al.* Thymic function as a predictor of immune recovery in chronically HIV-infected patients initiating antiretroviral therapy. *Front. Immunol.* 10(25), 1–13 (2019).
14. Leone A, Picker L, Sodora D. IL-2, IL-7 and IL-15 as immuno-modulators during SIV/HIV vaccination and treatment. *Curr. HIV Res.* 7(1), 83–90 (2009).
15. Hoang TN, Paiardini M. Role of cytokine agonists and immune checkpoint inhibitors toward HIV remission. *Curr. Opin. HIV AIDS* 14(2), 121–128 (2019).
16. Peraire J, Vilades´ C, Pacheco YM *et al.* Evaluation of the pharmacogenetics of immune recovery in treated HIV-infected patients. *Expert Opin. Drug Metab. Toxicol.* 10(1), 81–101 (2014).
17. Haas DW, Geraghty DE, Andersen J *et al.* Immunogenetics of CD4 lymphocyte count recovery during antiretroviral therapy: an AIDS Clinical Trials Group study. *J. Infect. Dis.* 194(8), 1098–1107 (2006).
18. Vijayan KKV, Karthigeyan KP, Tripathi SP, Hanna LE. Pathophysiology of $CD4^+$ T-cell depletion in HIV-1 and HIV-2 infections. *Front. Immunol.* 8(580), 1–8 (2017).
19. Younas M, Psomas C, Reynes J, Corbeau P. Immune activation in the course of HIV-1 infection: causes, phenotypes and persistence under therapy. *HIV Med.* 17(2), 89–105 (2016).

20. Darcis G, Berkhout B, Pasternak AO. The quest for cellular markers of HIV reservoirs: any color you like. *Front. Immunol.* 10, 2251 (2019).

21. Sokoya T, Steel HC, Nieuwoudt M, Rossouw TM. HIV as a cause of immune activation and immunosenescence. *Mediators Inflamm.* 2017, 6825493 (2017).

22. Boulougoura A, Sereti I. HIV infection and immune activation: the role of coinfections. *Curr. Opin. HIV AIDS* 11(2), 191–200 (2016).

23. Singh S, Arora SK. Impact of cytokine gene polymorphism on the HIV-1 disease progression and response to therapy. *J. AIDS Clin. Res.* 6(10), 1000506 (2015).

24. Doitsh G, Greene WC. Dissecting how CD4 T cells are lost during HIV infection. *Cell Host Microbe.* 19(3), 280–291 (2016).

25. Cummins NW, Badley AD. Mechanisms of HIV-associated lymphocyte apoptosis: 2010. *Cell Death Dis.* 1(11), e99 (2010).

26. Andrade-Santos JL, Carvalho-Silva WHV, Coelho AVC *et al. IL18* gene polymorphism and its influence on CD4+ T-cell recovery in HIV-positive patients receiving antiretroviral therapy. *Infect. Genet. Evol.* 75, 103997 (2019).

27. WHO. Consolidated guidelines on the use of antiretroviral drugs for treating and preventing HIV infection: what's new. (2015). https://apps.who.int/iris/bitstream/handle/10665/198064/9789241509893 eng.pdf ?sequence=1

28. Qi J, Ding C, Jiang X, Gao Y. Advances in developing CAR T-cell therapy for HIV cure. *Front. Immunol.* 11(361), 1–13 (2020).

3.2 ARTIGO 2 – *CCR5* GENOTYPE AND PRE-TREATMENT CD4+ T-CELL COUNT INFLUENCE IMMUNOLOGICAL RECOVERY OF HIV-POSITIVE PATIENTS DURING ANTIRETROVIRAL THERAPY

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Short communication

CCR5 genotype and pre-treatment CD4+ T-cell count influence immunological recovery of HIV-positive patients during antiretroviral therapy

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ABSTRACT

This study was performed to assess the association of *CCR5*Δ32 and *SDF1*-3′A polymorphisms with immunological recovery failure and to investigate the influence of sociodemographic and clinical data on immune reconstitution in human immunodeficiency virus (HIV)-positive patients during antiretroviral therapy (ART). Two hundred and forty-eight HIV-positive patients under ART with undetectable plasma viral load (< 40 co-pies/mL) were enrolled in this study and classified into two groups according to their CD4+ T-cell count changes: immunological responders (CD4+ T-cell count gain ≥ 200/μL or ≥ 30% compared with baseline) and immunological non-responders (CD4+ T-cell count gain < 200/μL or < 30% compared with baseline). DNA extraction was performed followed by *CCR5*Δ32 and *SDF1*-3′A genotyping. Sociodemographic and clinical data were evaluated from medical records. The logistic regression model showed that heterozygosity for *CCR5*Δ32 allele and lower pre-treatment CD4+ T-cell count (< 500 cells/μL) were statistically associated with immunological recovery failure (OR = 5.873, 95%CI = 1.204–28.633, $P = 0.028$ and OR = 10.00, 95%CI = 3.224–31.016, $P = 0.028$, respectively). No association of *SDF1*-3′A polymorphism with immune reconstitution failure was found. Additionally, we observed that there was a statistically significant difference between lower CD4+ T-cell count and INR status than the IR group ($Z = 4.687$, $P < 0.001$). Our results demonstrated, through a logistic regression model, that CCR5 32 polymorphism and pre-treatment CD4+ T-cell count have significant influence on immune reconstitution of HIV-positive patients during ART. These findings highlight some immunological factors associated with poor CD4+ T-lymphocytes recovery, which affect immune response level of ART-treated HIV-positive patients.

1. Introduction

Since the discovery of human immunodeficiency virus type 1 (HIV-1), there was an intensive research effort from the worldwide scientific community to develop anti-HIV drugs (Barré-Sinoussi et al., 2013).

Currently, there are several available antiretrovirals used in combination, consisting in the antiretroviral therapy (ART) regimens, which can maximally suppress HIV replication in plasma thus preventing further transmission, disease progression and acquired immunodeficiency syndrome (AIDS)-related deaths (Tseng et al., 2014; WHO, 2016).

Abbreviations: AIDS, acquired immunodeficiency syndrome; ART, antiretroviral therapy; AZT, zidovudine; bp, base pair; CCR5, C-C chemokine receptor 5; CI, confidence interval; CMV, cytomegalovirus; CXCL10, C-X-C chemokine ligand 10; CXCR4, C-X-C chemokine receptor 4; DNA, deoxyribonucleic acid; HBV, hepatitis B virus; HCV, hepatitis C virus; HIV, human immunodeficiency virus; HTLV-I, human T-cell lymphotropic virus types I; HTLV-II, human T-cell lymphotropic virus types II; INR, immunological non-responders; IP-10, induced protein 10; IQR, interquartile range; IR, immunological responders; NNRTI, non–nucleoside reverse transcriptase inhibitor; NRTI, nucleoside reverse transcriptase inhibitor; OR, odds ratio; PCR, polymerase chains reaction; PI/r, ritonavir-"boosted" protease inhibitor; PVL, plasma viral load; RFLP, restriction fragment length polymorphism; SDF-1, stromal-derived factor type 1; TDF, tenofovir; UTR, untranslated region; VDRL, venereal disease research laboratory; wt, wild type

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As virus levels decrease during treatment, it is expected that CD4+ T-cell count return to quasi-normal values in peripheral blood. However, approximately 30% of ART-treated patients do not significantly increase their CD4+ T-cell count even though achieving undetectable plasma viral concentration. These patients are defined as immunological non-responders since they are into a condition known as immunological recovery failure (Cenderello and De Maria, 2016; Corbeau and Reynes, 2011). Some studies have demonstrated two main mechanisms that may result in the poor immunological reconstitution during ART: insufficient production and excessive destruction of CD4+ T cells (Corbeau and Reynes, 2011; Gaardbo et al., 2012). Furthermore, immunological recovery failure has been reported in the literature as a multifactorial condition, having several risk factors involved in poor CD4+ T-lymphocytes reconstitution, such as: Male sex, older age at ART start, lower pre-treatment CD4+ T-cell count, high baseline HIV RNA levels, immune activation, co-infections during therapy and genetic alterations, such as in genes encoding chemokines and chemokine receptors (Pacheco et al., 2014; Pinzone et al., 2012).

The C–C motif chemokine receptor 5 (CCR5), besides being the main HIV-1 coreceptor, is also recognized as a receptor of molecules involved in the co-activation of CD4+ T-lymphocytes, and, thus, it could be involved in the immunological reconstitution (Camargo et al., 2009; Flanagan, 2014; Pinzone et al., 2012). Therefore, several studies have investigated possible associations of *CCR5* polymorphisms with the immune recovery in HIV-infected patients under ART (Pacheco et al., 2014). The most studied *CCR5* genetic polymorphism is a 32 bp deletion (*CCR5*Δ32, rs333) at exon 3, which results in a non-functional CCR5 protein (Arenzana-Seisdedos and Parmentier, 2006; Flanagan, 2014).

The stromal-derived factor (SDF-1) is so far the only ligand known for C-X-C chemokine receptor 4 (CXCR4), another HIV-1 coreceptor widely expressed by immune cells (Arenzana-Seisdedos and Parmentier, 2006; Karin, 2010). Consequently, SDF-1 is a molecule with strong chemotactic activity for lymphocytes and the interaction with its ligand could result in immunomodulation and hematopoiesis (Kucia et al., 2004). A polymorphism located at the 3′-untranslated region (3′UTR) in the *SDF1* gene, a guanine-to-adenine transition (G > A) at position 801 (*SDF1*-3′A, rs1801157), has been described as able to affect the SDF-1 protein production (Garcia-moruja et al., 2009). This polymorphism is presented only in the SDF-1β isoform transcript and its impact, apparently, increases mRNA stability, leading to higher protein production (Arenzana-Seisdedos and Parmentier, 2006; Liu and Zhu, 2011).

Thus, the present report was designed to assess whether there is association of *CCR5*Δ32 and *SDF1*-3′A polymorphisms with the immunological recovery failure and to investigate the influence of socio-demographic and clinical data on immunological reconstitution in HIV-positive patients receiving ART.

2. Material and methods

2.1. Study population

The subjects for this study were recruited at Instituto de Medicina Integral Prof. Fernando Figueira (IMIP) between 2011 and 2016. These patients came from regions of Pernambuco state (Northeast Brazil). General inclusion criteria: age over 18 years, being on ART for at least a year, good adherence to therapy, and achieved prolonged viral suppression (< 40 RNA copies/mL) during the first year of ART. General exclusion criteria: pregnancy and history of intravenous drug use. Sociodemographic and clinical data (see Supplementary Material 1) were collected from medical records. Thus, two hundred and forty-eight patients (107 males and 141 females) meet the criteria, and they were included in this study. All individuals answered standard questionnaires, gave written informed consent and provided blood samples for genetic analysis. The IMIP Research Ethics Committee (protocol number: 3629-13) approved this study.

2.2. Immunological classification of patients

These ART-treated patients, who had persistently undetectable plasma viral loads (< 40 copies/mL) during the first year of ART and maintained it at all clinical appointments, were classified according to CD4+ T-cell count changes. Patients that gained less than 200 CD4+ T-cells/μL during the first year of ART, when compared with pre-treatment count, were defined as immunological non-responders (INR) (adapted from (Li et al., 2011)). If absolute count were not available in medical records, CD4+ T-cell percentages in relation to total lymphocytes were used instead. Thus, if an individual had < 30% of CD4+ T-cells, he/she was also classified as INR. All other patients with CD4+ T-cell count or percentages above these thresholds were defined as immunological responders (IR). Therefore, 126 subjects (62 males and 64 females) were classified as INR, whereas the remaining 122 patients (45 males and 77 females) were classified as IR.

2.3. Genomic DNA preparation

Genomic DNA was extracted from blood samples using mini salting out method (Miller et al., 1988) with “in-house” modifications.

2.4. *CCR5Δ32* and *SDF1-3′A* genotyping

*CCR5*Δ32 genotyping was performed by polymerase chain reaction (PCR) and the products were then loaded in 3% agarose gel electrophoresis with ethidium bromide staining. For SDF1-3′A, genotyping was performed by PCR followed by restriction fragment length polymorphism (RFLP) using restriction enzyme HpaII. The banding patterns was observed in 2% agarose gel electrophoresis. See Supplementary Material 2 for more details.

2.5. Statistical analyses

The allelic and genotypic frequencies of *CCR5*Δ32 and *SDF1*-3′A polymorphisms were assessed by direct counting, and conformity with Hardy-Weinberg equilibrium was evaluated using the X^2 test. Fisher exact test were used to assess whether genetic, sociodemographic and clinical variables were associated with INR status. Wilcoxon-Mann-Whitney test was used to compare the groups for variables that do not follow a normal distribution (according to Shapiro-Wilk test). Cochran-Armitage test was used to assess whether there was any trend in the HIV-infected patients’ distribution in the groups according to pre-treatment CD4+ T-cell count. The variables deemed to have clinical importance or reached a p-value ≤ 0.20 during univariate analysis were included in the logistic regression analysis. The significance level (α) for the tests was set at 0.05. All analyses were performed using R software.

3. Results

3.1. Analysis of sociodemographic and clinical data

No statistical differences were observed when comparing IR and INR groups during univariate analyses regarding sex ($P = 0.055$), age and body mass at ART start date ($P = 0.212$ and $P = 0.293$, respectively), time until ART starting post-diagnosis ($P = 0.101$), ART regimen containing Zidovudine (AZT) versus Tenofovir (TDF) ($P = 1.00$), any ART regimen changes ($P = 0.070$) or pre-treatment plasma viral load ($P = 0.648$). See Table 1 for detailed breakdown. Moreover, the coinfections also had no influence over immunological response (syphilis: $P = 0.663$; CMV: $P = 0.352$ and toxoplasmosis: $P = 0.295$; Supplementary Material 3). Initially, the antiretroviral regimen containing ritonavir- “boosted” protease inhibitor (PI/r)

Table 1
Association tests with sociodemographic and clinical data among the ART-treated groups.

Variables		INR n = 126 (%)	IR n = 122 (%)	*P*
Sex	Male	62 (49.2)	45 (36.9)	0.055[a]
	Female	64 (50.8)	77 (63.1)	
Age (years old) at ART start date, median (IQR)		32.0 (29.0–39.0)	32.0 (28.0–36.0)	0.212[b]
Body mass (kg) at ART start date, median (IQR)		61.5 (55.0–70.5)	64.0 (54.0–75.0)	0.293[b]
Time (months) until ART starting post-diagnosis, median (IQR)		5.0 (1.0–30.0)	11.0 (3.0–29.0)	0.101[b]
PI/r-containing ART (n = 219/248)	Yes	38 (34.2)	52 (48.2)	0.040[a]
	No (NNRTI use instead)	73 (65.8)	56 (51.8)	
AZT-containing ART (n = 212/248)	Yes	92 (86.8)	91 (85.8)	1.00[a]
	No (TDF use instead)	14 (13.2)	15 (14.2)	
ART regimen change (n = 229/248)	Yes	12 (10.1)	4 (3.6)	0.070[a]
	No	107 (89.9)	106 (96.4)	
Pre-treatment CD4+ T-cell count (n = 207/248)	< 500 cells/μL	102 (93.6)	69 (70.4)	< 0.001[a]
	≥500 cells/μL	7 (6.4)	29 (29.6)	
Pre-treatment PVL (log_{10} RNA copies/mL)		4.51 (3.25–5.07)	4.43 (3.71–5.10)	0.648[b]

ART: antiretroviral therapy; AZT: zidovudine; INR: immunological non-responders; IR: immunological responders; IQR: interquartile range; NNRTI: non–nucleoside reverse transcriptase inhibitor; PI/r: ritonavir- "boosted" protease inhibitor; PVL: plasma viral load; TDF: Tenofovir.
[a] Fisher exact test.
[b] Wilcoxon-Mann-Whitney test (Shapiro Wilk: < 0.05).

was statistically associated with a better immunological recovery (OR = 0.562, 95%CI = 0.313–1.00, *P* = 0.040; Table 1). Nevertheless, after it is included in the logistic regression analysis, this statistical significance was lost.

Patients with pre-treatment CD4+ T-cell count < 500 cells/μL presented a six-times higher risk to immunological recovery failure occurrence than individuals with pre-treatment CD4+ T-cell count ≥500 cells/μL (OR = 6.072, 95%CI = 2.428–17.368, *P* < 0.001, Table 1). Additionally, we observed that there was a statistically significant difference between lower CD4+ T-cell count and INR status than the IR group, according to Cochran-Armitage trend test (Z = 4.687, *P* < 0.001). More than 40% of INRs patients group showed a very low pre-treatment CD4+ T-cell count (< 200 cells/μL) whereas they were approximately 20% of IRs patients (Fig. 1).

3.2. Genetic association analysis

The genotype distribution of the *CCR5*Δ32 and *SDF1*-3′A polymorphisms were in conformity to Hardy-Weinberg equilibrium in the IR and INR groups. We observed that among the *CCR5*Δ32-genotyped individuals, 205 (92.3%) did not have the deletion, 17 (7.7%) were heterozygous and no homozygosity for the *CCR5*Δ32 allele was found.

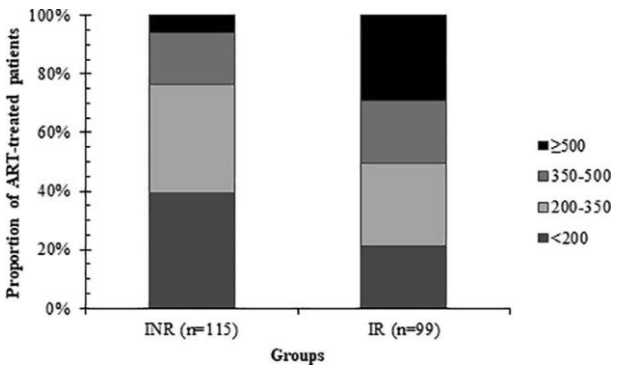

Fig. 1. Comparison of pre-treatment CD4+ T-cells count to immunological response groups. The proportion of ART-treated patients in each group (immunological non-responders (INR) and immunological responders (IR)) in relation to CD4+ T-cell count (cells/μL) stratification. The proportion of patients with very low CD4+ T-cell count (< 200) at ART start date was 40% in INR and only 20% in IR group, showing a significant trend between low CD4+ T-cell count and immunological non-response (Cochran-Armitage test for trend: Z = 4.687; *P* < 0.001).

Table 2
Allele and genotype frequencies of the CCR5 32 and SDF1-3′A polymorphisms, and comparison among ART-treated groups to assess genetic association with immunological recovery failure.

Genes		ART-treated patients		Genetic association (Fisher exact test)	
		INR n (%)	IR n (%)	OR [95% CI]	*P*
CCR5	*Alleles*				
	wt	218 (94.8)	209 (97.7)	2.297	0.140
	32	12 (5.2)	5 (2.3)	[0.737–8.469]	
	Genotypes				
	wt/wt	103 (89.6)	102 (95.3)	*Reference*	
	wt/Δ32	12 (10.4)	5 (4.7)	2.368 [0.743–8.897]	0.132
	Δ32/Δ32	0 (0.0)	0 (0.0)	–	–
SDF1	*Alleles*				
	wt	164 (85.4)	152 (82.6)	0.811	0.484
	3′A	28 (14.6)	32 (17.4)	[0.448–1.464]	
	Genotypes				
	wt/wt	72 (75.0)	64 (69.6)	*Reference*	
	wt/3′A	20 (20.8)	24 (26.1)	0.742 [0.352–1.549]	0.488
	3′A/3′A	4 (4.2)	4 (4.3)	0.890 [0.159–4.983]	1

CI: confidence interval; INR: immunological non-responders; IR: immunological responders; OR: odds ratio; wt: wild type; 3′A: G > A allele at 3′UTR position; Δ32: 32 base-pairs deletion allele.

Although the *CCR5*Δ32 variant allele was more frequent in the INR group (5.2%) than in the IR group (2.3%), this difference was not statistically significant according to univariate analysis (OR = 2.297, 95%CI = 0.747–8.469, *P* = 0.140, Table 2). Similarly, heterozygosity for *CCR5*Δ32 allele was more frequent in immunological non-responders (10.4%) compared with immunological responders (4.7%), but no statistically significant difference in the univariate analysis was observed (OR = 2.368, 95%CI = 0.743–8.897, *P* = 0.132). In relation to *SDF1*-3′A polymorphism, its frequency was 17.4% in the IR group and 14.6% among the non-responders with no statistically significant difference (OR = 0.811, 95%CI = 0.448–1.464, *P* = 0.484). In addition, the heterozygous individuals showed a similar distribution among the ART-treated groups (INR = 20 (20.8%) and IR = 24 (26.1%), *P* = 0.488, Table 2), and homozygosity for the *SDF1*-3′A variant allele was present in four immunological no-responders (4.2%) and in four immunological responders (4.3%).

Table 3
Variables included in a fitted logistic regression model to explain immunological recovery failure.

Variables	Estimate (β)*	OR	95% CI	P
SDF1 wt/wt genotype	−0.6162	0.540	0.235–1.243	0.148
SDF1 wt/3′A genotype	−0.7880	0.455	0.171–1.210	0.114
SDF1 3′A/3′A genotype	−0.5358	0.585	0.098–3.512	0.558
*CCR5*Δ32 allele presence	1.7703	5.873	1.204–28.633	0.028
Male sex	0.4266	1.532	0.816–2.875	0.250
ART regimen change	0.8572	2.357	0.346–16.068	0.381
IP/r-containing ART	−0.3902	0.677	0.328–1.395	0.290
Pre-treatment CD4+ T-cell count (< 500 cells/μL)	2.3026	10.000	3.224–31.016	< 0.001
(intercept)	−1.7438	–	–	0.007

ART: antiretroviral therapy; CI: confidence interval; OR: odds ratio; PI/r: ritonavir-"boosted" protease inhibitor.

* Model's internal validation: AUROC = 0.708; Z = -0.831; P = 0.406.

3.3. Logistic regression analysis

Although some clinical variables have not been associated with immunological recovery failure in this study, they were included in the logistic regression analysis along with associated variables (PI/r-containing ART and lower pre-treatment CD4+ T-cell count) and the *CCR5*Δ32 and *SDF1*-3′A polymorphisms because of their clinical importance and p-values. The results of the logistic regression analysis maintained significant association between lower pre-treatment CD4+ T-cell count (< 500 cells/μL), with ten-times higher risk to immunological recovery failure occurrence (OR = 10.000, 95%CI = 3.224–31.016, $P < 0.001$). In addition, heterozygosity for *CCR5*Δ32 polymorphism was associated with immunological reconstitution failure in this analysis, being linked with a five-times higher risk of poor immune recovery (OR = 5.873, 95%CI = 1.204–28.633, $P = 0.028$; Table 3). The model's internal validation showed that the analysis had good quality and adherence (AUROC = 0.708, Z = -0.831, $P = 0.406$) thus being appropriated to do predictions for immunological recovery failure occurrence.

4. Discussion

Since the advent of ART, survival rate and the quality of life of HIV-infected individuals have dramatically improved because of the powerful effect on viral suppression (Tseng et al., 2014; UNAIDS, 2018). However, despite the efficiency of ART in reducing HIV-1 load to un-detectable levels, significant number of patients fail to restore their peripheral blood CD4+ T-cell count (Cenderello and De Maria, 2016). In this context, the present study sought to assess whether the *CCR5*Δ32 and *SDF1*-3′A polymorphisms along with sociodemographic and clinical data could influence on immunological recovery in HIV-positive patients under ART.

According to clinical data, lower pre-treatment CD4+ T-cell count (< 500 cells/μL) at ART start date was statistically associated with immunological recovery failure in both analyses (univariate and multivariate logistic regression), and it is in agreement with previous studies (He et al., 2015; Hunt et al., 2003; Li et al., 2011; Pinzone et al., 2012). We also observed a statistically significant trend to immunological non-responders group have more HIV-infected patients with lower pre-treatment CD4+ T-cell count than the immunological responders group. Similarly, previous evidence pointed to a negative correlation among pre-treatment cell count. The lower the CD4+ T-cell numbers, the greater will be the time until immunological reconstitution, varying from little as six months to seven years (Kelley et al., 2009). According to some investigators, immune recovery is less apparent in HIV-positive patients who started treatment with severe immunodeficiency (He et al., 2015; Okoye and Picker, 2013). Moreover, some cytokines that stimulate HIV-1 replication and progression to AIDS such as IP-10 (CXCL10), a marker of viremia, have been correlated with low CD4+ T-cell counts in early HIV infection and during treatment (Freeman et al., 2016; Valverde-Villegas et al., 2018), corroborating with our results about the influence of pre-treatment CD4-cell counts on the immunological recovery of ART-treated HIV-positive patients. This poor immune reconstitution rate seems to be consequence of a process induced by HIV infection that limit the T cells production in the thymus and increase mortality rate of CD4+ T cells in the peripheral circulation (Corbeau and Reynes, 2011; Okoye and Picker, 2013). Therefore, when these patients start ART, sometimes with low CD4+ T-cell count as observed in this study, they do not show adequate immunological reconstitution because of their likely thymic exhaustion, a result of long HIV infection without treatment (He et al., 2015). Since these previous investigations have been published, the evidences to support earlier initiation of ART have progressively increased. As a result, in 2015, World Health Organization (WHO) started to re-commend ART initiation as soon as the individuals are diagnosed with HIV/AIDS regardless their CD4+ T-cell count (WHO, 2015). It has reduced morbimortality, disease progression, HIV transmission, and has improved ART outcomes. Although Brazil had adopted this strategy for initiating ART, called treat all, it is still in progress and HIV testing needs to be extended since late diagnosis continues to undermine the impact of treatment efforts (Ministério da Saúde, 2013; UNAIDS, 2018).

Some studies have demonstrated that the lack of expression of CCR5 on the cell membrane, consequence of the 32 bp deletion, influences the immune reconstitution, being associated with insufficient recovery of CD4+ T-cells (Camargo et al., 2009; Dolan et al., 2007). Ahuja et al. (2008), investigating the influence of *CCR5* genotypes on immunological response, observed that HIV-positive patients, carrying the *CCR5*Δ32 allele, showed impaired CD4+ T-cell recovery during treatment, especially when initiated ART at < 350 CD4+ T-cell/μL (Ahuja et al., 2008). Consisting with these findings, the present study observed an association of heterozygosity for *CCR5*Δ32 polymorphism with immunological recovery failure. This suggests that the *CCR5*Δ32 allele presence has significant influence on restoration of CD4+ T-lymphocytes in ART-treated patients since CCR5 have the ability to co-stimulate activation of CD4+ T-cells, inducing T-lymphocytes proliferation and differentiation (Camargo et al., 2009; Pacheco et al., 2014).

In our study, no influence was observed of *SDF1*-3′A allele on immunological recovery. The results concerning *SDF1*-3′A polymorphism and its effect on CD4+ T-cell restoration during ART is still unclear (Pacheco et al., 2014). However, it is believed that since the *SDF1*-3′A allele presence may increase the protein production, this polymorphism, consequently, could induce higher T cells chemoattraction with subsequent activation and proliferation, leading to a rise in peripheral blood CD4+ T-cell count (Nanki and Lipsky, 2000).

Our results demonstrated, through a logistic regression model, that *CCR5*Δ32 allele presence and pre-treatment CD4+ T-cell count influence the immune reconstitution of HIV-positive patients during ART. These findings highlight some immunological factors associated with poor CD4 recovery, which affect immune response level of ART-treated HIV-positive patients. Since the detection *CCR5*Δ32 variation is easy (conventional PCR and agarose gel detection) and cheap (around 3 USD with DNA extraction costs), and based on our findings also considering the literature, we propose routine *CCR5*Δ32 variation genotyping in ART-treated patients.

Credit authorship contribution statement

Wlisses Henrique Veloso Carvalho-Silva: Conceptualization, Formal analysis, Writing - original draft, Writing - review & editing. **José Leandro Andrade-Santos**: Methodology, Writing - review & editing. **Maria Carolina dos Santos Guedes**: Investigation, Writing - review & editing. **Sergio Crovella**:. **Rafael Lima Guimarães**: Conceptualization, Writing - review & editing.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.gene.2020.144568.

References

Ahuja, S.K., Kulkarni, H., Catano, G., Agan, B.K., Camargo, F.J., He, W., Connell, R.J.O., Marconi, V.C., Eron, J., Clark, R.A., Frost, S., Martin, J., Ahuja, S.S., Deeks, S.G., Little, S., Richman, D., Hecht, F.M., Dolan, M.J., 2008. CCL3L1-CCR5 genotype inffluences durability of immune recovery during antiretroviral therapy of HIV-1-in-fected individuals. Nat. Med. 14, 413–420. https://doi.org/10.1038/nm1741. CCL3L1-CCR5.

Arenzana-Seisdedos, F., Parmentier, M., 2006. Genetics of resistance to HIV infection: role of co-receptors and co-receptor ligands. Semin. Immunol. 18, 387–403. https://doi.org/10.1016/j.smim.2006.07.007.

Barré-Sinoussi, F., Ross, A.L., Delfraissy, J.-F., 2013. Past, present and future: 30 years of HIV research. Nat. Rev. Micro. 11, 877–883. https://doi.org/10.1038/nrmicro3132.

Camargo, J.F., Quinones, M.P., Mummidi, S., Srinivas, S., Gaitan, A., Begum, K., Jimenez, F., Vancompernolle, S., Unutmaz, D., Ahuja, S.S., Ahuja, S.K., 2009. CCR5 Expression levels influence NFAT translocation, IL-2 production, and subsequent signaling events during T lynphocyte activation. J. Immunol.

Cenderello, G., De Maria, A., 2016. Discordant responses to cART in HIV-1 patients in the era of high potency antiretroviral drugs: Clinical evaluation, classification, manage-ment prospects. Expert Rev. Anti. Infect. Ther. 14, 29–40. https://doi.org/10.1586/14787210.2016.1106937.

Corbeau, P., Reynes, J., 2011. Immune reconstitution under antiretroviral therapy: the new challenge in HIV-1 infection. Blood 117, 5582–5590. https://doi.org/10.1182/ blood-2010-12-322453.

Dolan, M.J., Kulkarni, H., Camargo, J.F., He, W., Smith, A., Anaya, J.-M., Miura, T., Hecht, F.M., Mamtani, M., Pereyra, F., Marconi, V., Mangano, A., Sen, L., Bologna, R., Clark, R.A., Anderson, S.A., Delmar, J., O'Connell, R.J., Lloyd, A., Martin, J., Ahuja, S.S., Agan, B.K., Walker, B.D., Deeks, S.G., Ahuja, S.K., 2007. CCL3L1 and CCR5 influence cell-mediated immunity and affect HIV-AIDS pathogenesis via viral entry-independent mechanisms. Nat. Immunol. 8, 1324–1336. https://doi.org/10.1038/ni1521.

Flanagan, C.A., 2014. Receptor conformation and constitutive activity in CCR5 chemokine receptor function and HIV infection. In: Advances in Pharmacology. Elsevier Inc., pp. 215–263. https://doi.org/10.1016/B978-0-12-417197-8.00008-0.

Freeman, M.L., Shive, C.L., Nguyen, T.P., Younes, S.-A., Panigrahi, S., Lederman, M.M., 2016. Cytokines and T-cell homeostasis in HIV infection. J. Infect. Dis. 214, S51–S57. https://doi.org/10.1093/infdis/jiw287.

Gaardbo, J.C., Hartling, H.J., Gerstoft, J., Nielsen, S.D., 2012. Incomplete immune recovery in HIV infection: mechanisms, relevance for clinical care, and possible solutions. Clin. Dev. Immunol. 67, 1–17. https://doi.org/10.1155/2012/670957.

Garcia-moruja, C., Rueda, P., Torres, C., Alcamí, J., Luque, F., Caruz, A., 2009. Molecular phenotype of CXCL12B 3'UTR G181A polymorphism (rs1801157) associated to HIV-1 disease progression polymorphism. Curr. HIV Res. 7, 384–389. https://doi.org/10.2174/157016209788680543.

He, S., Zhang, Z., Fu, Y., Qin, C., Li, S., Han, X., Xu, J., Liu, J., Jiang, Y., Shang, H., 2015. Thymic function is most severely impaired in chronic HIV-1 infection, but individuals with faster disease progression during early HIV-1 infection expressed lower levels of RTEs. JAIDS 70, 472–478. https://doi.org/10.1097/QAI.0000000000000801.

Hunt, P.W., Deeks, S.G., Rodriguez, B., Valdez, H., Shade, S.B., Abrams, D.I., Kitahata, M.M., Krone, M., Neilands, T.B., Brand, R.J., Lederman, M.M., Martin, J.N., 2003. Continued CD4 cell count increases in HIV-infected adults experiencing 4 years of viral suppression on antiretroviral therapy. AIDS 17, 1907–1915. https://doi.org/10. 1097/00002030-200309050-00009.

Karin, N., 2010. The multiple faces of CXCL12 (SDF-1α) in the regulation of immunity during health and disease. J. Leukoc. Biol. 88, 463–473. https://doi.org/10.1189/jlb. 0909602.

Kelley, C.F., Kitchen, C.M.R., Hunt, P.W., Rodriguez, B., Hecht, F.M., Kitahata, M., Crane, H.M., Willig, J., Mugavero, M., Saag, M., Martin, J.N., Deeks, S.G., 2009. Incomplete peripheral CD4+ cell count restoration in HIV-infected patients receiving long-term antiretroviral treatment. Clin. Infect. Dis. 48, 787–794. https://doi.org/10.1086/ 597093.

Kucia, M., Jankowski, K., Reca, R., Wysoczynski, M., Bandura, L., Allendorf, D.J., Zhang, J., Ratajczak, J., Ratajczak, M.Z., 2004. CXCR4-SDF-1 signalling, locomotion, che-motaxis and adhesion. J. Mol. Histol. 35, 233–245.

Li, T., Wu, N., Dai, Y., Qiu, Z., Han, Y., Xie, J., Zhu, T., Li, Y., 2011. Reduced thymic output is a major mechanism of immune reconstitution failure in HIV-infected patients after long-term antiretroviral therapy. Clin. Infect. Dis. 53, 944–951. https://doi.org/10.1093/cid/cir552.

Liu, S., Zhu, H., 2011. Association between polymorphism of SDF1 (CXCL12) gene and HIV-1 susceptibility: a meta-analysis. Curr. HIV Res. 9, 112–119. https://doi.org/10.2174/157016211795569113.

Miller, S.A., Dykes, D.D., Polesky, H.F., 1988. A simple salting out procedure for extracting DNA from human nucleated cells. Nucleic Acids Res. 16 (3), 1215. https://doi.org/10.1093/nar/16.3.1215.

Ministério da Saúde, 2013. Protocolo Clínico e Diretrizes Terapêuticas para Manejo da Infecção pelo HIV em Adultos. Ministério da Saúde-Secretaria Vigilância em Saúde.

Nanki, T., Lipsky, P.E., 2000. Cutting edge: stromal cell-derived factor-1 is a costimulator for CD4+ T cell activation. J. Immunol. 164, 5010–5014. https://doi.org/10.4049/JIMMUNOL.164.10.5010.

Okoye, A.A., Picker, L.J., 2013. CD4(+) T-cell depletion in HIV infection: mechanisms of immunological failure. Immunol. Rev. 254, 54–64. https://doi.org/10.1111/imr. 12066.

Pacheco, Y.M., Peraire, J., Vilade, C., Domingo, P., Gutie, M., Rosado, I., Vidal, F., Leal, M., 2014. Evaluation of the pharmacogenetics of immune recovery in treated HIV-infected patients. Expert Opin. Drug Metab. Toxicol. 10, 81–101.

Pinzone, M.R., Di Rosa, M., Cacopardo, B., Nunnari, G., 2012. HIV RNA suppression and immune restoration: can we do better? Clin. Dev. Immunol. 51, 1–12. https://doi.org/10.1155/2012/515962.

Tseng, A., Seet, J., Phillips, E.J., 2014. The evolution of three decades of antiretroviral therapy: challenges, triumphs and the promise of the future. Br. J. Clin. Pharmacol. 79, 182–194. https://doi.org/10.1111/bcp.12403.

UNAIDS, 2018. UNAIDS DATA 2018. Jt. United Nations Program. HIV/AIDS. Valverde-Villegas, J.M., de Medeiros, R.M., Ellwanger, J.H., Santos, B.R., de Melo, M.G., de Almeida, S.E.M., Chies, J.A.B., 2018. High CXCL10/IP-10 levels are a hallmark in the clinical evolution of the HIV infection. Infect. Genet. Evol. 57, 51–58. https://doi.org/10.1016/j.meegid.2017.11.002.

WHO, 2016. Consolidated Guidelines on the Use of Antiretroviral Drugs for Treating and Preventing HIV Infection: Recommendations for a Public Health Approach. World Health Organization.

WHO, 2015. Guidelines Guideline on When to Start Antiretroviral Therapy and on Pre-Exposure Prophylaxis for HIV, World Health Organization. https://doi.org/ 9789241509565.

Supplementary Material 1

Sociodemographic and clinical data collected from medical records: Age and body mass at ART start date; time until ART starting after HIV infection test for diagnosis, ART regimens received (2 nucleoside reverse transcriptase inhibitor (NRTI) + ritonavir-“boosted” protease inhibitor (PI/r) or 2 NRTI + non-nucleoside reverse transcriptase inhibitor (NNRTI) regimens, as recommended by the Brazilian guidelines available at the time of patient recruitment (Ministério da Saúde, 2013)); pre- and post-treatment viral loads as well as CD4+ T-cell counts; and serological data regarding co-infections (hepatitis B virus – HBV, hepatitis C virus – HCV, syphilis, cytomegalovirus – CMV, toxoplasmosis, and human T-cell lymphotropic virus types I and II (HTLV-I/II)).

Ministério da Saúde, 2013. Protocolo Clínico e Diretrizes Terapêuticas para Manejo da Infecção pelo HIV em Adultos. Ministério da Saúde-Secretaria Vigilância em Saúde.

Supplementary Material 2

*CCR5*Δ32 and *SDF1*-3'A polymorphisms genotyping

The Supplementary Table S1 summarize primer sequences and reaction conditions of the polymorphisms genotyping assay, including banding patterns of each genotype.

Supplementary Table S1. Primer sequences and reaction conditions for CCR5Δ32 and SDF1-3'A genotyping.

Polymorphisms / Primers	Ta (ºC)	PCR product	Restriction enzyme	Genotype
*CCR5*Δ32				wt/wt: 185 (bp)
F: GTCTTCATTACACCTGCAGCTCT	65	185pb	-	wt/Δ32: 185 + 153 (bp)
R: CACAGCCCTGTGCCTCTT				Δ32/Δ32: 153 (bp) *not observed*
SDF1-3'A				wt/wt: 303 (bp)
F: AGCTTTGGTCCTGAGAGTCC	50	303pb	HpaII	wt/3'A: 303 + 203 (bp)
R: CAGTCAACCTGGGCAAAGCC				3'A/3'A: 303 + 203 + 100 (bp)

Ta: anneling temperature; wt: wild type; pb: base pair.

*CCR5*Δ32

The ART-treated patients were genotyped for *CCR5*Δ32 using polymerase chain reaction (PCR). The PCR was performed with the following primers: 5'-GTCTTCATTACACCTGCAGCTCT-3' (forward) e 5'-CACAGCCCTGTGCCTCTT-3' (reverse). The final volume reaction was 25-µL containing: 2µL genomic DNA solution (100ng), 2.5µL 10x PCR buffer, 0.8µL dNTPS (2mM), 0.4µL forward primer (10µM), 0.4µL reverse primer (10µM), 1µL $MgCl_2$ (25mM), 0.2µL Taq polymerase (5U/µL, Affymetrix USB products) and 17.7µL ultrapure water. The temperature cycling conditions were: initial denaturation at 95ºC for 10 minutes, followed by 38 cycles (denaturation at 95ºC for 30s, annealing at 65ºC for 30s and extension at 72ºC for 60s) and 7 minutes at 72ºC for final extension. The PCR products were then loaded in 3% agarose gel electrophoresis with ethidium bromide staining (10mg/mL of gel buffered solution). See Figure S1 and Table S1 for banding patters of *CCR5* genotypes.

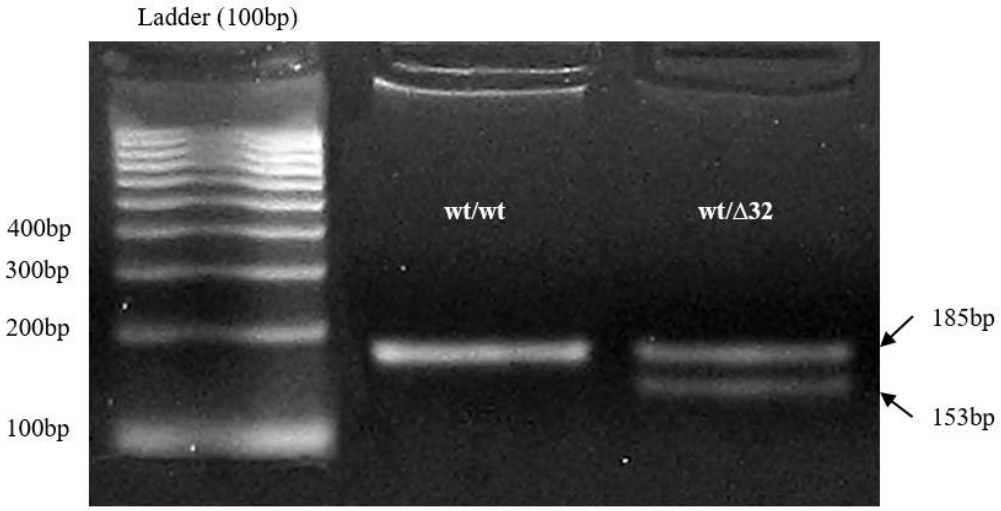

Figure S1. Polymerase chain reaction assay for analyzing the CCR5Δ32 polymorphism. The PCR products were separated in 3% agarose gel electrophoresis. It was possible to observe the following genotypes: wt/wt (wild-type homozygous) and wt/Δ32 (heterozygous). Homozygous for 32bp deletion was not observed in the studied population. A 100 base pair ladder (molecular markers) was used.

***SDF1*-3'A**

The SDF1-3'A polymorphism was genotyped by PCR-RFLP (polymerase chain reaction-restriction fragment length polymorphism. The PCR primers for amplification were: 5'-AGCTTTGGTCCTGAGAGTCC-3' (forward) and 5'-CAGTCAACCTGGGCAAAGCC-3' (reverse). The final PCR volume reaction was 25μL, containing: 2μL genomic DNA solution (100ng), 2.5μL 10x PCR buffer (Tris-HCL (350mM), KCL (250mM) and $MgCl_2$ (35mM)), 2.0μL dNTPS (2mM); 0.3μL primer forward (10 μM), 0.3μL primer reverse (10 μM), 0.2μL Taq polymerase (50U/μL, Uniscience Co.) and 17.7μL ultrapure water. For the cycling conditions: initial denaturation at 95°C for 10 minutes, followed by 40 cycles (denaturation at 95°C for 30s, annealing at 50°C for 30s and extension at 72°C for 30s), and 7 minutes at 72 ° C for final extension. The PCR products were confirmed by electrophoresis in 2% agarose gel with ethidium bromide staining (10mg/mL of gel buffered solution). The amplified fragments were formed by 303 bp.

The PCR products was then cleaved by restriction enzyme HpaII (50U/μL, New England BioLabs), which loss its sensitivity to the restriction site because of the nucleotide change (G>A). The final volume reaction for digestion was 10 μL: 2.0μL CutSmart Buffer (10x), 0.2μl HpaII and 7.8 μL ultrapure water. The cycling conditions

were according to the manufacturer's instructions (50min at 37°C for digestion followed by 20min at 80°C for inactivation). The digested products were loaded in 2% agarose gel electrophoresis with ethidium bromide staining. See Figure S2 and Table S1 for banding patters of *SDF1* genotypes.

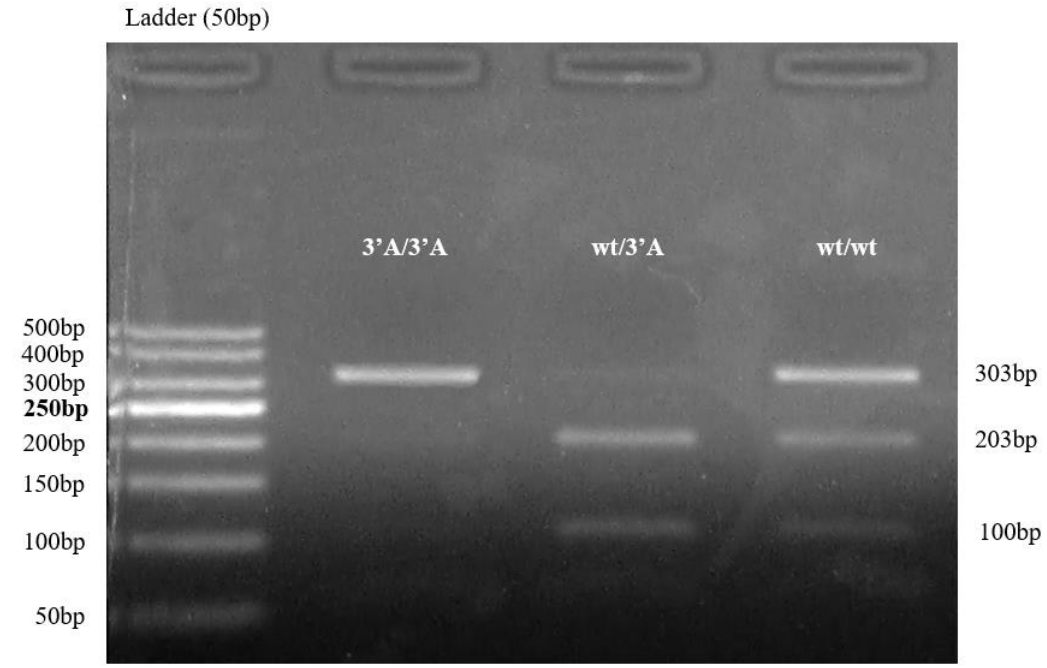

Figure S2. Polymerase chain reaction–restriction fragment length polymorphism assay for analyzing the SDF1-3'A polymorphism. The PCR products were digested by restriction enzyme HpaII and separated in 2% agarose gel electrophoresis. It was possible to observe the following genotypes: 3'A/3'A (variant homozygous), wt/3'A (heterozygous) and wt/wt (wild-type homozygous). A 50 base pair ladder (molecular markers) was used.

Supplementary Material 3

Coinfections

The patients did not present active hepatitis B virus (HBV) infection (3.2% (INR) and 1.6% (IR) were immune by natural infection; 22.2% and 31.2% were immune by vaccination in INR and IR groups, respectively; and 22.2% (INR) and 14.8% (IR) were susceptible – did not present evidence for contact with HBV). Furthermore, no patients were co-infected by hepatitis C virus (HCV) or human T-cell lymphotropic virus types I and II (HTLV-I/II). Evidence for syphilis infection was present in 10.3% (n=13) of patients in the INR group and 8.2% (n=10) in the IR group, as detected by VDRL test. Notably, past coinfections with cytomegalovirus (CMV) and toxoplasmosis were more frequent in immunological responders (15.6% and 12.3%, respectively) compared to immunological non-responders (11.1% and 7.9%, respectively), as revealed by immunoglobulin G tests. However, the coinfections had no influence over immunological response in this study (syphilis: *P*=0.663; CMV: *P*=0.352 and toxoplasmosis: *P*=0.295, Supplementary Table).

Supplementary Table S2. Coinfections serology status of HIV-positive patients under ART enrolled in our study.

Coinfections Serology Status	INR n=126 (%)	IR n=122 (%)	*P* *
Syphilis (VDRL test)	13 (10.3)	10 (8.2)	0.663
Toxoplasmosis[b] (IgG test)	10 (7.9)	15 (12.3)	0.295
CMV[b] (IgG test)	14 (11.1)	19 (15.6)	0.352

* Fisher exact test.

[b] Chronic infection – Immunoglobulin G tests.

CMV: cytomegalovirus; INR: immunological non-responders; IR: immunological responders; VDRL: venereal disease research laboratory.

3.3 ARTIGO 3 – IMMUNOLOGICAL RECOVERY FAILURE IN cART-TREATED HIV-POSITIVE PATIENTS IS ASSOCIATED WITH REDUCED THYMIC OUTPUT AND RTE CD4+ T CELL DEATH BY PYROPTOSIS

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ARTICLE

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Immunological recovery failure in cART-treated HIV-positive patients is associated with reduced thymic output and RTE CD4+ T cell death by pyroptosis

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Abstract

Despite more than three decades of studies and advances in combination antiretroviral therapy (cART) against human immunodeficiency virus (HIV), the mechanisms that precisely determine immune reconstitution failure have not been completely elucidated yet. Thus, this study aimed to investigate the thymic function, immune activation, and cell death by pyroptosis and apoptosis in virologically suppressed HIV-positive patients receiving cART. Immunophenotyping analyses were performed in 57 cART-treated HIV-infected patients with undetectable plasma viral load, who were classified as immunological nonresponders (INR = 29) and immunologic responders (IR = 28). Sociodemographic and clinical data were also assessed from medical records. Twelve healthy volunteers were also included in this study. The INR showed lower pretreatment CD4+ T cell count that remained low even after 1 yr of treatment, lower CD4/CD8 ratio, lower per-centage of recent thymic emigrant (RTE) CD4+ T cell (CD45RA+CD31+) and naïve CD4+ T cell (CD45RA+CD62L+), higher levels of effector memory CD4+ T cells (CD45RA-CD62L-), and higher pyroptosis levels of RTE CD4+ T cells (CD31+FLICA-Caspase1+) when compared with IR. Our findings indicate that reduced thymic function and RTE CD4+ T cell death by pyroptosis are the major mechanisms of immunological recovery failure in HIV-infected patients receiving cART.

KEYWORDS
AIDS, antiretroviral therapy, HIV-1, poor CD4+ T cell reconstitution, thymic function

1 | INTRODUCTION

Combination antiretroviral therapy (cART) has revolutionized the treatment against human immunodeficiency virus type 1 (HIV-1) infection by reducing plasma viral concentration to undetectable levels (<50 RNA copies/mL).[1,2] Therefore, cART has prevented further trans-mission, disease progression, and AIDS-related deaths.[3,4]

Currently, there are several available anti-HIV drugs used in combination to maximally suppress HIV replication.[5] As virus levels decrease during treatment, it is expected that CD4+ T cell count returns to quasi-normal values in peripheral blood. However, even though achieving suppressed plasma viral load, 15–30% of patients who start cART do not significantly increase their CD4+ T cell count.[6–8] This condition is defined as immunological recovery failure because these patients are known as virological responders but immunological nonresponders (INR).[9–11]

Despite more than three decades of studies and advances in anti-HIV therapy, the mechanisms that precisely determine immune reconstitution failure have not been completely elucidated yet, consisting one of the main areas of research on HIV/AIDS. [3,12,13] To date, immunological recovery failure has been reported as a multifactorial condition, having several risk factors involved in this poor recovery of CD4+ T cells, such as male sex, older age at cART start, advanced HIV infection with lower pretreatment CD4+ T cell count, coinfections during therapy, and genetic background.[6,8,10,11,14]

Furthermore, dysregulation of CD4+ T cell homeostasis is a determining factor for immunological recovery in cART-treated patients, and it is regulated by a dynamic balance between CD4+

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T-lymphocytes destruction, production and trafficking between peripheral circulation and lymphoid organs. [11,13] Thus, If the cell destruction exceeds production or there is reduced production, the CD4+ T cell count decrease. [15,16] Some studies have demonstrated that poor immune restoration during cART is linked to two main processes: insufficient CD4+ T-lymphocytes production, represented by reduced recent thymic emigrants (RTE), and/or excessive destruction of CD4+ T cells, mainly by pyroptosis, a highly inflammatory form of programmed cell death that is induced in HIV-1 infection via inflammatory caspase-1.[11,15,17,18]

For this reason, it is of major importance to study and understand these various factors involved in immunological recovery to propose an appropriate therapy to HIV-infected individuals or improve the treatment for INR. Therefore, in order to shed light on the mechanisms of poor CD4+ T cell reconstitution, we carried out immunophenotypic analyses of T cell subsets to investigate the thymic function, immune activation, and cell death by apoptosis and pyroptosis in virologically suppressed HIV-positive patients receiving cART. In addition, because it has been shown that INR may present alterations in the thymic output and destruction of several CD4+ T cell subset, we also evaluated cell death processes in these recent emigrants from the thymus.

2 | METHODS

2.1 | Study population

Fifty-seven HIV-1 positive patients (25 males and 32 females) were recruited at Instituto de Medicina Integral Prof. Fernando Figueira (IMIP) between 2016 and 2018. These patients came from regions of Pernambuco state (northeast Brazil). The general inclusion criteria were age over 18 yr, being on cART for at least a year, good adherence to therapy, and having achieved prolonged viral suppression (<50 copies/mL) during the first year of treatment. The general exclusion criteria were pregnancy and history of intravenous drug use. Sociodemographic and clinical data were collected from medical records: age and body mass at cART start date; time until cART start after HIV infection tests; cART regimens received (2 nucleoside reverse transcriptase inhibitor (NRTI) + protease inhibitor/ritonavir (IP/r) or 2 NRTI + nonnucleoside reverse transcriptase inhibitor (NNRTI) regimens, as recommended by the Brazilian guidelines available at the time of patient recruitment[19]); pre- and post-treatment viral loads as well as CD4+ T cell counts; and serological data regarding co-infections (hepatitis B virus—HBV; hepatitis C virus—HCV; syphilis, cytomegalovirus—CMV; toxoplasmosis, human T cell lymphotropic virus types I and II [HTLV-I/II]). In addition, 12 HIV-negative, clinically healthy volunteers, were also included in this study as control group (see Supplementary Data, Table S1). All individuals answered standard questionnaires, gave written informed consent and provided blood samples for immunological analysis. The IMIP Research Ethics Committee approved this study (protocol number: 3629-13).

2.2 | Patient classification

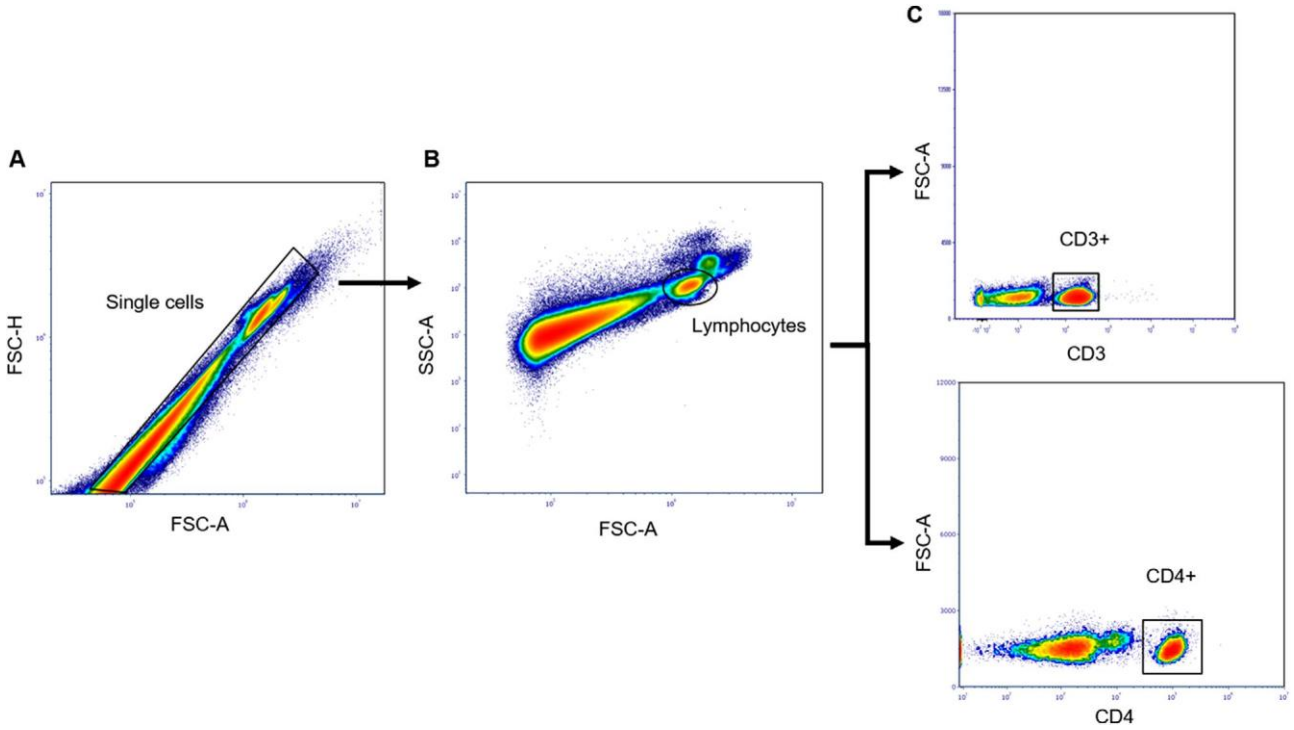
These 57 patients (25 males and 32 females), who had persistently undetectable plasma viral loads (<50 copies/mL) during the first year of cART, were classified according to CD4+ T cell count changes. Patients who gained <200 CD4+ T cells/μL when compared with pretreatment count or presented T cell percentages in relation to total lymphocytes <20% (if absolute counts were not available) after the first year of treatment were classified as INR (adapted from Li et al.[9]). Consequently, all other patients with CD4+ T cell count or percentages above these thresholds were defined as immunological responders (IR; CD4+ T cell count ≥200/μL or ≥20% compared with baseline). Therefore, 29 individuals (17 males and 12 females) were classified as INR, and the remaining 28 patients (8 males and 20 females) were classified as IR.

2.3 | Peripheral blood mononuclear cell isolation

Blood samples were collected from all subjects in EDTA tubes (4 mL). Peripheral blood mononuclear cells (PBMC) were separated by Ficoll-Paque Plus density gradient centrifugation and washed twice in PBS (1x) then resuspended in FACS buffer (3% BSA, 0.01% NaN3—sodium azide, PBS 1×). The number of viable leukocytes was determined by the Trypan blue (0.4%) exclusion test. The cellular viability was >90% in average.

2.4 | Flow cytometric analysis

PBMCs were stained with combinations of different fluorescent monoclonal antibodies, washed, fixed with PBS-formaldehyde 1%, and then analyzed by flow cytometry using an Accuri C6 cytometer (BD Biosciences). For pyroptosis detection (a caspase-1-dependent cell death pathway in HIV-1 infection), we employed FAM-FLICA Caspase-1 (FAM-YVAD-FMK) Assay kit (containing propidium iodide—PI) following the manufacturer's instructions (ImmunoChemistry Technologies). In each analysis, 50,000 events were acquired and gated (Fig. 1) to detect the following T lymphocytes subsets: CD4+ T cells (CD4+CD3+), CD8+ T cells (CD8+CD3+), activated CD8+ T cells (CD8+CD3+CD38+), RTE CD4+ T cells (CD4+CD45RA+CD31+), naive CD4+ T cells (CD4+CD45RA+CD62L+), central memory CD4+ T cells (CD4+CD45RA-CD62L+), effector memory CD4+ T cells (CD4+CD45RA-CD62L-), and effector CD4+ T cells (CD4+CD45RA+CD62L-). Moreover, we also detected apoptotic CD4+ T cells (early: CD4+AnnexinV+PI-and late: CD4+AnnexinV+PI+), pyroptotic T cells (CD4+FLICA-Caspase1+),[20] and dead RTE CD4+ T cells by apoptosis (early: CD4+CD31+AnnexinV+PI- and late: CD4+CD31+AnnexinV+PI+) and by pyroptosis (CD4+CD31+FLICA-Caspase1+). Immunofluorescent monoclonal antibodies FITC-CD3, APC-CD4, BB515-CD4, PE-CD31, PercpCy5.5-CD45RA, APC-CD62L, PECy7-CD8, PE-CD38, and FITC-AnnexinV were obtained from BD Biosciences. Acquired data were analyzed using FCS Express 6 Plus software.



F I G U R E 1 Representative flow cytometry plots illustrating the gating strategy for T-lymphocytes. (**A**) Initial gating was performed to identify single cells. (**B**) Lymphocytes were selected from singles cells based on forward scatter (FSC) and side scatter (SSC). (**C**) Cells expressing CD3+ and CD4+ were selected to further analyses.

2.5 | Statistical analyses

Shapiro-Wilk test was used to check whether variables followed a normal distribution and were displayed as mean ± SD and means of groups were compered using Student's *t*-test for independence. Similarly, Wilcoxon-Mann-Whitney test was used to compare two groups for variables, which did not follow a normal distribution, displaying values as median and interquartile range (IQR). Categorical variables (sociodemographic and clinical data) were assessed through Fisher's exact test. Correlation coefficients for normal variables were calculated by Pearson's correlation test. Linear regression analysis was performed to correct possible confounding factors in relation to thymic output. The significance level (α) for all tests was set at 0.05, and tests were 2-tailed. All analyses were performed using R software.

3 | RESULTS

3.1 | Analysis of sociodemographic and clinical data

We observed that men were more frequent in the INR group (58.6%) than among IR (28.6%), showing a 3 times higher risk of immunological recovery failure compared to women (OR = 3.46; 95%CI = 1.04– 12.45; *P* = 0.033). Age at cART start date was slightly different in the groups (means: 36 yr old—INR group and 33 yr old—IR group), but it was not statistically significant (*P* = 0.151). Both cART-treated groups presented similar pretreatment plasma viral load (INR = 4.13 $\log_{10}$copies/mL and IR = 4.03 $\log_{10}$copies/mL, *P* = 0.772). Furthermore, there were no statistical differences regarding body mass at cART start data, time to cART starting, NNRTI versus PI/r-based cART regimens, zidovudine- versus tenofovir-containing regimens or any cART regimen changes (such as switching from an NNRTI drug to a PI/r drug). The coinfections also had no influence over immunological response in this study (see Supplementary Data, Table S2).

HIV-infected patients with lower pretreatment CD4+ T cell count (<350 cells/μL) presented a 4-fold higher risk to immunological recovery failure occurrence than individuals with pretreatment CD4+ T cell count ≥350 cells/μL (OR = 4.71; 95%CI = 1.37–7.94; *P* = 0.007). Additionally, we observed that there was a statistically significant difference of CD4+ percentage between the two groups (*P* = 0.010), showing higher CD4% in IR group (25.0%) than in the INR group (19.9%). These cART-treated patients with poor immunological recovery were also characterized by significantly lower CD4+ T cell count (INR = 346 cells/μL vs. IR = 528 cells/μL, *P* < 0.001) and lower CD4/CD8 ratio (INR = 0.47 vs. IR = 0.70, *P* = 0.020) even after 1 yr of treatment. Table 1 summarizes the sociodemographic and clinical characterization of the cART-treated groups.

3.2 | Thymic output and T cell subsets

Regarding thymic function, we analyzed the RTE CD4+ T cells subset quantifying CD31+ percentage (CD4+/CD45RA+CD31+, Fig. 2A) and naïve CD4+ T cells (CD4+/CD45RA+CD62L+, Fig. 2C). CD31% was significantly lower in the INR group than in the IR group (*P* = 0.001, Fig. 2B), indicating a lower frequency of RTE cells in these cART-treated patients with immunological recovery failure. Noticeably, the CD31% in

TABLE 1 Sociodemographic and clinical characteristics of cART-treated HIV-positive patients enrolled in our study

Variables		INR *n* = 29 (%)	IR *n* = 28 (%)	*P*
Sex	Male	17 (58.6)	8 (28.6)	**0.033**
	Female	12 (41.4)	20 (71.4)	
Age (years old) at cART start date, mean ± SD*		36.1 ± 8.4	33.2 ± 8.4	0.151
Body mass (kg) at cART start date, mean ± SD*		61.8 ± 13.9	64.6 ± 14.0	0.221
Time (weeks) to cART starting, median (IQR)**		14.5 (5.0–105.0)	34.0 (8.0–138.5)	0.328
PI/r-containing cART (n = 49/57)	Yes	11 (47.8)	14 (53.8)	0.778
	No (NNRTI instead)	12 (52.2)	12 (46.2)	
AZT-containing ART (n = 49/57)	Yes	22 (95.6)	24 (92.3)	1.00
	No (TDF instead)	1 (4.4)	2 (7.7)	
cART regimen change[a]		5 (17.2)	0 (0.0)	0.051
Pretreatment CD4+ T cell count	<350 cells/μL	22 (75.9)	11 (39.3)	**0.007**
	≥350 cells/μL	7 (24.1)	17 (60.7)	
Most recent CD4+ T cell count**		346 (260–458)	528 (346–741)	**<0.001**
CD4/CD8 ratio**		0.47 (0.30–0.65)	0.70 (0.46–0.90)	**0.020**
Most recent CD4+ (%)*		19.9 ± 7.7	25.0 ± 9.6	**0.010**
Most recent CD8+ (%)*		24.5 ± 11.9	22.3 ± 9.3	0.413
Pretreatment PVL (log10 RNA copies/mL)*		4.13 ± 1.12	4.03 ± 1.54	0.772

**t*-test (Shapiro-Wilk test: > 0.05). ***Wilcoxon-Mann-Whitney test (Shapiro-Wilk: <0.05).
[a]Change from a NNRTI-containing cART to a PI/r-containing cART regimen.
AZT: zidovudine; cART: combination antiretroviral therapy; INR: immunological nonresponders; IR: immunologic responders; IQR: interquartile range; NNRTI: nonnucleoside reverse transcriptase inhibitor; PI/r: ritonavir-"boosted" protease inhibitor; PVL: plasma viral load; TDF: tenofovir.

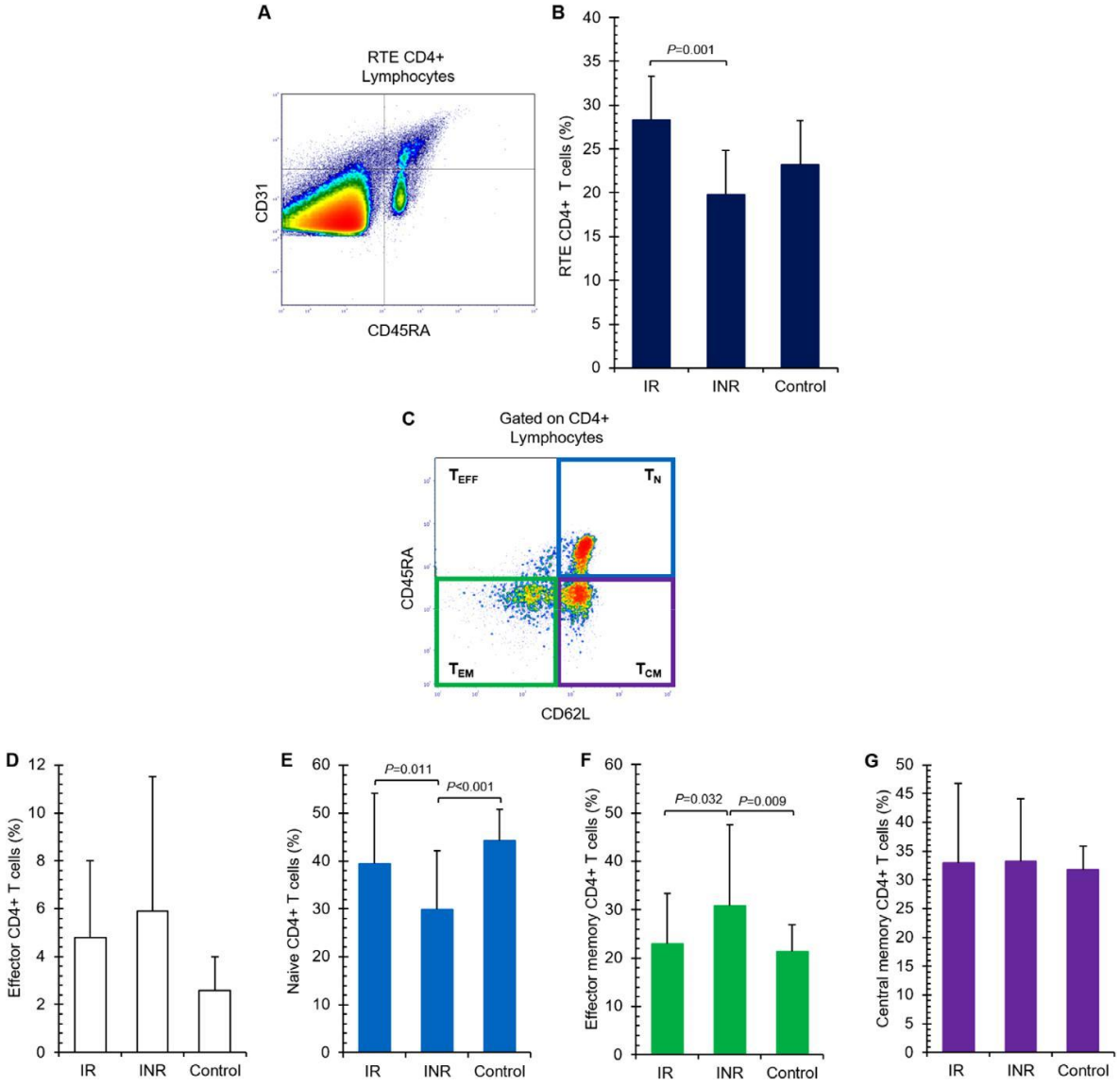
control group (23.2 ± 5.1%) was closer to INR group (20 ± 4.5%) than to IR group (28.3 ± 4.8%) with no statistically significant differences (control vs. IR: $P = 0.161$ and control vs. INR: $P = 0.324$).

After gated on CD4+ cells, we performed analyses on gates of CD45RA and CD62L CD4+ T cell subsets (Fig. 2C). The CD4+ T-lymphocytes repertoire among the three groups mainly consisted of naïve CD4 T cells (T_N = 37.8%) followed by central memory CD4 T cells (T_{CM} = 32.7%), effector memory CD4 T cells (T_{EM} = 25.1%), and effector CD4 T cells (T_{EFF} = 4.4%). The T_N fraction was significantly lower in the INR group (29.9 ± 12.3%) than the other groups, IR (39.4 ± 14.7%, $P = 0.011$), and controls (44.2 ± 6.9%, $P < 0.001$, Fig. 2E), suggesting that thymic function of these individuals may have declined as evidenced by CD31%. Moreover, the INR showed statistically significant higher levels of T_{EM} (30.9 ± 16.6%) compared to IR (22.9 ± 10.5%) and control group (21.4 ± 5.5%) ($P = 0.032$ and $P = 0.009$, respectively, Fig. 2F). There was no statistically significant difference among the groups in relation to effector and central memory CD4+ T-lymphocytes (Figs. 2D and G, respectively). Because age and sex may influence thymic function, we performed linear regression analyses among these variables and CD31% and CD45-CD62L% to account for these confounding factors. Thus, according to a linear regression model for CD31% and CD45-CD62L%, sex (male sex as a risk factor) and age at cART start date have no significant influence on thymic function in the cART-treated HIV-positive patients. Linear regression models are provided in the Supplementary Data (Tables S3–S4).

3.3 | Pyroptosis and apoptosis of T cells

According to pyroptosis of CD4+ T cells (CD4+FLICA-Caspase1+, Fig. 3A), the cART-treated groups showed significantly higher dead cells percentage (IR = 25.1 ± 10.8% and INR = 23.3 ± 7.1%) than the control group (1.5 ± 2%, $P < 0.001$). No statistical difference was found between IR and INR groups. Similarly, for pyroptotic RTE CD4+ T-lymphocytes (CD4+/CD31+FLICA-Caspase1+, Fig. 3B), the IR and INR groups had higher percentages than the control group ($P < 0.001$, Fig. 3C). In addition, INR patients showed higher dead RTE CD4+ T cells percentage by pyroptosis than IR patients (68.6 ± 9.6% and 46 ± 11.4%, respectively; $P = 0.032$).

Both CD4+ T cells in early apoptosis (AnnexinV+PI-) and in late apoptosis (AnnexinV+PI+, Fig. 4A) were statistically higher in the cART-treated groups than control group ($P < 0.001$, Fig. 4B). No significant difference between IR and INR groups was found ($P = 0.935$ and $P = 0.311$, respectively). Similar apoptotic cells percentages and distribution among the groups was observed in relation to apoptotic RTE CD4+ T-lymphocytes (Fig. 4C and D), where controls showed lower apoptotic RTE CD4+ T cells ($P < 0.001$) than cART-treated groups. In addition, INR and IR groups had no statistical differences for RTE CD4+ T cells in early ($P = 0.877$) and late ($P=0.261$) apoptosis. Interestingly, in INR, RTE CD4+T cell death



F I G U R E 2 CD4+ T-lymphocytes production and differentiation in cART-treated HIV-positive patients (INR and IR groups) and healthy, HIV-1 negative individuals (control group). (**A**) Representative plot showing the gating strategy for RTE CD4+ T cells (CD4+/CD45RA+CD31+). (**B**) RTE CD4+ T cells percentages among the groups. (**C**) Representative plot illustrating the gating strategy for CD4+ T cells subsets. Lymphocytes (selected according to FSC and SSC, Fig. 1) were gated on CD4+ cells and then on CD45RA and CD62L. Percentages of CD4+ T cells subsets in IR, INR and control groups: (**D**) T_{EFF} = effector CD4+ T cells (CD45RA+CD62L-); (**E**) T_N = naïve CD4+ T cells (CD45RA+CD62L+); (**F**) T_{EM} = effector memory CD4+ T cells (CD45RA-CD62L-); (**G**) T_{CM} = central memory CD4+ T cells (CD45RA-CD62L+). Mean values, SD, and *P*-values (according to *t*-test) are shown. INR: immunological nonresponders. IR: immunological responders. RTE: recent thymic emigrants.

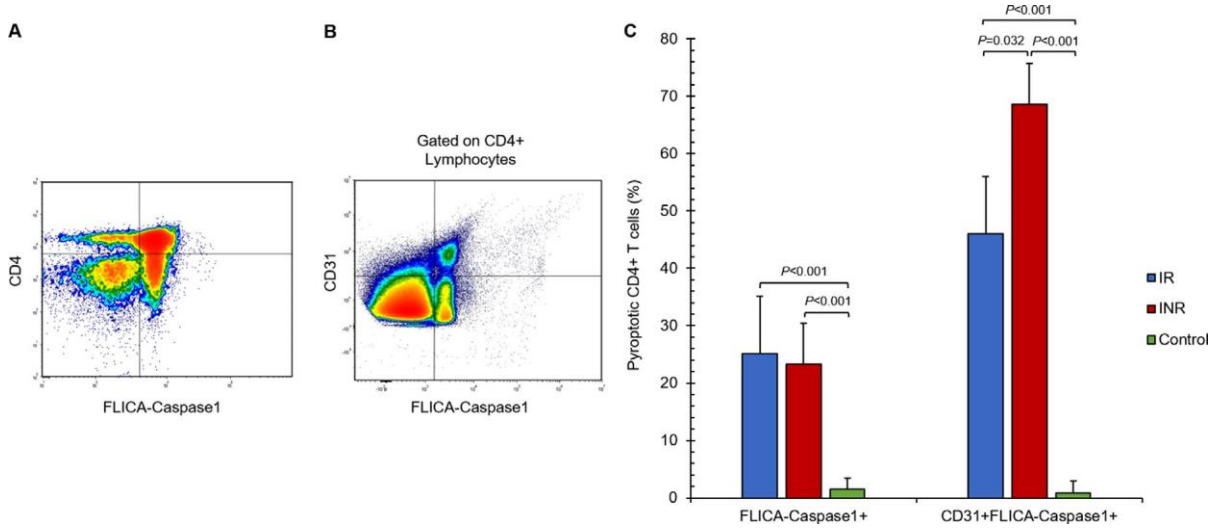
by pyroptosis (68.6 ± 7.1%, Fig. 3C) was significantly higher than by apoptosis (38.6 ± 13.0%, Fig. 4D) (P = 0.002).

3.4 | Immune activation

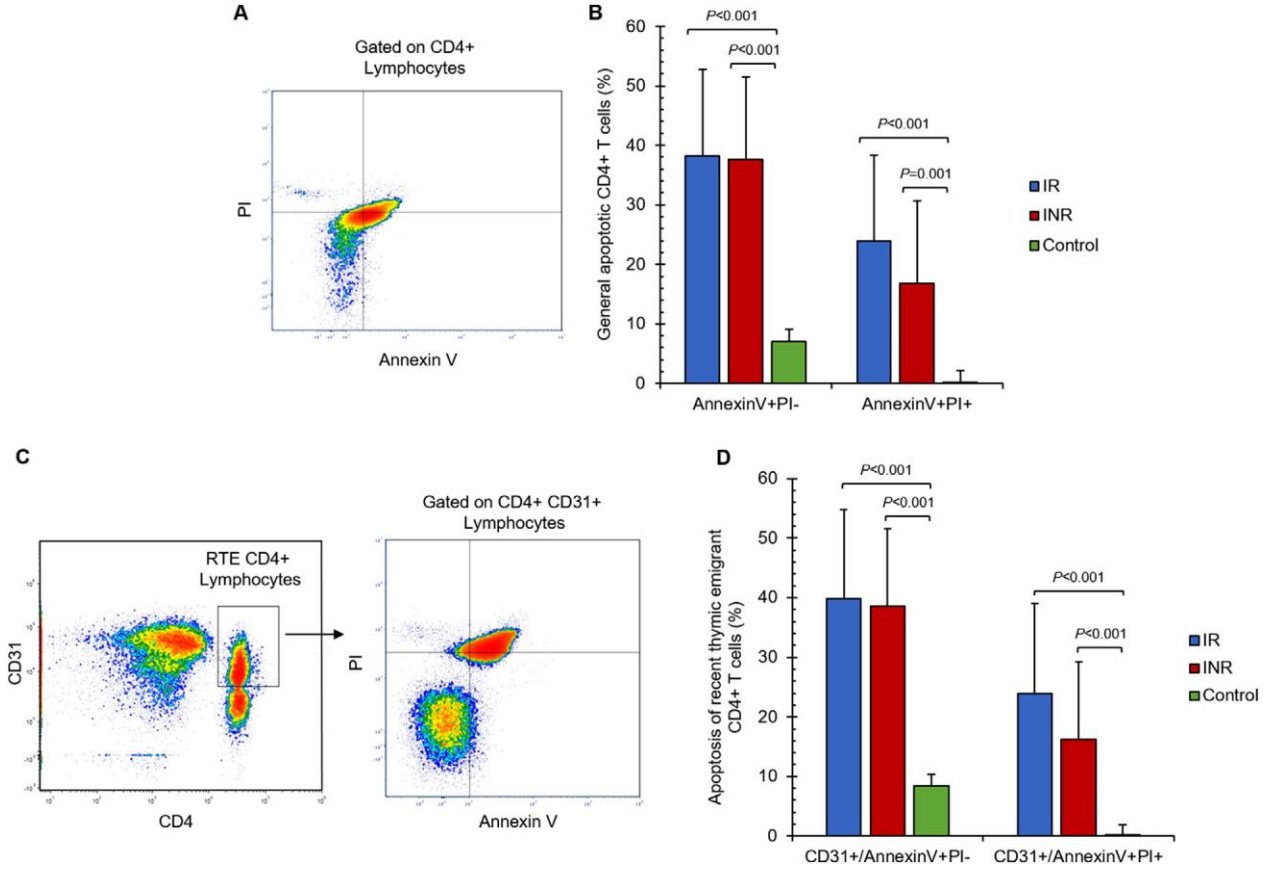
Percentages of CD8+ T cells were similar between INR (24.5 ± 11.9%) and IR (22.3 ± 9.3%) groups (P = 0.413, Table 1), but they were significantly higher than the control group (15.6 ± 4.9%, P = 0.002, see supplemental table). Activated CD8+ T cells (CD8+CD38+, Fig. 5A) percentage was slightly higher in the INR group (2.7 [1.3-4.5]) than in the IR group (1.3 [0.9-2.4]) but with no statistical difference (P = 0.094, Fig. 5B).

3.5 | Correlations

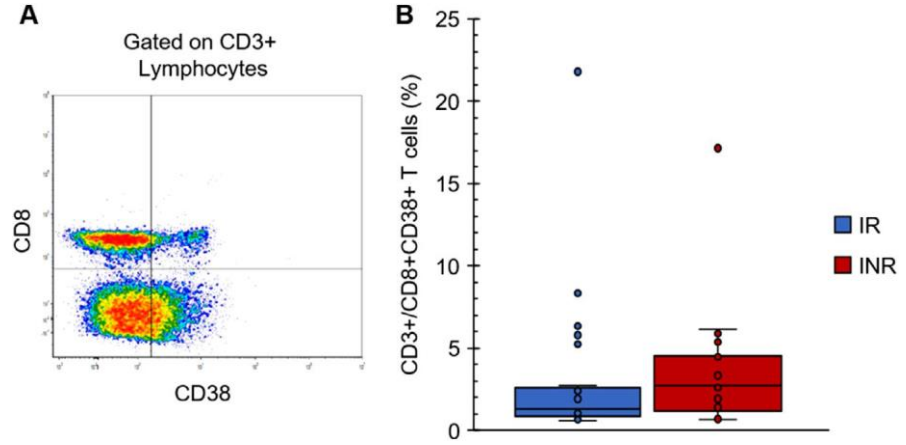
Correlations between RTE CD4+ T cells (CD31+CD45RA+) and pyroptotic RTE CD4+ T cells (CD31+Flica-Caspase1+) were investigated in the IR and INR groups. Significant negative correlation was observed among these RTE CD4-cells in the IR group (r = −0.502; P = 0.011; Fig. 6A), suggesting that these HIV-infected individuals besides having higher percentages of RTE CD4+ T-lymphocytes, they show reduced pyroptosis levels of these cells. On the other hand, a weak positive correlation was observed between RTE and pyroptotic RTE CD4+ T cells in the INR group (r = 0.306; Fig 6B), but it was not statistically significant (P = 0.250).



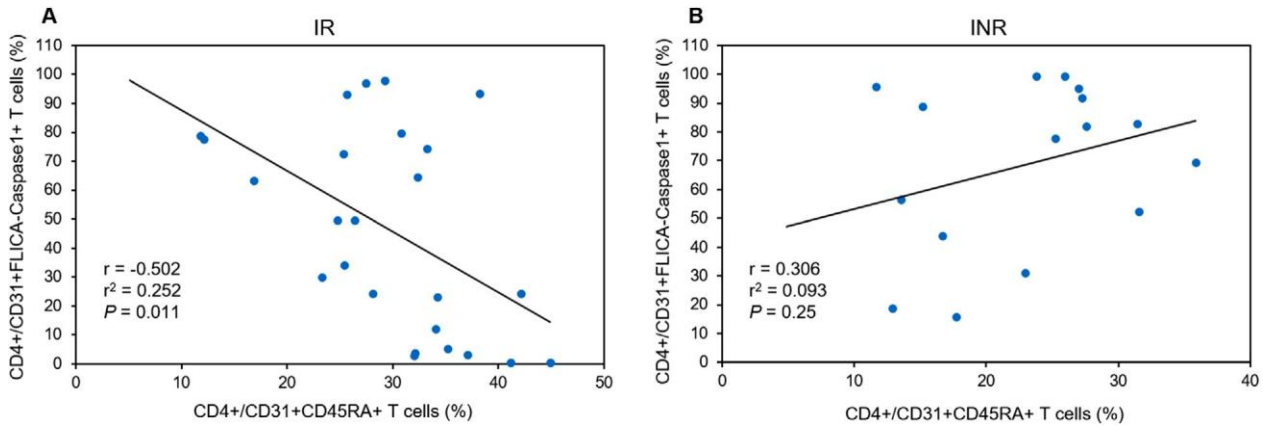
F I G U R E 3 Pyroptosis levels of CD4+ T cells based on FLICA-Caspase1 activity in cART-treated HIV-positive patients (INR and IR groups) and healthy, HIV-1 negative individuals (control group). Representative plots showing the gating strategy for (**A**) general pyroptotic CD4+ T cells (CD4+FLICA-Caspase1+) and (**B**) pyroptotic RTE CD4+ T cells (CD4+/ CD31+FLICA-Caspase1+) from lymphocytes gated on CD4+ (see Fig. 1). (**C**) Percentages of pyroptotic CD4+ T cells among the groups. Mean values, SD, and *P*-values (according to *t*-test) are shown. FLICA: fluorescent-labeled inhibitors of caspases. INR: immunological nonresponders. IR: immunological responders. RTE: recent thymic emigrants.



F I G U R E 4 CD4+ T cells undergoing apoptosis in cART-treated HIV-positive patients (INR and IR groups) and healthy, HIV-1 negative individuals (control group). (**A**) Representative plot illustrating the gating strategy for general apoptotic CD4+ T cells. Lymphocytes were gated on CD4+ cells (see Fig. 1) and then on PI and AnnexinV. (**B**) Apoptosis levels of general CD4+ T cells: early apoptosis (CD4+/AnnexinV+PI-) and late apoptosis (CD4+/AnnexinV+PI+). (**C**) Representative plot showing the gating strategy for apoptotic RTE CD4+ T-lymphocytes. Cells were gated on CD4 and CD31 and then on PI and AnnexinV. (**D**) Percentages of apoptotic RTE CD4+ T cells: early apoptosis (CD4+CD31+/ AnnexinV+PI-) and late apoptosis (CD4+CD31+/AnnexinV+PI+). Mean values, SD, and *P*-values (according to *t*-test) are shown. INR: immunological nonresponders. IR: immunological responders. RTE: recent thymic emigrants.



F I G U R E 5 Immune activation levels represented by CD8+ T cells expressing CD38 surface marker in the cART-treated HIV-positive patients (INR and IR groups). (**A**) Representative plot illustrating the gating strategy for activated CD8+ T-lymphocytes (CD3+/CD8+CD38+). Initial gating was performed on CD3+ population (see Fig. 1), and then on CD8 and CD38. (**B**) Percentage of CD8+CD38+ T cells in INR versus IR group. The median is represented by the line inside the box and its lower and upper borders represent the interquartile range (IQR). There was no statistically significant difference between the groups according to Wilcoxon-Mann-Whitney test (P = 0.094). INR: immunological nonresponders; IR: immunological responders.



F I G U R E 6 Correlations between RTE CD4+ T cells (CD31+CD45RA+) and pyroptotic RTE CD4+ T cells (CD31+FLICA-Caspase1+) in the cART-treated HIV-positive patients—IR (**A**) and INR (**B**) groups. Coefficient r and P-values (according to Pearson's correlation test) are shown. INR: immunological nonresponders. IR: immunological responders. RTE: recent thymic emigrants.

4 | DISCUSSION

Since the introduction of cART into clinical routine, survival of HIV-infected patients has improved considerably.[21] However, despite the efficiency of cART, a significant number of patients present impaired rise in peripheral blood CD4+ T cell count under treatment, even with fully suppressed HIV-1 replication. [11,13,15,16] In this context, the present study sought to investigate thymic output, immune activation and CD4 T cell death pathways in cART-treated individuals with suppressed viremia to better understand the mechanisms involved in poor CD4+ T cell reconstitution.

Regarding some risk factors, we found association between male sex and risk to immunological recovery failure in the univariate analysis. Some studies have demonstrated that sex may influence the therapeutic outcome in relation to the immune system thymic output during therapy because thymic function appear to be not compromised by a more advanced infection compared with men, leading a better immune outcome[23,24] although it was not observed in this study.

Consisting with other findings,[7,9,10] low pretreatment CD4+ T cell count (<350 cells/μL) at cART start was significantly associated with immunological recovery failure. We also observed a statistically significant difference of CD4 percentage and CD4/CD8 ratio among the ART-treated groups, evidencing a persistently reduced CD4+ T-lymphocytes in these INR, as observed in other studies.[8,12,25,26] Previous evidence pointed to a negative correlation among pretreatment cell count. The lower the pretreatment CD4+ T cell numbers, the greater will be the time until immunological reconstitution, varying from 6 mo to 7 yr.[8] According to some investigators, immune recovery is less apparent in patients who started cART with severe immunodeficiency.[16,27]

This low immune reconstitution rate seems to be consequence of a process induced by HIV infection that limit T lymphocytes production in the thymus and increase mortality rate of CD4+ T cells in the peripheral circulation. [11,15,23,28] It is known that thymus begins to atrophy following ageing and, consequently, its function in adults has been assumed to be limited or unnecessary. In fact, during childhood the thymus produces a wide variety of T lymphocytes, which would be sufficient for homeostatic proliferation and maintaining a large compartment of naïve CD4+ T cells. [29,30] Thymic activity is again required when a massive exhaustion of the T cell pool occurs, and accelerated T cell regeneration is then required, as observed in HIV infection. [28,31] These previous observations may explain the finding that HIV-1 negative individuals in the control group showed a similar CD31% with the INR because they do not have any infection or inflammatory chronic disease and simply showed a residual thymic activity, which can persist beyond the fifth decade of life. [30] Besides progressive CD4+ T cells destruction, during HIV-1 infection thymopoiesis is also compromised. HIV-1 infects and kills the developing CD4+ thymocytes, inhibits their maturation by affecting thymic stromal cells, and appears to impair CD34+ progenitors, thus influencing the inflow of stem cell for thymic function. [28,32,33] Therefore, when these HIV-positive individuals start cART, sometimes with low CD4+ T cell count as observed in this study, they do not present adequate immune reconstitution because of their thymic exhaustion, a result of long HIV infection without treatment. These findings were also confirmed by the immunological analyses of thymic output based on RTE and naïve CD4+ T cell percentages (CD31% and CD45RA-CD62L%, respectively), which were significantly lower in the INR group than in the IR group, showing a reduced central immune activity.

In the context of cell death in HIV-positive patients, it had been initially believed that most of CD4+ T cell depletion was due to cell death by apoptosis. [34,35] However, a study developed by Doitsh et al. using lymphoid tissues showed that only 5% of dead CD4+ T cells die by apoptosis whereas the remained 95% were due to pyroptotic processes. [20] Although we did not observe the same results, because our study was based on PBMCs, CD4+ T cell death by pyroptosis was almost twice higher compared to apoptosis in the INR. Pyroptosis is a natural cellular defense mechanism against pathogens, but in HIV-1 infection, it becomes excessive and creates a vicious cycle in which dying CD4+ T-lymphocytes (called abortive cells) release inflammatory signals that attract more cells to die, exacerbating cell death pathways. [17,36] It is important to note that although the HIV-infected patients involved in our analyses presented suppressed viral loads according to clinical practice tests, the possibility of residual viral production by reservoir cells should not be excluded. [7,11] Most of the analyses involving death by pyroptosis use lymphoid tissue cultures because some studies have demonstrated that CD4+ T-lymphocytes in peripheral blood retain resistance to pyroptosis as they migrate to the peripheral blood. [37,38] It is believed that there is a decreased T lymphocyte activation during trafficking because of absence of cell-cell interactions, which may prevent pyroptosis because virological synapses is not observed. [39] However, as observed in this study, the cell death pattern does not appear to change during T cell trafficking, being pyroptosis the main pathway of cell death in HIV-positive patients under cART, independent of the microenvironment. Another study also using PBMCs observed higher expression of pyroptotic genes in individuals with immunological recovery failure compared to those with adequate response.[40]

In relation to dying RTE CD4+ T cell by pyroptosis, higher levels of pyroptotic cells were observed in INR compared to IR. This difference may be related to the immune modulation in response of released cytokines and cellular contents during pyroptosis pathway.[18] After Caspase-1 activation, pro-interleukins IL-18 and IL-1 are cleaved into their active form, which modulate inflammatory responses and promote cell activation and migration to the infection site.[36] Studies have shown that IL-1 release during pyroptotic death plays an important role in Th1 response, being associated with memory CD4+ T cell activation. [41,42] This could explain why the INR showed higher levels of effector memory CD4+ T cells than IR group. Therefore, in lymphoid tissues, pyroptotic cells are responsible to generate higher release of IL-1 , which leads to CD4+ T cell exhaustion, decreasing in their numbers and impairing their functions.[42] Researches have also demonstrated that IL-18 is able to play functions in synergy with other interleukins inducing *CCR5* expression, which acts as the main coreceptor for HIV-1 cell entry.[43] An increased expression of this gene could be related to a higher abortive cell number in the thymus. As a result, it may stimulate pyroptosis as the T lymphocytes migrate to maturate in the secondary lymphoid organs, as observed in our study according to pyroptotic RTE CD4+ T cells percentage, which was higher in the INR group. Another explanation is based on cellular contents released by pyroptosis. Besides proinflammatory cytokines, pyroptotic cells also release pathogen- and damage-associated molecular patterns (PAMPs and DAMPs, respectively), stimulating the NLRP3 inflammasome activation and assembly.[44] In the thymus, lipid molecules from membrane destruction or age-related lipid depositions act as triggers for NLRP3 activation, stimulating cell death by pyroptosis in T lymphocytes that migrate to peripheral blood.[45,46]

Comparing immune activation according to CD38%, both cART-treated groups displayed decreased activated CD8+ T cells and, despite no significant difference, the INR showed slightly higher CD38% compared with the IR group. Although the immune activation was not associated with influence on immunological recovery in our study, other authors suggest that it indeed might play a role in immunological reconstitution failure.[16,47,48]

Several factors are described in the literature as involved in poor immunological reconstitution of HIV-positive patients under cART[6,11–13,15] and, based on these immunological factors also observed in this study, we could predict who would become INR and suggest therapeutic strategies. For instance, if a male HIV-positive patient starts a cART regimen with low CD4+ T cell count and reduced thymic function (low CD31% and CD45RA-CD62%), he might need additional treatment such as an immunological adjuvant that may improve his immune outcome. Among all suggested strategies to increase peripheral CD4+ T cell count in cART-treated patients, IL-7, IL-2, or IL-15, which regulate T cell development, activation, proliferation, and survival, have been intensely investigated.[15,49–51] Moreover, these results even more confirm that

the antiretroviral therapy should be started early, as recommended by World Organization Healthy guidelines.[4] The later the patient starts cART, the more impaired will be his/her thymus; and this damage would not be improved over time even under anti-HIV therapy. [27,52,53] Besides having reduced thymic function, INR show high pyroptosis levels of RTE cells, making immunological recovery even more difficult. Therefore, there is no reason to wait for treatment starting.

Although there are some limitations, our study suggests that reduced thymic function and cell death by pyroptosis are the major mechanisms of immunological recovery failure in cART-treated HIV-positive patients. In addition, to the best of our knowledge, this is the first study to report high levels of pyroptotic RTE CD4+ T cells being associated with immunological reconstitution failure in cART-treated patients. Our findings, together with the literature, highlight the strong need of additional studies to provide a suitable immune outcome to patients who are now considered INR.

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AUTHORSHIP

W.H.V.C.-S. and J.L.A.-S. were responsible for the study design, immunologic analyses, and writing of the manuscript; F.O.S. did the flow cytometry analyses; A.V.C.C., S.C., and R.L.G. were responsible for the study design. All authors were involved with the manuscript review and editing. W.H.V.C.-S. and J.L.A.-S. authorship section to this work and should be considered as co-first authors.

DISCLOSURES

The authors declare no conflicts of interest.

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REFERENCES

1. Barré-Sinoussi F, Ross AL, Delfraissy J-F. Past, present and future: 30 years of HIV research. *Nat Rev Micro*. 2013;11:877-883.
2. Arts EJ, Hazuda DJ. HIV-1 antiretroviral drug therapy. *Cold Spring Harb Perspect Med*. 2012;2:a007161.
3. Tseng A, Seet J, Phillips EJ. The evolution of three decades of antiretro-viral therapy: challenges, triumphs and the promise of the future. *Br J Clin Pharmacol*. 2014;79:182-194.
4. WHO. *Consolidated Guidelines on the Use of Antiretroviral Drugs for Treat-ing and Preventing HIV Infection: Recommendations for a Public Health Approach*. Geneva: World Health Organization; 2016:1-480.
5. De Clercq E, Li G. Approved antiviral drugs over the past 50 years. *Clin Microbiol Rev*. 2016;29:695-747.
6. Pacheco YM, Peraire J, Vilade C, et al. Evaluation of the pharmaco-genetics of immune recovery in treated HIV-infected patients. *Expert Opin Drug Metab Toxicol*. 2014;10:81-101.
7. Benveniste O, Flahault A, Rollot F, et al. Mechanisms involved in the low-level regeneration of CD4 + cells in HIV-1–infected patients receiving highly active antiretroviral therapy who have prolonged undetectable plasma viral loads. *J Infect Dis*. 2005;191:1670-1679.
8. Kelley CF, Kitchen CMR, Hunt PW, et al. Incomplete peripheral CD4+ cell count restoration in HIV-infected patients receiving long-term antiretroviral treatment. *Clin Infect Dis*. 2009;48:787-794.
9. Li T, Wu N, Dai Y, et al. Reduced thymic output is a major mechanism of immune reconstitution failure in HIV-infected patients after long-term antiretroviral therapy. *Clin Infect Dis*. 2011;53:944-951.
10. Aiuti F, Mezzaroma I. Failure to reconstitute CD4+ T-cells despite sup-pression of HIV replication under HAART. *AIDS Rev*. 2006;8:88-97.
11. Corbeau P, Reynes J. Immune reconstitution under antiretroviral therapy : the new challenge in HIV-1 infection. *Blood*. 2011;117: 5582-5590.
12. Pinzone MR, Di Rosa M, Cacopardo B, Nunnari G. HIV RNA suppres-sion and immune restoration: can we do better. *Clin Dev Immunol*. 2012;51:1-12.
13. Cenderello G, De Maria A. Discordant responses to cART in HIV-1 patients in the era of high potency antiretroviral drugs: clinical evalua-tion, classification, management prospects. *Expert Rev Anti Infect Ther*. 2016;14:29-40.
14. Castilho JL, Melekhin VV, Sterling TR. Sex differences in HIV outcomes in the highly active antiretroviral therapy era: a systematic review. *AIDS Res Hum Retroviruses*. 2014;30:446-456.
15. Gaardbo JC, Hartling HJ, Gerstoft J, Nielsen SD. Incomplete immune recovery in HIV infection: mechanisms, relevance for clinical care, and possible solutions. *Clin Dev Immunol*. 2012;67:1-17.
16. Okoye AA, Picker LJ. CD4(+) T-cell depletion in HIV infection: mecha-nisms of immunological failure. *Immunol Rev*. 2013;254:54-64.
17. Doitsh G, Greene WC. Dissecting how CD4 T cells are lost during HIV infection. *Cell Host Microbe*. 2016;19:280-291.
18. Man SM, Karki R, Kanneganti TD. Molecular mechanisms and func-tions of pyroptosis, inflammatory caspases and inflammasomes in infectious diseases. *Immunol Rev*. 2017;277:61-75.
19. Ministério da Saúde. *Protocolo Clínico e Diretrizes Terapêuticas para Manejo da Infecção pelo HIV em Adultos*. Brasília: Ministério da Saúde-Secretaria de Vigilância em Saúde; 2013:1-227.
20. Doitsh G, Galloway NLK, Geng X, et al. Cell death by pyroptosis drives CD4 T-cell depletion in HIV-1 infection. *Nature*. 2014;505:509-514.
21. UNAIDS. UNAIDS DATA 2018. Joint United Nations Programme on HIV/AIDS (UNAIDS). 2018:1-376.
22. Hunt PW, Deeks SG, Rodriguez B, et al. Continued CD4 cell count increases in HIV-infected adults experiencing 4 years of viral suppres-sion on antiretroviral therapy. *AIDS*. 2003;17:1907-1915.
23. Teixeira L, Valdez H, McCune JM, et al. Poor CD4 T cell restoration after suppression of HIV-1 replication may reflect lower thymic func-tion. *AIDS*. 2001;15:1749-1756.
24. Pido-Lopez J, Imami N, Aspinall R. Both age and gender affect thymic output: more recent thymic migrants in females than males as they age. *Clin Exp Immunol*. 2001;125:409-413.
25. Engsig FN, Zangerle R, Katsarou O, et al. Long-term mortality in HIV-positive individuals virally suppressed for >3 years with incomplete CD4 recovery. *Clin Infect Dis*. 2014;58:1312-1321.
26. Rosado-Sánchez I, Herrero-Fernández I, Genebat M, Ruiz-Mateos E, Leal M, Pacheco YM. Thymic function impacts the peripheral CD4/CD8 ratio of HIV-infected subjects. *Clin Infect Dis*. 2017;64:152-158.
27. He S, Zhang Z, Fu Y, et al. Thymic function is most severely impaired in chronic HIV-1 infection, but individuals with faster disease pro-gression during early HIV-1 infection expressed lower levels of RTEs. *JAIDS*. 2015;70:472-478.
28. Kolte L. Thymic function in HIV-infection. *Dan Med J*. 2013;60:44-52.

29. Goronzy JJ, Fang F, Cavanagh MM, Qi Q, Weyand CM. Naive T cell maintenance and function in human aging. *J Immunol*. 2015;194: 4073-4080.
30. Kumar BV, Connors TJ, Farber DL. Human T cell development, localization, and function throughout life. *Immunity*. 2018;48:202-213.
31. Ye P, Kirschner DE, Kourtis AP. The thymus during HIV disease : role in pathogenesis and in immune recovery. *Curr HIV Res*. 2004;2:177-183.
32. Fang HRT, Colantonio AD, Uittenbogaart CH. The role of the thymus in HIV infection : a 10 year perspective. *AIDS*. 2008;22:171-184.
33. Carter CC, Onafuwa-Nuga A, McNamara LA, et al. HIV-1 infects multi-potent progenitor cells causing cell death and establishing latent cellu-lar reservoirs. *Nat Med*. 2010;16:446-451.
34. Mbita Z, Hull R, Dlamini Z. Human immunodeficiency virus-1 (HIV-1)-mediated apoptosis: new therapeutic targets. *Viruses*. 2014;6: 3181-3227.
35. Negredo E, Massanella M, Puig J, et al. Nadir CD4 T cell count as predictor and high CD4 T cell intrinsic apoptosis as final mechanism of poor CD4 T cell recovery in virologically suppressed HIV-infected patients: clinical implications. *Clin Infect Dis*. 2010;50:1300-1308.
36. Jorgensen I, Miao EA. Pyroptotic cell death defends against intracellu-lar pathogens. *Immunol Rev*. 2015;265:130-142.
37. Muñoz-Arias I, Doitsh G, Yang Z, Sowinski S, Ruelas D, Greene WC. Blood-derived CD4 T cells naturally resist pyroptosis during abortive HIV-1 infection. *Cell Host Microbe*. 2015;18:463-470.
38. Doitsh G, Cavrois M, Lassen KG, et al. Abortive HIV infection mediates CD4 T cell depletion and inflammation in human lymphoid tissue. *Cell*. 2010;143:789-801.
39. Galloway NLK, Doitsh G, Monroe KM, et al. Cell-to-cell transmission of HIV-1 is required to trigger pyroptotic death of lymphoid-tissue-derived CD4 T cells. *Cell Rep*. 2015;12:1555-1563.
40. Bandera A, Masetti M, Fabbiani M, et al. The NLRP3 inflammasome is upregulated in HIV-infected antiretroviral therapy-treated individuals with defective immune recovery. *Front Immunol*. 2018;9:1-8.
41. Ben-Sasson SZ, Hu-Li J, Quiel J, et al. IL-1 acts directly on CD4 T cells to enhance their antigen-driven expansion and differentiation. *Proc Natl Acad Sci*. 2009;106:7119-7124.
42. Shive CL, Mudd JC, Funderburg NT, et al. Inflammatory cytokines drive CD4+ T-cell cycling and impaired responsiveness to inter-leukin 7: implications for immune failure in HIV disease. *J Infect Dis*. 2014;210:619-629.
43. Rodriguez-Galan MC, Bream JH, Farr A, Young HA. Synergistic effect of IL-2, IL-12, and IL-18 on thymocyte apoptosis and Th1/Th2 cytokine expression. *J Immunol*. 2005;174:2796-2804.
44. Guo H, Gao J, Taxman DJ, Ting JPY, Su L. HIV-1 infection induces interleukin-1 production via TLR8 protein-dependent and NLRP3 inflammasome mechanisms in human monocytes. *J Biol Chem*. 2014;289:21716-21726.
45. Chanpimol S, Seamon B, Hernandez H, Harris-love M, Blackman MR. Age-associated alterations in the levels of cytotoxic lipid molecular species and oxidative stress in the murine thymus are reduced by growth hormone treatment. *Mech Ageing Dev*. 2017;167: 46-55.
46. Harijith A, Ebenezer DL, Natarajan V. Reactive oxygen species at the crossroads of inflammasome and inflammation. *Front Physiol*. 2014;5:1-11.
47. Piconi S, Trabattoni D, Gori A, et al. Immune activation, apoptosis, and Treg activity are associated with persistently reduced CD4 R T-cell counts during antiretroviral therapy. *AIDS*. 2010;24:1991-2000.
48. van den Dries L, Claassen MAA, Groothuismink ZMA, van Gorp E, Boonstra A. Immune activation in prolonged cART-suppressed HIV patients is comparable to that of healthy controls. *Virology*. 2017;509:133-139.
49. Klatzmann D, Abbas AK. The promise of low-dose interleukin-2 therapy for autoimmune and inflammatory diseases. *Nat Rev Immunol*. 2015;15:283-294.
50. Mackall CL, Fry TJ, Gress RE. Harnessing the biology of IL-7 for thera-peutic application. *Nat Rev Immunol*. 2011;11:330-342.
51. Watson DC, Moysi E, Valentin A, et al. Treatment with native heterodimeric IL-15 increases cytotoxic lymphocytes and reduces SHIV RNA in lymph nodes. Silvestri G, editor. *PLOS Pathog*. 2018; 14:1-24.
52. Ferrando-Martinez S, De Pablo-Bernal RS, De Luna-Romero M, et al. Thymic function failure is associated with human immunodeficiency virus disease progression. *Clin Infect Dis*. 2017;64:1191-1197.
53. Rb-Silva R, Nobrega C, Azevedo C, et al. Thymic function as a predic-tor of immune recovery in chronically HIV-infected patients initiating antiretroviral therapy. *Front Immunol*. 2019;10:1-13.

SUPPORTING INFORMATION

Additional information may be found online in the Supporting Information section at the end of the article.

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Supplementary Data

Control Group

Supplementary Table S1. Basic characteristics of clinically healthy volunteers enrolled in our study.

Variables		Controls (n=12[a])
Sex	Male	5 (41.7%)
	Female	7 (58.3%)
Age (years old), mean ± SD		26.0 ± 4.7
Body mass (kg), mean ± SD		69.4 ± 13.9
CD4+ (%)		28.1 ± 9.8
CD8+ (%)*		15.6 ± 4.9

Variables followed a normal distribution (Shapiro-Wilk test: >0.05).

[a]Clinically healthy volunteers presented neither autoimmune or infectious diseases nor first-degree kinship with autoimmune diseases-carrying individuals.

*Percentage was significantly lower than the cART-treated groups: immunological responders (22.3±9.3%, *P*=0.0018) and immunological non-responders (24.5±11.9%, *P*=0.0025).

cART: combination antiretroviral therapy; SD: standard deviation.

Coinfections

The patients did not present active HBV infection (20.7% and 39.3% were immune by vaccination in INR and IR groups, respectively and 34.5% (INR) and 14.3% (IR) were susceptible – did not present evidence for contact with HBV). In addition, no patients were co-infected by HCV or HTLV-1/2. Evidence for syphilis infection was present in 17.2% (n=5) of patients in the INR group and only one patient in the IR group, as detected by VDRL test. Regarding CMV and toxoplasmosis infections, notably, the same patients presented both past coinfections (INR=10.3% and IR=14.3%), as revealed by immunoglobulin G tests. However, the coinfections also had no influence over immunological response in this study (syphilis: *P*=0.706; CMV: *P*=0.706 and toxoplasmosis: *P*=0.214, Supplementary Table).

Supplementary Table S2. Coinfections serology status of cART-treated HIV-positive patients enrolled in our study.

Coinfections Serology Status	INR n=29 (%)	IR n=28 (%)	*P* *
Coinfection: syphilis	5 (17.2)	1 (3.6)	0.214
Coinfection: toxoplasmosis[b]	3 (10.3)	4 (14.3)	0.706
Coinfection: CMV[b]	3 (10.3)	4 (14.3)	0.706

* Fisher exact test.

[b] Chronic infection – Immunoglobulin G tests.

CMV: cytomegalovirus; INR: immunological non-responders; IR: immunological responders.

Linear Regression Analyses

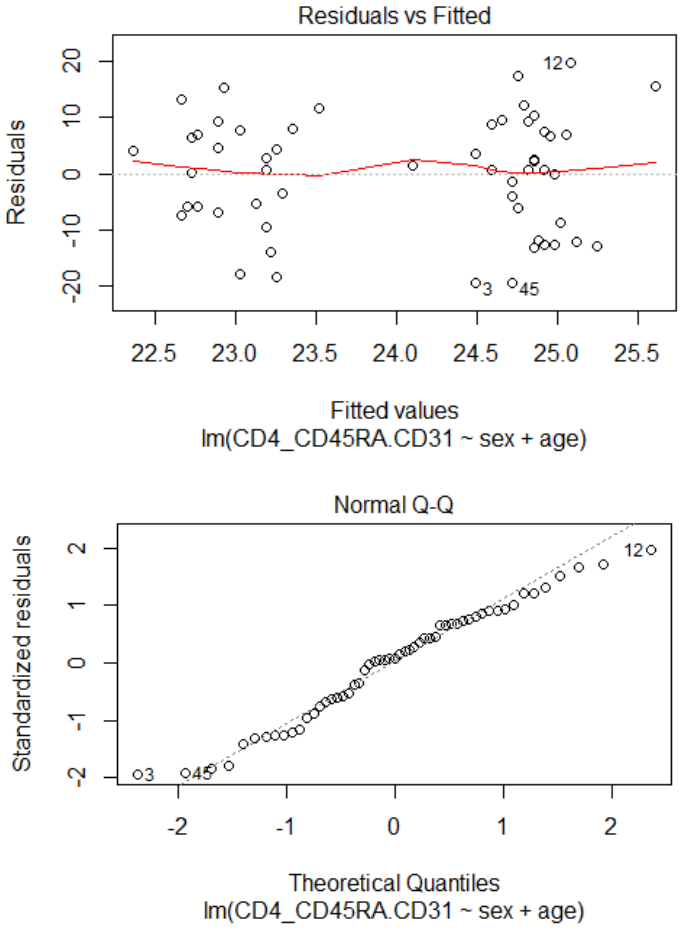
Supplementary Table S3. Linear regression model to correct confounding factors regarding to recent thymic emigrants CD4 T cells (CD4+/CD45RA+CD31+) output.

Variables	Estimate (β)*	t-value	*P*
Male sex	-2.123	-0.675	0.502
Age at cART start date	0.033	0.189	0.851
(Intercept)	23.869	4.362	<0.001

cART: combination antiretroviral therapy.

*Standard error: 10.35; R-squared: 0.009; Adjusted R-squared: -0.029; F-statistic: 0.236.

Distribution of the CD31% values based on sex and age at cART start in the linear logistic model:



Model's internal validation had good adherence (p-value=0.791).

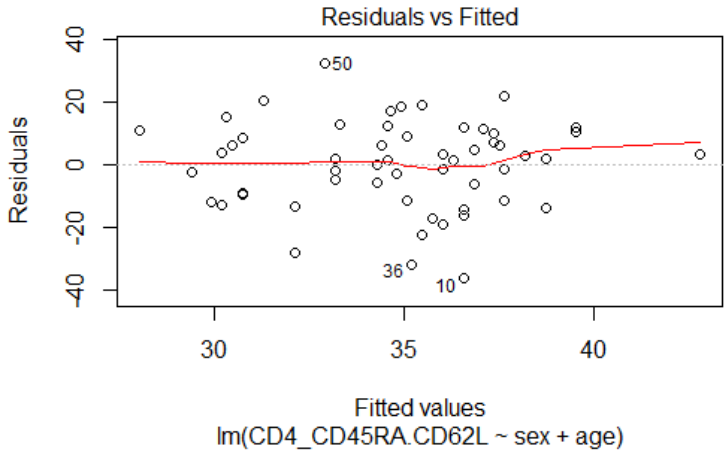
Supplementary Table S4. Linear regression model to correct confounding factors regarding to naïve CD4 T cells (CD4+/CD45RA+CD62L+) output.

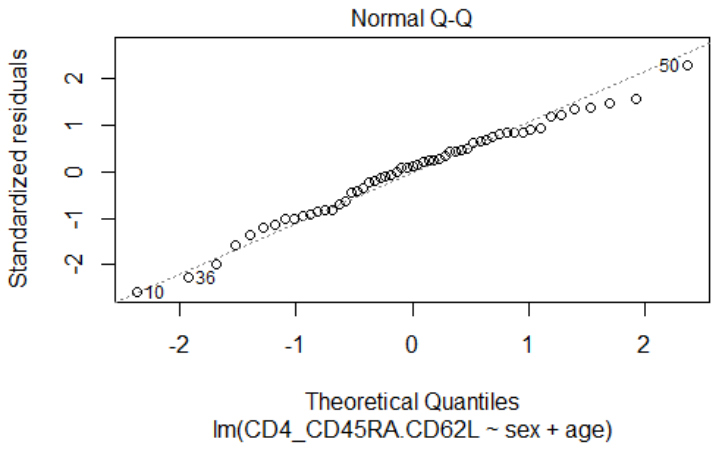
Variables	Estimate (β)*	t-value	*P*
Male sex	-2.008	-0.461	0.647
Age at cART start date	-0.271	-1.127	0.265
(Intercept)	44.685	5.896	<0.001

cART: combination antiretroviral therapy.

* Standard error: 14.34; R-squared: 0.045; Adjusted R-squared: 0.008; F-statistic: 1.213.

Distribution of the CD45RA-CD62L% values based on sex and age at cART start in the linear logistic model:





Model's internal validation had good adherence (p-value=0.306).

4 DISCUSSÃO GERAL

Desde a introdução da ART na prática clínica como principal forma de tratamento contra o HIV-1, a qualidade de vida e sobrevivência dos pacientes tem melhorado consideravelmente (UNAIDS, 2021a; WHO, 2016). Todavia, mesmo alcançando supressão da carga viral plasmática, existe um número significativo de pacientes tratados com ART que apresentam deficiência na reconstituição de células T CD4+ (CORBEAU; REYNES, 2011; GAARDBO et al., 2012; MINISTÉRIO DA SAÚDE, 2018b). Mais de duas décadas de ART já se passaram e os mecanismos que determinam essa reconstituição imune reduzida ainda não foram completamente esclarecidos. Consequentemente, ainda não foi possível estabelecer uma estratégia terapêutica eficaz para melhorar essa deficiência na recuperação imunológica nesses indivíduos, atualmente conhecidos como não-respondedores imunológicos (INR) (CENDERELLO; DE MARIA, 2016; PINZONE et al., 2012; YANG et al., 2020). Com base nisso, este estudo se propôs a investigar o perfil fenotípico de linfócitos T e citocinas dos pacientes HIV-positivos tratados com ART e avaliar alterações genéticas em genes envolvidos na resposta imune desses pacientes afim de melhor entender a condição clínica dos INR.

No primeiro artigo, buscamos investigar, a partir de uma pequena revisão na forma de editorial fatores genéticos que poderiam estar envolvidos na produção, ativação, proliferação e nos mecanismos de destruição das células T CD4+, visto que ainda pouco se conhece sobre a influência da genética na recuperação imunológica dos pacientes em ART. Sabe-se que existe uma considerável variabilidade interindividual com relação as repostas imunes durante a terapia, e que grande parte dessa variação é consequência das diferenças genéticas dos pacientes (CENDERELLO; DE MARIA, 2016; CORBEAU; REYNES, 2011; GREENBLATT et al., 2019). Alguns estudos têm demonstrado que o *background* genético pode predispor os indivíduos a reduzidas taxas de proliferação e diferenciação dos linfócitos T CD4+, ativação imunológica exacerbada e elevados níveis de mortes celular programada (como apoptose e piroptose) (GREENBLATT et al., 2019; HAAS et al., 2006; PACHECO et al., 2014; YOUNAS et al., 2016), assim também como evidenciado em nossas análises (Artigo II e Apêndice A). Porém, ainda são estudos genéticos insuficientes acerca dos fatores que possam estar envolvidos na reconstituição de linfócitos T CD4+ durante a ART, principalmente com relação à produção e

homeostase dessas células, onde a literatura ainda é limitada. Isso dificulta também na elaboração de estratégias terapêuticas satisfatórias para os INR, tornando-se essencial conhecer o perfil genético desses pacientes.

Dessa forma, no segundo artigo bem como no artigo do Apêndice A, procuramos avaliar alterações em genes que possuam funções relevantes nas respostas imunes dos pacientes em ART. Como resultado, observamos a influência imunológica de uma importante alteração genética nos estudos com suscetibilidade a infecção pelo HIV-1, a deleção de 32 pares de base no gene *CCR5* (*CCR5*Δ32). Por ser o principal correceptor do HIV-1 durante a infecção viral, a presença do Δ32 no *CCR5* resulta em uma proteína truncada que não é expressa na membrana celular, e consequentemente, confere aos indivíduos que apresentam esse polimorfismo resistência contra o HIV-1 de tropismo R5 (ARENZANA-SEISDEDOS; PARMENTIER, 2006; FLANAGAN, 2014). Contudo, o CCR5 é primordialmente um receptor de quimiocinas, expresso em diversas células do sistema imune, atuando no recrutamento de leucócitos e coestimulando a ativação, proliferação e diferenciação dos linfócitos T e macrófagos (ALKHATIB, 2009; FLANAGAN, 2014). Dessa maneira, sua ausência poderia influenciar na recuperação imunológica dos pacientes em ART, como observado no respectivo estudo e em outros na literatura (AHUJA et al., 2008; CAMARGO et al., 2009; DOLAN et al., 2007), onde o *CCR5*Δ32 se mostrou como um fator de risco associado com a baixa reconstituição de células T CD4+. Por outro lado, interessantemente, alguns estudos (CORBEAU; REYNES, 2011; PACHECO et al., 2014; VINCENT, 2006) têm evidenciado que níveis elevados do CCR5 na superfície celular podem ocasionar em um aumento na ativação imunológica, resultando em maiores níveis de mortes celular dos linfócitos T CD4+, e assim, redução dessas células durante a ART. Isso permitiu estabelecer algumas estratégias terapêuticas, ainda em estudo (GAARDBO et al., 2012; GRANDE et al., 2019; QI et al., 2020), que sugerem reduzir a expressão do CCR5 por antagonistas ou terapia genética, e como resultado, diminuir a persistente ativação imunológica nos pacientes em terapia. É notório que em um paciente HIV-positivo o CCR5 seja parte de um sistema complexo de interações envolvendo o vírus, o receptor e seus ligantes imunológicos, e que um desbalanço extremo na expressão desse gene pode desencadear alterações imunes que são desvantajosas nas respostas terapêuticas do indivíduo em ART (ELLWANGER; KAMINSKI; CHIES, 2019).

Levando em consideração os dados sociodemográficos, observamos que o sexo masculino se mostrou associado como um fator de risco com a deficiência na recuperação imunológica (Artigo 3). Além disso, foi observado também que esses pacientes HIV-positivos em tratamento apresentaram maiores níveis de morte celular por piroptose que as pacientes mulheres (Apêndice B), evidenciando que indivíduos do sexo masculino apresentam maior dificuldade na reconstituição de linfócitos T CD4+ durante a ART comparado aos do sexo feminino. Estudos têm demonstrado que o sexo tende a influenciar no resultado terapêutico quanto à reconstituição do sistema imune, onde indivíduos do sexo feminino apresentam durante a terapia melhor imunidade adaptativa com maiores contagens de linfócitos T CD4 e níveis mais elevadas de produção tímica e proliferação das células T, além de taxas de mortalidade reduzida (AIUTI; MEZZAROMA, 2006; CASTILHO; MELEKHIN; STERLING, 2014; HUNT et al., 2003; KLEIN; FLANAGAN, 2016; PIDO-LOPEZ; IMAMI; ASPINALL, 2001). Além disso, alguns estudos sugerem que os hormônios esteroides sexuais femininos são fortes fatores protetivos nas respostas imunológicas durante tratamentos, como os efeitos antiapoptóticos evidenciados em algumas células do sistema imune. Estudos observaram que esses hormônios atuam inibindo a ativação de algumas interleucinas pro-inflamatórias, como a IL-1β (CHEN et al., 2013; GRIMALDI et al., 2002; KLEIN; FLANAGAN, 2016; MOLLOY et al., 2003; STRAUB, 2007).

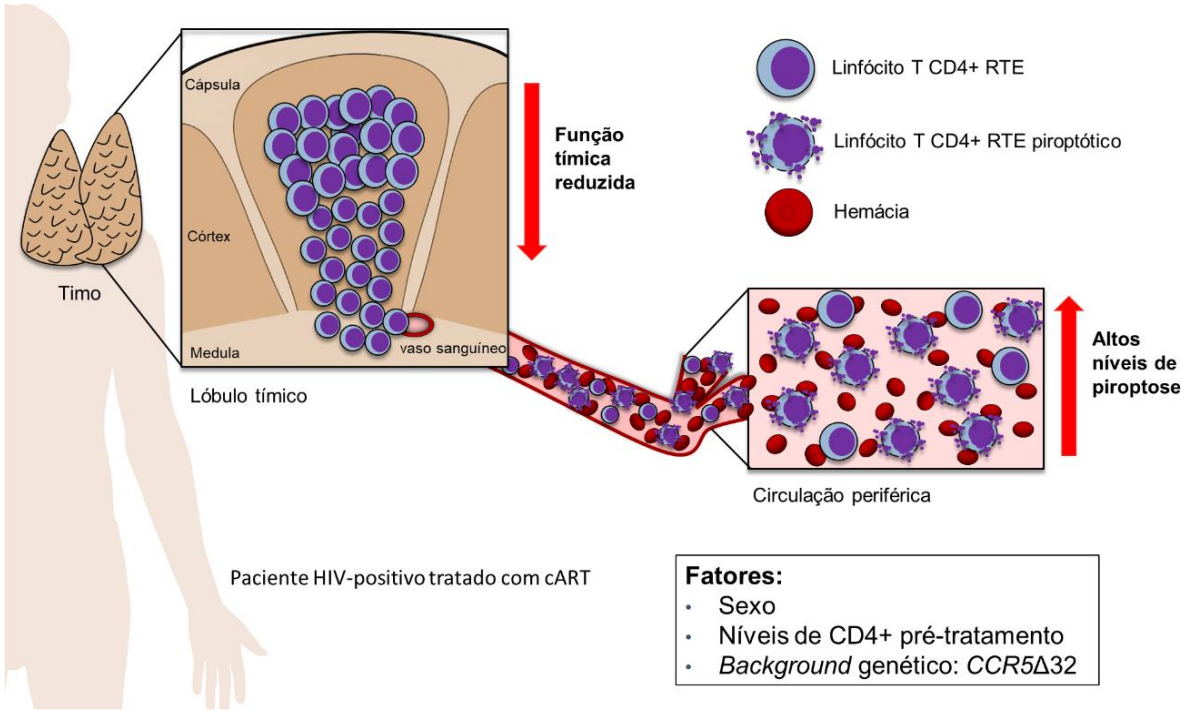
Com relação às variáveis clínicas, podemos perceber nos três artigos apresentados desse estudo que a contagem baixa pré-tratamento de linfócitos T CD4+ é um fator de risco importante, e bem descrito na literatura (CENDERELLO; DE MARIA, 2016; PINZONE et al., 2012; SONG et al., 2018; YANG et al., 2020), associado com a deficiência na recuperação imunológica. Essa é uma característica comum observada nos INR, que usualmente iniciam a ART com reduzidos níveis de células CD4+ (<500 céls/μL) e estágios avançados da doença, dificultando ainda mais a reconstituição imune (GAARDBO et al., 2012; KELLY et al., 2016; SONG et al., 2018). É com base nessas evidências, que atualmente recomenda-se o início da ART assim que os indivíduos são diagnosticados com HIV/AIDS, independentemente da contagem de linfócitos T CD4+ ou estágio clínico da doença (WHO, 2016).

Uma das principais consequências da infecção prolongada pelo HIV-1 é o enfraquecimento do sistema imune e consigo a exaustão do timo, órgão responsável pelo desenvolvimento e maturação dos linfócitos T. Como bem evidenciado em

nossas análises (Artigos I e III) e na literatura (FANG; COLANTONIO; UITTENBOGAART, 2008; FERRANDO-MARTINEZ et al., 2017; HE et al., 2015; KOLTE, 2013), além do timo sofrer involução ao longo da vida, reduzindo consideravelmente sua função, ele é extremamente prejudicado no curso da infecção pelo HIV-1. E quanto mais prolongada for essa infecção sem tratamento, mais debilitado pode ficar o timo do paciente. Além desses indivíduos continuarem a apresentar altos níveis de morte celular programada durante o tratamento, principalmente por piroptose (DOITSH; GREENE, 2016; PICONI et al., 2010; YOUNAS et al., 2016) (Artigos I e III, Apêndices A e B), a exaustão tímica tem sido demonstrada como sendo uma das principais causas de deficiência na recuperação imunológica dos pacientes em ART (CORBEAU; REYNES, 2011; HE et al., 2015; LI et al., 2011; RB-SILVA et al., 2019; ROSADO-SÁNCHEZ et al., 2017), principalmente nesses indivíduos que iniciam o tratamento com níveis baixos de células CD4+ e estágios avançado da doença. Isso também foi observado em nossas análises (Artigo III), reforçando ainda mais a necessidade do início da ART o mais cedo possível, pois uma vez debilitado o timo não consegue restabelecer os níveis de linfócitos T, mesmo o paciente em terapia e com CVP suprimida.

Como observado, dentre os diversos fatores e mecanismos envolvidos na baixa reconstituição imune, demonstrou-se que o perfil imune frequentemente regulado por variações genéticas seja a determinante para a condição de deficiência da recuperação imunológica. Principalmente aqueles fatores envolvidos na produção tímica, proliferação e diferenciação dos linfócitos T, ativação imune e morte celular periférica das células CD4+ (CORBEAU; REYNES, 2011; GAARDBO et al., 2012; YANG et al., 2020; YOUNAS et al., 2016), como observado também nos resultados do presente estudo (Figura 12). Esses podem ser os pontos chave para um entendimento mais claro sobre essa deficiência na reconstituição de linfócitos T CD4+ durante a ART, e podermos estabelecer estratégias terapêuticas eficazes para esses pacientes, agora conhecidos pelo *status* de INR.

Figura 12 – Mecanismos e fatores envolvidos na reconstituição imune dos pacientes HIV-positivos em ART da população estudada. Em destaque a função tímica reduzida e os altos níveis de morte celular por piroptose como principais mecanismos da deficiêna na recuperação imunológica.



Fonte: O autor (2022).

5 CONSIDERAÇÕES FINAIS

- Dos aspectos sociodemográficos e clínicos, o sexo masculino e a contagem baixa de células T CD4+ no início da ART (fatores de risco) mostraram-se influenciar significativamente na recuperação imunológica dos pacientes, sendo associados com a reduzida reconstituição de linfócitos T CD4+ durante a terapia;
- Baseado nos estudos da literatura acerca da influência de fatores genéticos na recuperação imunológica, pode-se perceber uma alta variabilidade interindividual nas respostas imune à ART e observar que os estudos disponíveis são fundamentados principalmente em alterações genéticas em genes de citocinas e de vias de morte celular programada;
- Com relação às variáveis genéticas avaliadas, foi encontrada associação do polimorfismo *CCR5*Δ32 com o ganho reduzido de células CD4+ durante a ART, tendo significante influência na reconstituição imune dos INR;
- A produção de linfócitos T CD4+ pelo timo (linfócitos T CD4+ naive e recém emigrados do timo) mostrou-se ser um mecanismo essencial para reconstituição imune dos pacientes em ART, onde a função tímica apresenta-se consideravelmente menor nos INR que nos indivíduos com boa resposta imunológica.
- Os níveis de morte celular programada, principalmente via piroptose, foi significativamente maior nos indivíduos INR, evidenciando ser também um importante mecanismo envolvido na deficiência de recuperação imunológica dos pacientes HIV-positivos em tratamento.

REFERÊNCIAS

ABBAS, A. K.; LICHTMAN, A. H.; PILLAI, S. HUMAN IMMUNODEFICIENCY VIRUS AND THE ACQUIRED IMMUNODEFICIENCY SYNDROME. Em: **Cellular and Molecular Immunology**. 9th. ed. Philadelphia: Elsevier, 2018. p. 475–87.

AHUJA, S. K. et al. CCL3L1-CCR5 genotype influences durability of immune recovery during antiretroviral therapy of HIV-1–infected individuals. **Nature Medicine**, v. 14, n. 4, p. 413–420, 2008.

AIDS.GOV. **A Timeline of HIV/AIDS**. Disponível em: <https://www.hiv.gov/hiv-basics/overview/history/hiv-and-aids-timeline>. Acesso em: 3 abr. 2020.

AIUTI, F.; MEZZAROMA, I. Failure to reconstitute CD4+ T-cells despite suppression of HIV replication under HAART. **AIDS Reviews**, v. 8, n. 2, p. 88–97, 2006.

ALDOUS, J. L.; HAUBRICH, R. H. Defining treatment failure in resource-rich settings. **Current Opinion in HIV and AIDS**, v. 4, n. 6, p. 459–466, 2009.

ALKHATIB, G. The biology of CCR5 and CXCR4. **Current Opinion in HIV and AIDS**, v. 4, n. 2, p. 96–103, 2009.

ALVES, B. M. et al. Estimating HIV-1 Genetic Diversity in Brazil Through Next-Generation Sequencing. **Frontiers in Microbiology**, v. 10, n. 749, p. 1–11, 9 abr. 2019.

ARAÚJO, L.; ALMEIDA, S. HIV-1 Diversity in the Envelope Glycoproteins: Implications for Viral Entry Inhibition. **Viruses**, v. 5, n. 2, p. 595–604, 2013.

ARENZANA-SEISDEDOS, F.; PARMENTIER, M. Genetics of resistance to HIV infection: Role of co-receptors and co-receptor ligands. **Seminars in Immunology**, v. 18, n. 6, p. 387–403, dez. 2006.

ARTS, E. J.; HAZUDA, D. J. HIV-1 antiretroviral drug therapy. **Cold Spring Harbor Perspectives in Medicine**, v. 2, n. 4, p. a007161, 2012.

AVERT. **HIV Timeline**. Disponível em: <https://timeline.avert.org/?531/North-America%E2%80%99s-first-AIDS-case>. Acesso em: 6 dez. 2022.

BACK, D.; MARZOLINI, C. The challenge of HIV treatment in an era of polypharmacy. **Journal of the International AIDS Society**, v. 23, n. 2, p. e25449, 3 fev. 2020.

BARRÉ-SINOUSSI, F. et al. Isolation of a T-lymphotropic retrovirus from a patient at risk for acquired immune deficiency syndrome (AIDS). **Science**, v. 220, n. 4599, p. 868–871, jan. 1983.

BBOSA, N.; KALEEBU, P.; SSEMWANGA, D. HIV subtype diversity worldwide. **Current Opinion in HIV and AIDS**, v. 14, n. 3, p. 153–160, 2019.

BLUMENTHAL, R.; DURELL, S.; VIARD, M. HIV entry and envelope glycoprotein-mediated fusion. **The Journal of Biological Chemistry**, v. 287, n. 49, p. 40841–40849, 2012.

BRANDENBERG, O. F. et al. The HIV-1 Entry Process: A Stoichiometric View. **Trends in Microbiology**, v. 23, n. 12, p. 763–774, 2015.

BRIGGS, J. A. G.; KRÄUSSLICH, H.-G. The Molecular Architecture of HIV. **Journal of Molecular Biology**, v. 410, p. 491–500, 2011.

CAMARGO, J. F. et al. CCR5 Expression Levels Influence NFAT Translocation, IL-2 Production, and Subsequent Signaling Events during T Lymphocyte Activation. **The Journal of Immunology**, n. 23, 2009.

CAMBOU, M. C.; LANDOVITZ, R. J. Novel Antiretroviral Agents. **Current HIV/AIDS Reports**, v. 17, n. 2, p. 118–124, 2020.

CARTER, C. A.; EHRLICH, L. S. Cell biology of HIV-1 infection of macrophages. **Annual Review of Microbiology**, v. 62, p. 425–43, 11 jan. 2008.

CASE, K. Nomenclature: Human immunodeficiency virus. **Annals of Internal Medicine**, v. 105, n. 1, p. 133, 1986.

CASTILHO, J. L.; MELEKHIN, V. V; STERLING, T. R. Sex differences in HIV outcomes in the highly active antiretroviral therapy era: a systematic review. **AIDS Research and Human Retroviruses**, v. 30, n. 5, p. 446–56, maio 2014.

CDC. Pneumocystis Pneumonia - Los Angeles. **Morbidity and Mortality Weekly Report**, v. 31, p. 1–3, 1981a.

CDC. Kaposi's sarcoma and Pneumocystis pneumonia among homosexual men--New York City and California. **Morbidity and Mortality Weekly Report**, v. 30, n. 25, p. 305–308, 3 jul. 1981b.

CDC. Opportunistic Infections and Kaposi's Sarcoma among Haitians in the United States. **Morbidity and Mortality Weekly Report**, v. 31, n. 26, p. 353–54, 1982a.

CDC. Epidemiologic Notes and Reports Pneumocystis carinii Pneumonia among Persons with Hemophilia A. **Morbidity and Mortality Weekly Report**, v. 31, n. 27, p. 365–67, 1982b.

CDC. Current Trends Update on Acquired Immune Deficiency Syndrome (AIDS) -- United States. **Morbidity and Mortality Weekly Report**, v. 31, n. 37, p. 507– 08, 1982c.

CENDERELLO, G.; DE MARIA, A. Discordant responses to cART in HIV-1 patients in the era of high potency antiretroviral drugs: Clinical evaluation, classification, management prospects. **Expert Review of Anti-Infective Therapy**, v. 14, n. 1, p. 29–40, 2016.

CHEN, F. P. et al. Effects of estradiol and progestogens on human breast cells: Regulation of sex steroid receptors. **Taiwanese Journal of Obstetrics and Gynecology**, v. 52, n. 3, p. 365–373, 1 set. 2013.

COHEN, M. S. et al. Acute HIV-1 Infection. **The New England Journal of Medicine**, v. 364, n. 20, p. 1943–1954, 2013.

CORBEAU, P.; REYNES, J. Immune reconstitution under antiretroviral therapy : the new challenge in HIV-1 infection. **BLOOD**, v. 117, n. 21, p. 5582–90, 2011.

DE CLERCQ, E.; LI, G. Approved Antiviral Drugs over the Past 50 Years. **Clinical Microbiology Reviews**, v. 29, n. 3, p. 695–747, 2016.

DEEKS, S. G. et al. HIV infection. **Nature Reviews Disease Primers**, v. 1, p. 1–22, 2015.

DOITSH, G.; GREENE, W. C. Dissecting How CD4 T Cells Are Lost during HIV Infection. **Cell Host and Microbe**, v. 19, n. 3, p. 280–291, 2016.

DOLAN, M. J. et al. CCL3L1 and CCR5 influence cell-mediated immunity and affect HIV-AIDS pathogenesis via viral entry-independent mechanisms. **Nature Immunology**, v. 8, n. 12, p. 1324–1336, 2007.

DONNELLY, M. R.; CIBOROWSKI, P. Proteomics, biomarkers, and HIV-1: A current perspective. **Proteomics - Clinical Applications**, v. 10, n. 2, p. 110–125, 2016.

DUMAS, F.; PREIRA, P.; SALOME, L. Membrane organization of virus and target cell plays a role in HIV entry. **Biochimie**, v. 107, p. 22–27, 2014.

EISINGER, R. W.; DIEFFENBACH, C. W.; FAUCI, A. S. HIV viral load and transmissibility of HIV infection undetectable equals untransmittable. **Journal of the American Medical Association**, v. 321, n. 5, p. 451–452, 5 fev. 2019.

ELLWANGER, J. H.; KAMINSKI, V. DE L.; CHIES, J. A. B. CCR5 gene editing - Revisiting pros and cons of CCR5 absence. **Infection, Genetics and Evolution**, v. 68, p. 218–220, 1 mar. 2019.

ENGELMAN, A.; CHEREPANOV, P. The structural biology of HIV-1: mechanistic and therapeutic insights. **Nature Reviews Microbiology**, v. 10, p. 279–90, 2012.

FANALES-BELASIO, E. et al. HIV virology and pathogenetic mechanisms of infection: A brief overview. **Annali dell'Istituto Superiore di Sanita**, v. 46, n. 1, p. 5–14, 2010.

FANG, H. R. T.; COLANTONIO, A. D.; UITTENBOGAART, C. H. The role of the thymus in HIV infection : a 10 year perspective. **AIDS**, v. 22, n. 2, p. 171–184, 2008.

FARIA, N. R. et al. The early spread and epidemic ignition of HIV-1 in human populations. **Science**, v. 346, n. 6205, p. 56–61, 2014.

FERRANDO-MARTINEZ, S. et al. Thymic Function Failure Is Associated With Human Immunodeficiency Virus Disease Progression. **Clinical Infectious Diseases**, v. 64, n. 9, p. 1191–1197, 1 maio 2017.

FIOCRUZ. **A Epidemia da AIDS Através do Tempo**. Disponível em: <https://www.ioc.fiocruz.br/aids20anos/linhadotempo.html>. Acesso em: 6 dez. 2022.

FLANAGAN, C. A. Receptor conformation and constitutive activity in CCR5 chemokine receptor function and HIV infection. Em: **Advances in Pharmacology**. 1. ed. [s.l.] Elsevier Inc., 2014. v. 70p. 215–263.

FRASER, C. et al. Virulence and pathogenesis of HIV-1 infection: an evolutionary perspective. **Science**, v. 343, n. 6177, p. 1243727, 2014.

FREED, E. O. HIV-1 assembly, release and maturation. **Nature Reviews Microbiology**, v. 13, n. 8, p. 484–496, 29 ago. 2015.

GAARDBO, J. C. et al. Incomplete immune recovery in HIV infection: Mechanisms, relevance for clinical care, and possible solutions. **Clinical and Developmental Immunology**, v. 67, n. 670957, p. 1–17, 2012.

GALLO, R. et al. Isolation of human T-cell leukemia virus in acquired immune deficiency syndrome (AIDS). **Science**, v. 220, n. 4599, p. 865–867, 1983.

GAO, F. et al. Origin of HIV-1 in the chimpanzee Pan troglodytes troglodytes. **Nature**, v. 397, n. 6718, p. 436–41, 1999.

GRANDE, F. et al. CCR5/CXCR4 Dual Antagonism for the Improvement of HIV Infection Therapy. **Molecules**, v. 24, n. 550, p. 1–11, 2 fev. 2019.

GREENBLATT, R. et al. Genetic and clinical predictors of CD4 lymphocyte recovery during suppressive antiretroviral therapy: Whole exome sequencing and antiretroviral therapy response phenotypes. **PLoS ONE**, v. 14, n. 8, p. e0219201, 2019.

GRIMALDI, C. M. et al. Estrogen alters thresholds for B cell apoptosis and activation. **The Journal of clinical investigation**, v. 109, n. 12, p. 1625–33, jun. 2002.

GUIHOT, A.; TUBIANA, R.; BRETON, G. ET AL. Immune and virological benefits of 10 years of permanent viral control with antiretroviral therapy. **AIDS**, v. 24, n. 4, p. 614–617, 2010.

HAAS, D. W. et al. Immunogenetics of CD4 lymphocyte count recovery during antiretroviral therapy: An AIDS Clinical Trials Group study. **The Journal of Infectious Diseases**, v. 194, n. 8, p. 1098–107, 15 out. 2006.

HE, S. et al. Thymic function is most severely impaired in chronic HIV-1 infection, but individuals with faster disease progression during early HIV-1 infection expressed lower levels of RTEs. **JAIDS**, v. 70, n. 5, p. 472–478, 2015.

HEMELAAR, J. The origin and diversity of the HIV-1 pandemic. **Trends in Molecular Medicine**, v. 18, n. 3, p. 182–192, 2012.

HILL, A.; POZNIAK, A. HIV treatment cascades : how can all countries reach the UNAIDS 90 – 90 – 90 target ? **AIDS**, v. 29, n. 18, p. 2523–25, 2015.

HLADIK, F.; MCELRATH, M. J. Setting the stage: Host invasion by HIV. **Nature Reviews Immunology**, v. 8, n. 6, p. 447–457, jun. 2008.

HUNT, P. W. et al. Continued CD4 cell count increases in HIV-infected adults experiencing 4 years of viral suppression on antiretroviral therapy. **AIDS**, v. 17, n. 13, p. 1907–1915, 2003.

HUTCHINSON, J. F. The Biology and Evolution of HIV. **Annual Review of Anthropology**, v. 30, n. May, p. 85–108, 2001.

HYMES, K. B. ET AL. Kaposi's sarcoma in homosexual man: A report of eight cases. **The Lancet**, v. 2, n. 8247, p. 598–600, 1981.

JAFFAR, S. et al. The natural history of HIV-1 and HIV-2 infections in adults in Africa: a literature review. **Bulletin of the World Health Organization**, v. 82, n. 6, p. 462–469, 2004.

KELLY, C. et al. Discordant Immune Response with Antiretroviral Therapy in HIV-1: A Systematic Review of Clinical Outcomes. **PLoS ONE**, v. 11, n. 6, p. e0156099, 2016.

KLEIN, S. L.; FLANAGAN, K. L. Sex differences in immune responses. **Nature Reviews Immunology**, v. 16, n. 10, p. 626–638, 2016.

KOLTE, L. Thymic function in HIV-infection. **Danish Medical Journal**, v. 60, n. 4, p. 44–52, 2013.

KONG, Y. et al. Two types of poor immunological responder showing distinct responses to long-term HAART. **International Journal of Infectious Diseases**, v. 86, p. 178–187, 1 set. 2019.

LEDERGERBER, B. et al. Predictors of trend in CD4-positive T-cell count and mortality among HIV-1-infected individuals with virological failure to all three antiretroviral-drug classes. **The Lancet**, v. 364, n. 9428, p. 51–62, 2004.

LI, G.; DE CLERCQ, E. HIV Genome-Wide Protein Associations: a Review of 30 Years of Research. **Microbiology and Molecular Biology Reviews (MMBR)**, v. 80, n. 3, p. 679–731, 2016.

LI, T. et al. Reduced thymic output is a major mechanism of immune reconstitution failure in HIV-infected patients after long-term antiretroviral therapy. **Clinical Infectious Diseases**, v. 53, n. 9, p. 944–51, nov. 2011.

LIHANA, R. W. et al. Update on HIV-1 diversity in Africa: A decade in review. **AIDS Reviews**, v. 14, n. 2, p. 83–100, 2012.

LINDEMANN, D.; STEFFEN, I.; PÖHLMANN, S. Cellular entry of retroviruses. **Advances in Experimental Medicine and Biology**, v. 790, p. 128–149, 2013.

MAARTENS, G.; CELUM, C.; LEWIN, S. R. HIV infection: Epidemiology, pathogenesis, treatment, and prevention. **The Lancet**, v. 384, n. 9939, p. 258–271, 2014.

MAEDA, K. et al. Discovery and Development of Anti-HIV Therapeutic Agents: Progress Towards Improved HIV Medication. **Current Topics in Medicinal Chemistry**, v. 19, n. 18, p. 1621–1649, 2019.

MANN, J. M.; CHIN, J. AIDS: A Global Perspective. **The New England Journal of Medicine**, v. 319, p. 302–303, 1988.

MASUR, H.; ET AL. An Outbreak of community acquired Pneumocystis carinii pneumonia: initial manifestation of cellular immune dysfunction. **The New England Journal of Medicine**, v. 305, n. 24, p. 1431–1438, 1981.

MELHUISH, A.; LEWTHWAITE, P. Natural history of HIV and AIDS. **Medicine**, v. 46, n. 6, p. 356–361, 1 jun. 2018.

MINISTÉRIO DA SAÚDE. **Manual Técnico para o Diagnóstico da Infecção pelo HIV em Adultos e Crianças**. Brasília: Ministério da Saúde-Secretaria de Vigilância em Saúde, 2018a.

MINISTÉRIO DA SAÚDE. **Protocolo Clínico e Diretrizes Terapêuticas para Manejo da Infecção pelo HIV em Adultos.** Brasília: Ministério da Saúde-Secretaria de Vigilância em Saúde, 2018b.

MINISTÉRIO DA SAÚDE. **Boletim Epidemiológico HIV/AIDS.** Brasília: Ministério da Saúde-Secretaria de Vigilância em Saúde, 2021

MOLLOY, E. J. et al. Sex-specific alterations in neutrophil apoptosis: the role of estradiol and progesterone. **BLOOD**, v. 102, n. 7, p. 2653–59, 2003.

MOUREZ, T.; SIMON, F.; PLANTIER, J.-C. Non-M variants of human immunodeficiency virus type 1. **Clinical microbiology reviews**, v. 26, n. 3, p. 448–61, 2013.

NAIF, H. M. Pathogenesis of HIV infection. **Infectious Disease Reports**, v. 5, n. supl.1, p. 26–30, 2013.

OKOYE, A. A.; PICKER, L. J. CD4(+) T-cell depletion in HIV infection: mechanisms of immunological failure. **Immunological Reviews**, v. 254, n. 1, p. 54–64, 2013.

PACHECO, Y. M. et al. Evaluation of the pharmacogenetics of immune recovery in treated HIV-infected patients. **Expert Opin. Drug Metab. Toxicol.**, v. 10, n. 1, p. 81–101, 2014.

PANTAZIS, N. et al. Long-term evolution of CD4+ cell count in patients under combined antiretroviral therapy. **AIDS**, v. 33, n. 10, p. 1645–1655, 2019.

PAU, A. K.; GEORGE, J. M. Antiretroviral Therapy: Current Drugs. **Infectious Disease Clinics of North America**, v. 28, n. 3, p. 371–402, 2014.

PEETERS, M.; JUNG, M.; AYOUBA, A. The origin and molecular epidemiology of HIV. **Expert review of anti-infective therapy**, v. 11, n. 9, p. 885–896, 2013.

PICONI, S. et al. Immune activation, apoptosis, and Treg activity are associated with persistently reduced CD4 R T-cell counts during antiretroviral therapy. **AIDS**, v. 24, n. 13, p. 1991–2000, 2010.

PIDO-LOPEZ, J.; IMAMI, N.; ASPINALL, R. Both age and gender affect thymic output: More recent thymic migrants in females than males as they age. **Clinical and Experimental Immunology**, v. 125, n. 3, p. 409–413, 2001.

PINZONE, M. R. et al. HIV RNA suppression and immune restoration: Can we do better? **Clinical and Developmental Immunology**, v. 51, n. 515962, p. 1–12, 2012.

QI, J. et al. Advances in Developing CAR T-Cell Therapy for HIV Cure. **Frontiers in Immunology**, v. 11, n. 361, p. 1–13, 10 mar. 2020.

RB-SILVA, R. et al. Thymic Function as a Predictor of Immune Recovery in Chronically HIV-Infected Patients Initiating Antiretroviral Therapy. **Frontiers in Immunology**, v. 10, n. 25, p. 1–13, 5 fev. 2019.

ROSADO-SÁNCHEZ, I. et al. Thymic Function Impacts the Peripheral CD4/CD8 Ratio of HIV-Infected Subjects. **Clinical Infectious Diseases**, v. 64, n. 2, p. 152–158, 15 jan. 2017.

SHARP, P. M.; HAHN, B. H. Origins of HIV and the AIDS pandemic. **Cold Spring Harbor Perspectives in Medicine**, v. 1, n. 1, p. 1–22, 2011.

SHAW, G. M.; HUNTER, E. HIV Transmission. **Cold Spring Harbor Perspectives in Medicine**, v. 2, n. 11, p. a006965, 2012.

SILICIANO, R. F.; GREENE, W. C. HIV latency. **Cold Spring Harbor Perspectives in Medicine**, v. 1, n. 1, p. a007096, 1 set. 2011.

SLEASMAN, J. W.; GOODENOW, M. M. HIV-1 infection. **The Journal of allergy and clinical immunology**, v. 111, n. 2 Suppl, p. S582-92, 1 fev. 2003.

SMITH, C. J. et al. The potential for CD4 cell increases in HIV-positive individuals who control viraemia with highly active antiretroviral therapy. **AIDS**, v. 17, n. 7, p. 963–969, 2003.

SONG, A. et al. From CD4-based initiation to treating all HIV-infected adults immediately: An evidence-based meta-analysis. **Frontiers in Immunology**, v. 9, n. 212, p. 1–9, 2018.

STEVENSON, M. HIV-1 pathogenesis. **Nature Medicine**, v. 9, n. 7, p. 853–60, 2003.

STRAUB, R. H. The Complex Role of Estrogens in Inflammation. **Endocrine Reviews**, v. 28, n. 5, p. 521–574, 1 ago. 2007.

SWANSTROM, R.; COFFIN, J. HIV-1 pathogenesis: the virus. **Cold Spring Harbor Perspectives in Medicine**, v. 2, n. 12, p. a007443, dez. 2012.

TAYLOR, B. S. et al. The Challenge of HIV-1 Subtype Diversity Origin of HIV and Mechanisms of HIV Diversity. **The New England Journal of Medicine**, v. 358, p. 1590–602, 2008.

TSENG, A.; SEET, J.; PHILLIPS, E. J. The Evolution of Three Decades of Antiretroviral Therapy: Challenges, Triumphs and the Promise of the Future. **British Journal of Clinical Pharmacology**, v. 79, n. 2, p. 182–194, 2014.

UNAIDS. **Ending AIDS: Progress towards the 90-90-90 targets.** Joint United Nations Programme on HIV/AIDS (UNAIDS), 2017.

UNAIDS. **UNAIDS DATA 2021.** Joint United Nations Programme on HIV/AIDS (UNAIDS), 2021a.

UNAIDS. **Global AIDS Update: CONFRONTING INEQUALITIES Lessons for pandemic responses from 40 years of AIDS.** Joint United Nations Programme on HIV/AIDS (UNAIDS), 2021b.

VIJAYAN, K. K. V. et al. Pathophysiology of CD4+ T-Cell Depletion in HIV-1 and HIV-2 Infections. **Frontiers in Immunology**, v. 8, n. 580, p. 1–8, 23 maio 2017.

VINCENT, T. ET AL. The Immunological Response to Highly Active Antiretroviral Therapy Is Linked to CD4 + T-Cell Surface CCR5 Density. **JAIDS**, v. 43, n. 3, p. 376–381, 2006.

VUILLEUMIER, S.; BONHOEFFER, S. Contribution of recombination to the evolutionary history of HIV. **Current Opinion in HIV and AIDS**, v. 10, n. 2, p. 84–89, 2015.

WANG, H. et al. Estimates of global, regional, and national incidence, prevalence, and mortality of HIV, 1980-2015: the Global Burden of Disease Study 2015. **Lancet HIV**, v. 3, n. 8, p. e361-87, 2016.

WATTS, J. M. et al. Architecture and secondary structure of an entire HIV-1 RNA genome. **Nature**, v. 460, n. 7256, p. 711–716, 6 ago. 2009.

WHO. **Consolidated Guidelines on the Use of Antiretroviral Drugs for Treating and Preventing HIV Infection: Recommendations for a Public Health Approach.** World Health Organization, 2016.

WHO. **Transition to new antiretrovirals in HIV programmes.** World Health Organization, 2017.

WHO. **Global HIV Programme: HIV data and statistics**. World Health Organization, 2022. Disponível em: <https://www.who.int/teams/global-hiv-hepatitis-and-stis-programmes/hiv/strategic-information/hiv-data-and-statistics>. Acesso em: 7 jun. 2022.

WILEN, C. B.; TILTON, J. C.; DOMS, R. W. HIV: Cell Binding and Entry. **Cold Spring Harbor Perspectives in Medicine**, v. 2, n. 8, 2012.

WOLFF, M. J. et al. A decade of HAART in Latin America: Long term outcomes among the first wave of HIV patients to receive combination therapy. **PLoS ONE**, v. 12, n. 6, p. 1–15, 2017.

WOROBEY, M. et al. Direct evidence of extensive diversity of HIV-1 in Kinshasa by 1960. **Nature**, v. 455, n. 7213, p. 661–664, 2 out. 2008.

YANG, X. et al. Incomplete immune reconstitution in HIV/AIDS patients on antiretroviral therapy: Challenges of immunological non-responders. **Journal of Leukocyte Biology**, v. 107, n. 4, p. 597–612, 1 abr. 2020.

YOUNAS, M. et al. Immune activation in the course of HIV-1 infection: Causes, phenotypes and persistence under therapy. **HIV Medicine**, v. 17, n. 2, p. 89–105, 1 fev. 2016.

ZHU, T. et al. An African HIV-1 sequence from 1959 and implications for the origin of the epidemic. **Nature**, v. 391, n. 6667, p. 594–597, 5 fev. 1998.

APÊNDICE A – ARTIGO PUBLICADO NA *INFECTION, GENETICS AND EVOLUTION*: POLIMORFISMO DO *IL18* E A RECUPERAÇÃO IMUNE

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Research Paper

IL18 gene polymorphism and its influence on CD4+ T-cell recovery in HIV-positive patients receiving antiretroviral therapy

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ABSTRACT

Background: Pyroptosis has been reported to be critical in human immunodeficiency virus type 1 (HIV-1) pathogenesis and acquired immunodeficiency syndrome (AIDS) progression. Even after achieving viral suppression to undetectable levels during antiretroviral therapy (ART), exacerbated CD4+ T-cell death by pyroptosis has been suggested as one of the main causes of immunological non-response. Thus, variants in genes of pyroptosis pathway were studied in individuals with poor CD4+ T-cell reconstitution under antiretroviral therapy against HIV-1.

Methods: 248 virologically suppressed ART-treated patients, 126 immunological non-responders (INR) and 122 immunological responders (IR) were recruited. Genotyping was performed using TaqMan probe-based realtime PCR platform. Genotype-guided flow cytometry analysis with general and recent thymic emigrant (RTE) CD4+ T-cells in pyroptosis was performed based on associated polymorphisms.

Results: Both IL18 rs187238 G allele and GG genotype were associated as protection factors against poor CD4+ T-cell recovery (OR = 0.22; 95%CI = 0.50–0.77; P = .010 and OR = 0.58; 95%CI = 0.36–0.93; P = .022, respectively). It was demonstrated a statistical association between IL18 rs187238 genotypes of ART-treated patients and death by Caspase-1 levels (P = .020). The GG genotype showed lower pyroptotic RTE CD4+ T-lymphocytes levels in the ART-treated groups comparing with CC (P = .029) and CG (P = .018) genotypes, suggesting that the G allele presence may be related to a lower IL-18 production and thus reduced dead CD4+ T-cells levels by Caspase-1.

Conclusion: We observed that IL18 G variant allele and genotype were associated with a better immunological response, which may influence on immunological recovery of HIV-positive patients receiving antiretroviral therapy, and low Caspase-1 activity levels was observed on GG genotype when compared CC genotypes.

1. Introduction

It is widely known that approximately 30% of patients achieving plasma human immunodeficiency virus (HIV) load suppression do not recover their CD4+ T-cell levels during antiretroviral therapy (ART). These ART-treated patients are defined as immunological non-responders (Aiuti and Mezzaroma, 2006; Li et al., 2011). Despite being considered a multifactorial condition, poor CD4+ T-cell reconstitution has been associated with two main mechanisms: reduced CD4+ T-cells production and exacerbated cell death of these lymphocytes (Corbeau and Reynes, 2011; Gaardbo et al., 2012).

Pyroptosis is inflammatory programmed cell death, mainly mediated by caspase-1, that has emerged as an important mechanism of innate immunity against pathogens (Boucher et al., 2016; Jorgensen and Miao, 2015). The main activation pathway of caspase-1 occurs through inflammasome, a signaling protein complex assembled in response to cell disorders or intracellular pathogens. Several types of inflammasome are able to activate caspase-1 but, specifically in HIV-1 infection, the NLRP3 and IFI16 inflammasomes are the major signaling pathways into cells that recognize this virus in the early stages of its

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replicative cycle and prevent cell infection, called non-permissive cells. Both IFI16 and NLRP3 sensors connect to caspase-1 via ASC, an adapter protein containing a CARD (caspase activation and recruitment domain) region that promote the interaction. Among CARDs variety, studies have demonstrated that CARD8 acts as a negative regulator of the NLRP3 inflammasome, thus dampening NF-κβ, with consequent absence of caspase-1 activation (Ito et al., 2014; Kesavardhana and Kanneganti, 2017; Miao, 2011). Once caspase-1 is activated, the cleavage of the pro-IL-1B and pro-IL-18 molecules occurs, converting them into active proinflammatory cytokines, resulting in an inflammatory process following HIV infection (Kesavardhana and Kanneganti, 2017; Man et al., 2017; Miao, 2011).

In HIV-1 infection, it is believed that pyroptosis presents as a pathogenic vicious cycle, in which inflammatory stimuli are released, recruiting more cells to the sites of infection thus promoting augmented cell death (Doitsh et al., 2014). Hence, the activation of the pyroptosis pathway turns out to be a form of chronic immune activation in lymphoid tissues, contributing to the progression to AIDS (Doitsh and Greene, 2016; Gaiha and Brass, 2014).

In this study, we evaluated SNPs in *CARD8, NLRP3, IL1B, IL18* and *IFI16* genes involved in the pyroptosis pathway, aimed at finding a possible association with poor immune recovery of CD4 + T cells in subjects living with HIV submitted to antiretroviral therapy.

2. Methodology

2.1. Study population

The study population consisted of 248 HIV-positive patients (107 males and 141 females) under ART enrolled at Instituto de Medicina Integral Professor Fernando Figueira (IMIP), Pernambuco state (Northeast Brazil), between 2011 and 2014. The subjects were recruited according to the following inclusion criteria: age over 18 years old, on ART for at least one year with prolonged undetectable viral load (< 50 copies/mL), good adherence to treatment; and exclusion criteria: pregnancy, autoimmune diseases and history of injecting drug use. Sociodemographic and clinical data were collected from medical records: age and body mass at ART start date; time until ART start after HIV infection tests; ART regimens received (2NRTI + PI/r or 2NRTI + NNRTI regimens, as recommended by the Brazilian guidelines available at the time of patient recruitment); pre- and post-treatment viral loads as well as CD4+ T-cell counts; and serological data regarding co-infections (hepatitis B virus – HBV, hepatitis C virus – HCV, syphilis, cytomegalovirus – CMV, toxoplasmosis, human T-cell lymphotropic virus types I and II (HTLV-I/II)). All patients answered standard questionnaires and signed written informed consent, providing blood samples for genetic and immunological analyses. The IMIP Research Ethics Committee approved this study (protocol number: 3629-13)

2.2. Determination of study groups

The ART-treated patients, who had persistently undetectable plasma HIV concentration (< 50 copies/mL) during the first year of therapy, were classified according to gains in CD4+ T cell counts or percentages. Patients that gained < 200 CD4+ T-cells/μL compared with pretreatment count or presented T-cell percentages in relation to total lymphocytes < 30% (if absolute counts were not available) after the first year of ART were classified as immunological non-responders (INR) (adapted from (Li et al., 2011). All others subjects were defined as immunological responders (IR).

2.3. Selection of single base polymorphisms (SNPs)

The SNPs selection was based on the literature, functional characteristics and minor allele frequency (MAF) > 10% in European, Amerindian and African populations, which reflect the Brazilian genetic admixture (Coelho et al., 2015). Thus, five SNPs in pyroptosis pathway genes were evaluated: *NLRP3* (rs10754558 C > G, MAF: 0.35) inflammasome activator; *CARD8* (rs2043211 A > T, MAF: 0.32) – molecular adapter; *IL1B* (rs1143634 G > A, MAF: 0.13) and *IL18* (rs187238 C > G, MAF: 0.21) – effector molecules; and *IFI16* (rs6940 A > T, MAF: 0.23) – intracellular DNA sensor. SNPs selection was performed using NCBI dbSNP (https://www.ncbi.nlm.nih.gov/projects/SNP/) and 1000 Genomes Project Browser (http://browser.1000genomes.org).

2.4. Sampling and DNA extraction

Peripheral blood sample (4 mL) was collected from all ART-treated patients in EDTA tubes for genomic DNA extraction using *mini salting out* protocol (Miller et al., 1988) with "in-house" modifications. DNA quantification and purity were assessed using the Thermo Scientific™ NanoDrop 2000 (ThermoFisher) spectrophotometer, considering absorbance values: 260/280 nm and 260/230 nm ratios.

Genotyping was performed using TaqMan® allele-specific probes: *NLRP3* rs10754558 (C__26052028_10), *CARD8* rs2043211 (C__11708080_1), *IL18* rs187238 (C__2408543_10), *IL1B* rs1143634 (C__9546517_10) and *IFI16* rs6940 (C__7483779_10); on ABI® real-time platform 7500 (Applied Biosystems) using protocols recommended by the manufacturer.

2.5. Genotype-guided flow cytometry analysis

Flow cytometric analysis was performed according to *IL18* rs187238 polymorphism genotypes, the only one associated with immunological non-response. The Peripheral blood mononuclear cells (PBMCs) were separated by Ficoll-Paque density gradient technique and washed twice in phosphate-buffered saline (PBS 1×), following manufacturer's guidelines (Healthcare, 2007). Cell viability (> 90% in average) was determined by Trypan blue (0.4%) exclusion test. For pyroptosis detection, we employed FAM-FLICA Caspase-1 (FAM-YVAD-FMK) Assay kit following the manufacturer's instructions (ImmunoChemistry Technologies), and PBMCs were also stained with immunofluorescent monoclonal antibodies APC-CD4 and PE-CD31 (BD Biosciences) and analyzed by flow cytometry using BD Accuri C6 cytometer (BD Biosciences). In this analysis, 20,000 events were acquired and gated to detect death by Caspase-1 CD4+ T-cells (CD4 + FLICA-Caspase1+) and dead recent thymic emigrant CD4+ T-cells by Caspase-1 activation (CD4 + CD31 + FLICA-Caspase1+). Acquired data were analyzed using FCS Express 6 Plus software.

2.6. Statistical analysis

Genotypic and allelic frequencies were calculated by direct counting, and $\chi 2$ test was used to verify the conformity with Hardy-Weinberg equilibrium. Sample size analyses were performed in the G*Power® software using post-roc power test. Student's *t*-test was used to compare means of groups for variables that followed a normal distribution (according to Shapiro-Wilk test), and Wilcoxon-Mann-Whitney test for variables that do not follow a normal distribution. Fisher exact test was used to assess whether genetic, sociodemographic and clinical variables were associated with INR status. The variables deemed to have clinical importance or reached a *p*-value ≤.20 during univariate analysis were included in the logistic regression analysis. The statistical significance level (α) was set at 0.05 for all tests. Statistical analyses were performed using R program, version 3.5.0.

Table 1
Allelic and genotype frequencies of polymorphisms among ART-treated groups (immunological non-responders and immunological responders).

Gene (SNP)		INR n (%)	IR n (%)	OR (95%CI)	*p-Value*[a]
NLRP3	*Genotypes*				
rs10754558	CC	39 (38)	40 (39)	Reference	–
(*n* = 206/248)	CG	48 (47)	46 (45)	0.93 (0.49–1.77)	0.88
	GG	16 (15)	17 (16)	1.03 (0.42–2.53)	1.00
	Alelles				
	C	126 (61)	126 (61)	1 (0.66–1.51)	1.00
	G	80 (39)	80 (39)		
CARD8	*Genotypes*				
rs2043211	AA	49 (56)	43 (46)	Reference	–
(*n* = 181/248)	AT	32 (36)	44 (47)	1.56 (0.81–3.02)	0.16
	TT	7 (8)	6 (7)	0.97 (0.25–3.69)	1.00
	Alelles				
	A	130 (74)	130 (70)	1.21	0.41
	T	46 (26)	56 (30)	(0.74–1.98)	
IL1B	*Genotypes*				
rs1143634	GG	59 (60)	61 (64)	Reference	–
(*n* = 195/248)	GA	35 (35)	33 (34)	0.91 (0.48–1.73)	0.88
	AA	5 (5)	2 (2)	0.39 (0.03–2.5)	0.44
	Alelles				
	G	153 (77)	155 (81)	0.81	0.46
	A	45 (23)	37 (19)	(0.48–1.36)	
IL18	*Genotypes*				
rs187238	CC	60 (60)	46 (49.5)	Reference	–
(*n* = 193/248)	CG	36 (36)	33 (35.5)	0.84 (0.44–1.61)	0.64
	GG	4 (4)	14 (15)	0.22 (0.50–0.77)	0.010
	Alelles				
	C	156 (78)	125 (67)	0.58	0.022
	G	44 (22)	61 (33)	(0.36–0.93)	
IFI16	*Genotypes*				
rs6940	AA	62 (64)	66 (73)	Reference	–
(*n* = 187/248)	AT	32 (33)	21 (23)	0.62 (0.30–1.23)	0.19
	TT	3 (3)	3 (4)	0.94 (0.12–7.28)	1.00
	Alelles				
	A	156 (80)	153 (85)	0.72	0.27
	T	38 (19)	27 (15)	(0.40–1.29)	

INR = Immunological non-responders; IR = Immunological Responders; OR = Odds Ratio; SNP = Single Nucleotide Polymorphism.
[a] Fisher's Exact Test.

3. Results

3.1. Genotype analysis

A total of 248 virologically suppressed patients living with HIV were recruited for this study. Thus, 126 ART-treated patients (62 males and 64 females) were classified as INR, whereas the remaining 122 subjects (45 males and 77 females) were included in the IR group; the median age at the beginning of therapy in both groups was 32 years (28.5–37.5). Population characterization data are shown in Supplementary Table 1.

Five variants of genes involved in cell death pathway by pyroptosis (*NLRP3*, *CARD8*, *IL1B*, *IL18* and *IFI16*) were genotyped (Table 1). Genotypes distribution was consistent with the Hardy-Weinberg equilibrium for all the variants analyzed. The post-hoc power tests for all genes was > 80%, where the sampled N of the population has shown representative for the analysis.

The *NLRP3* polymorphism (rs10754558) analysis included 206 subjects (INR = 103 and IR = 103 groups). The G allele frequency in the both groups was 39%. Fisher's exact test showed that there was no association of neither G variant allele (OR = 1.00; 95%CI = 0.66–1.51; $P = 1.00$) nor GG genotype (OR = 1.03; CI-95% = 0.42–2.53; $P = 1.00$) with immunological recovery failure in the analyzed population. The *CARD8* rs2043211 polymorphism also showed no association with poor CD4 + T-cell reconstitution, either the T allele (OR = 0.97, 95%CI = 0.25–3.69; $P = 1.00$) or the TT genotype (OR = 1.21,95%CI = 0.74–1.98, $P = .41$). The polymorphism frequencies observed in the INR and IR groups were 26% and 30% respectively, being analyzed in 181 individuals (88 immunological non-responders and 93 immunological responders).

The *IL1B* (rs1143634) and *IFI16* (rs6940) polymorphisms were analyzed in 195 and 187 individuals, respectively. The allelic frequency of variant A (rs1143634 G > A) was 23% in the INRs and 19% in the IR group. Association analysis for this polymorphism did not show correlation between the AA genotype (OR = 0.39; 95%CI = 0.03–2.5; $P = .44$) and A variant allele (OR = 0.81; 95%CI = 0.48–1.36; $P = .46$) with immunological recovery failure. For *IFI16* gene, the observed T allele (rs6940 A > T) frequencies were 19% in the INR group and 15% in the IR group. The hypothesis that this variant could be associated with immunological recovery failure was not confirmed in our study population: TT genotype (OR = 0.94; 95%CI = 0.12–7.28; $P = 1.00$) or T variant allele (OR = 0.72; 95%CI = 0.40–1.29; $P = .27$).

The *IL18* (rs187238 C > G) polymorphism analysis demonstrated a G allele frequency of 22% in the immunological non-responders group and 33% in the immunological responders group, among a total of 193 individuals genotyped (100 in the case group and 93 in the control group). According to Fisher test both the GG genotype (OR = 0.22; 95%CI = 0.50–0.77; $P = .010$) and the G variant allele (OR = 0.58; 95%CI = 0.36–0.93; $P = .022$) were statistically associated with immune reconstitution as a protection factor against the occurrence of immunological non-response in the ART-treated groups.

3.2. Genotype-guided immunophenotypic analysis

The genotype-guided analysis consisted of 29 individuals, 13 immunological non-responders (CC = 01; CG = 05; GG = 07) and 16 immunological responders (CC = 02; CG = 04; GG = 10) (Fig. 1).

When comparing the CD4 + /FLICA-Caspase1 + T-cell population, no statistical association ($P = .67$) was found among the ART-treated groups regarding genotype presence. Despite this, it was observed a lower death CD4 + T-cell levels in the ART-treated individuals carrying the G variant allele.

Regarding recent thymic emigrants (RTE) CD4 + T-cell death by Caspase-1 (CD4 + /CD31 + FLICACaspase1 +) it was demonstrated statistical association between *IL18* genotypes of ART-treated patients and pyroptosis levels ($P = .020$). An immunological non-responder individual with CC genotype showed higher Caspase-1 RTE CD4 + T-cell levels (99.3%) than individuals carrying GG genotype in the same group (48.1%), as well as comparing with the immunological responders for these genotypes (CC = 53.9%; GG = 50.3%; $P = .018$). Considering CG and GG genotypes in the ART-treated groups (INR = CG (62.5%) and GG (48.1%) vs IR = CG (32.8%) and GG (50.3%), it was also observed statistically significant difference ($P = .029$) in relation RTE CD4 + T-cell death by Caspase-1, suggesting that the G allele presence may be related to a lower IL-18 production and thus reduced level of Caspase-1 detection in CD4 + T-cells.

3.3. Logistic regression analysis

Even though some clinical variables have not been associated with immunological recovery failure in univariate tests, they were included in the logistic regression analyses together with associated rs187238 (*IL18)* genotype because of their clinical importance and *p*-values (Table 2). Others were not included because they showed either not

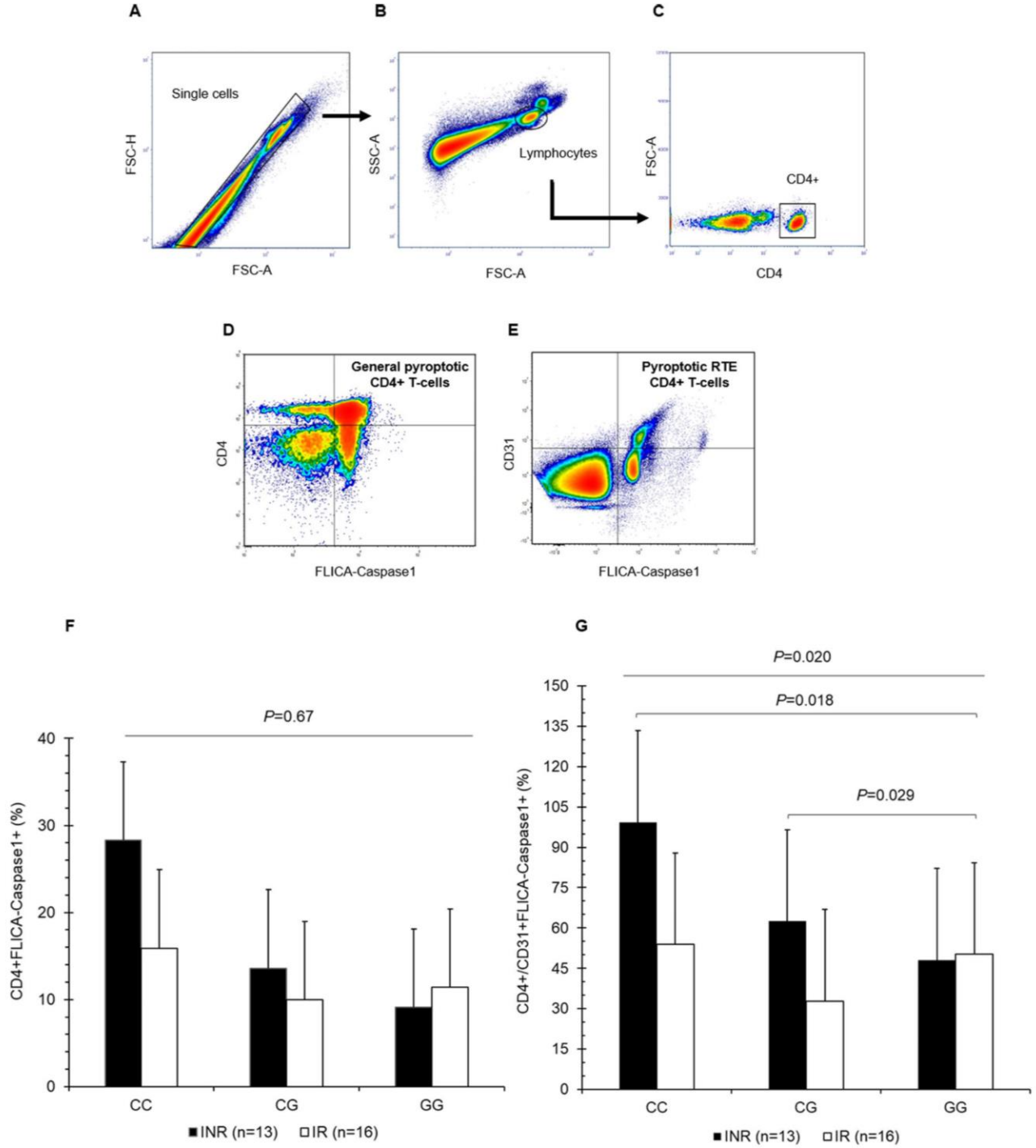

Fig. 1. Pyroptosis levels of CD4 T-cells based on FLICA-Caspase1 activity in ART-treated patients (immunological non-responders – INR and immunological responders – IR). (A) Initial gating was performed to identify single cells. (B) Lymphocytes were selected from singles cells based on forward scatter (FSC) and side scatter (SSC). (C) Cells expressing CD4+ were selected to genotype-guided pyroptosis analyses. (D) Representative flow cytometry plots illustrating the gating strategy for (D) general pyroptotic CD4+ T-lymphocytes (CD4 + FLICA-Caspase1 +) and (E) pyroptotic recent thymic emigrants (RTE) CD4+ T-cells (CD4 +/CD31 + FLICA-Caspase1 +). Pyroptosis levels of general CD4+ T-cells and RTE CD4+ T-cells are shown in (F) and (G) bar plots, respectively. Mean values, standard deviation, and *P* value (according to *t*-test) are shown. FLICA: Fluorescent-Labeled Inhibitors of CAspases.

significant p-values or sparse data, making not possible the logistic regression model calculate the internal parameters. The results of the logistic regression analysis maintained significant association between rs187238 GG genotype and immune reconstitution, as a protection factor to immunological recovery failure occurrence (OR = 0.157, 95%CI = 0.027–0.921, $P < .040$). The model's internal validation showed that the analysis had good quality and adherence, fitting adequately the data (AUROC = 0.699; Z = -0.452; $P = .651$) and, thus,

Table 2
Variables included in a fitted logistic regression model to explain immunological recovery failure of HIV-positive patients receiving antiretroviral therapy.

Variables	Estimate (β)[a]	OR	95% CI	*P*
rs187238 *(IL18)* CC genotype	0.0968	1.102	0.425–2.856	0.842
rs187238 *(IL18)* CG genotype	0.0113	1.011	0.345–2.966	0.984
rs187238 *(IL18)* GG genotype	−1.8540	0.157	0.027–0.921	**0.040**
Male sex	0.7233	2.061	0.848–5.011	0.110
ART regimen change	−0.4697	0.625	0.060–6.486	0.694
IP/r-containing ART	−0.7905	0.454	0.204–1.011	0.053
Pre-treatment CD4+ T-cell count	0.0006	1.001	0.998–1.003	0.680
(intercept)	0.1485	-	-	0.783

ART: antiretroviral therapy; CI: confidence interval; OR: odds ratio; PI/r: ritonavir-"boosted" protease inhibitor.

[a] Model's internal validation: AUROC = 0.699; Z = −0.452; *P* = .651.

being appropriated to do predictions for immunological recovery failure occurrence.

4. Discussion

Evidence shows that only 5% of the cells are permissive to HIV-1 infection, generating new viral particles and culminating in apoptosis. The remaining 95% of cells are non-permissive and provoke an abortive infection through the recognition of the new synthetized HIV-1 cDNA by cytoplasmic DNA sensors, eliciting cell death by pyroptosis, suggested as death by activation of caspase-1 (Doitsh et al., 2010; Doitsh and Greene, 2016).

Functional analysis evidenced that the SNP located at the position −137 of the promoter region of the *IL18* gene alters the binding site of the H4TF-1 nuclear transcription factor. Thus, the G allele variant may decrease the bindings site strength, decreasing *IL18* gene expression (Giedraitis et al., 2001). Increase in the transcriptional level of some genes, among them the *IL18* gene, in the pyroptosis pathway, suggested as Caspase-1 activation cell death in immunological non-responders was observed in some studies, while lower levels of expression were observed in the group of immunologic responders (Bandera et al., 2018). Thus, considering more complete analyzes, the presence of the G allele may present as an influencing aspect that corroborates both studies.

Once interleukin-18 is released after activation via Caspase 1, it recruits cells to the infection site, stimulating the differentiation of naive T cells into Th1 response cells (Arimitsu et al., 2006), which in turn can be infected and the death via pyroptosis process activated, generating a chronic inflammatory cycle as demonstrated by (Doitsh and Greene, 2016). Thus, a decrease in the transcriptional level and IL-18 protein production would entail a functional reduction in recruitment of cells, resulting in the possible protective character of the polymorphism.

We observed higher levels of Caspase-1 activation RTE CD4+ T cells in INRs compared to the IR group. This difference may be related to the immune modulation in response of released cytokines and cellular contents during pyroptosis pathway (Man et al., 2017). After caspase-1 activation, pro-interleukins IL-18 is cleaved into their active form, which modulate inflammatory responses and promote cell activation and migration to the infection site (Boucher et al., 2016). Researches have also demonstrated that IL-18 is able to play functions in synergy with other interleukins inducing *CCR5* expression, which acts as the main co-receptor in the process of HIV-1 infection (Li et al., 2004; Rodriguez-Galan et al., 2005). Although the observed results were methodologically concise, these results may be observed carefully, since the scarce number of individuals with CC genotype.

An increased expression of this gene could be related to higher level of abortive infections in the thymus. As a result, it may indicate stimulate pyroptosis as the T lymphocytes migrate to mature in the secondary lymphoid organs, as observed in our study according to pyroptotic by Caspase-1 RTE CD4+ T cells, which was higher in the INR group. Another explanation is based on cellular contents released by pyroptosis. Besides pro-inflammatory cytokines, pyroptotic cells also release pathogen- and damage-associated molecular patterns (PAMPs and DAMPs, respectively), stimulating the NLRP3 inflammasome activation and assembly (Guo et al., 2014). In the thymus, lipid molecules from membrane destruction or age-related lipid depositions act as triggers for NLRP3 activation, stimulating cell death by pyroptosis in T lymphocytes that migrate to peripheral blood (Chanpimol et al., 2017; Harijith et al., 2014; Lecossier et al., 2001).

Despite our study we did not find any association, some studies have shown the importance of *IFI16*, *NLRP3*, *CARD8*, and *IL1B* genes to HIV-1 infection and CASP-1 cell death pathway. IFI16 was described as the main DNA sensor that triggers inflammasome pathways and pyroptosis in HIV-1 infection. It was suggested that HIV-1 has the capacity to increase the expression of NLRP3 inflammasome (Bandera et al., 2018; Pontillo et al., 2010) and CARD8 protein is suggested as NLRP3 inhibitor, while IL1β is correlated with inflammatory process (Langmia et al., 2016; Pontillo et al., 2013). However, the association of these polymorphisms observed in other studies was not observed in our population. So, new studies should be performed in other polymorphisms and populations, given the functional importance of these genes in pyroptosis pathway.

Thus, based on the analyzed data, the present study demonstrated a possible association of the *IL18* rs187238 polymorphism with immunological recovery failure in ART-treated patients.

Supplementary data to this article can be found online at https://doi.org/10.1016/j.meegid.2019.103997.

Declaration of Competing Interest

There is no conflict interest on this paper.

References

Aiuti, F., Mezzaroma, I., 2006. Failure to reconstitute CD4+ T-cells despite suppression of HIV replication under HAART. AIDS Rev. 8, 88–97.

Arimitsu, J., Hirano, T., Higa, S., Kawai, M., Naka, T., Ogata, A., Shima, Y., Fujimoto, M., Yamadori, T., Hagiwara, K., Ohgawara, T., Kuwabara, Y., Kawase, I., Tanaka, T., 2006. IL-18 gene polymorphisms affect IL-18 production capability by monocytes. Biochem. Biophys. Res. Commun. 342, 1413–1416. https://doi.org/10.1016/j.bbrc.2006.02.096.

Bandera, A., Masetti, M., Fabbiani, M., Biasin, M., Muscatello, A., Squillace, N., Clerici, M., Gori, A., Trabattoni, D., 2018. The NLRP3 inflammasome is upregulated in HIV-infected antiretroviral therapy-treated individuals with defective immune recovery. Front. Immunol. 9, 1–8. https://doi.org/10.3389/fimmu.2018.00214.

Boucher, D., Chen, K.W., Schroder, K., 2016. Burn the house, save the day: pyroptosis in pathogen restriction. Inflammasome 2, 1–6. https://doi.org/10.1515/infl-2015-0001.

Chanpimol, S., Seamon, B., Hernandez, H., Harris-love, M., Blackman, M.R., 2017. Age-associated alterations in the levels of cytotoxic lipid molecular species and oxidative stress in the murine thymus are reduced by growth hormone treatment. Mech. Ageing Dev. 167, 46–55. https://doi.org/10.1186/s40945-017-0033-9.Using.

Coelho, A., Moura, R.R., Cavalcanti, C., Guimarães, R.L., Sandrin-Garcia, P., Crovella, S., Brandão, L.A.C., 2015. A rapid screening of ancestry for genetic association studies in an admixed population from Pernambuco, Brazil. Genet. Mol. Res. 14, 2876–2884. https://doi.org/10.4238/2015.March.31.18.

Corbeau, P., Reynes, J., 2011. Review article immune reconstitution under antiretroviral therapy : the new challenge in HIV-1 infection. Therapy 117, 5582–5590. https://doi.org/10.1182/blood-2010-12-322453.

Doitsh, G., Greene, W.C., 2016. Dissecting how CD4 T cells are lost during HIV infection. Cell Host Microbe 19, 280–291. https://doi.org/10.1016/j.chom.2016.02.012.

Doitsh, G., Cavrois, M., Lassen, K.G., Zepeda, O., Yang, Z., Santiago, M.L., Hebbeler, A.M., Greene, W.C., 2010. Abortive HIV infection mediates CD4 T cell depletion and inflammation in human lymphoid tissue. Cell 143, 789–801. https://doi.org/10.1016/j.cell.2010.11.001.

Doitsh, G., Galloway, N.L.K., Geng, X., Yang, Z., Monroe, K.M., Zepeda, O., Hunt, P.W., Hatano, H., Sowinski, S., Muñoz-Arias, I., Greene, W.C., 2014. Cell death by pyroptosis drives CD4 T-cell depletion in HIV-1 infection. Nature 505, 509–514. https://doi.org/10.1038/nature12940.

Gaardbo, J.C., Hartling, H.J., Gerstoft, J., Nielsen, S.D., 2012. Incomplete immune recovery in HIV infection: mechanisms, relevance for clinical care, and possible solutions. Clin. Dev. Immunol. 2012. https://doi.org/10.1155/2012/670957.

Gaiha, G.D., Brass, a.L., 2014. The fiery side of HIV-induced T cell death. Science (80-)

343, 383–384. https://doi.org/10.1126/science.1250175.
Giedraitis, V., He, B., Huang, W., Hillert, J., 2001. Cloning and mutation analysis of the human IL-18 promoter : a possible role of polymorphisms in expression regulation. J. Neuroimmunol. 112, 146–152.
Guo, H., Gao, J., Taxman, D.J., Ting, J.P.Y., Su, L., 2014. HIV-1 infection induces interleukin-1β production via TLR8 protein-dependent and NLRP3 inflammasome mechanisms in human monocytes. J. Biol. Chem. 289, 21716–21726. https://doi.org/10.1074/jbc.M114.566620.
Harijith, A., Ebenezer, D.L., Natarajan, V., 2014. Reactive oxygen species at the crossroads of inflammasome and inflammation. Front. Physiol. 5, 1–11. https://doi.org/10.3389/fphys.2014.00352.
Healthcare GE, 2007. Ficoll-Paque PLUS.
Ito, S., Hara, Y., Kubota, T., 2014. CARD8 is a negative regulator for NLRP3 inflammasome, but mutant NLRP3 in cryopyrin-associated periodic syndromes escapes the restriction. Arthritis Res. Ther. 16, R52. https://doi.org/10.1186/ar4483.
Jorgensen, I., Miao, E.A., 2015. Pyroptotic cell death defends against intracellular pathogens. Immunol. Rev. 265, 130–142. https://doi.org/10.1111/imr.12287.
Kesavardhana, S., Kanneganti, T., 2017. Mechanisms governing inflammasome activation, assembly and pyroptosis induction. Int. Immunol. 29, 201–210. https://doi.org/10.1093/intimm/dxx018.
Langmia, I.M., Apalasamy, Y.D., Omar, S.Z., Mohamed, Z., 2016. Impact of IL1B gene polymorphisms and interleukin 1B levels on susceptibility to spontaneous preterm birth. Pharmacogenet. Genomics 26, 505–509. https://doi.org/10.1097/FPC.0000000000000243.
Lecossier, D., Bouchonnet, F., Schneider, P., Clavel, F., Hance, A.J., 2001. Discordant increases in CD4+ T cells in human immunodeficiency virus-infected patients experiencing virologic treatment failure: role of changes in thymic output and T cell death. J. Infect. Dis. 183, 1009–1016. https://doi.org/10.1086/319285.
Li, L., Hsu, H.-C., Stockard, C.R., Yang, P., Zhou, J., Wu, Q., Grizzle, W.E., Mountz, J.D., 2004. IL-12 inhibits thymic involution by enhancing IL-7- and IL-2-induced thymocyte proliferation. J. Immunol. 172, 2909–2916. https://doi.org/10.4049/jimmunol.172.5.2909.
Li, T., Wu, N., Dai, Y., Qiu, Z., Han, Y., Xie, J., Zhu, T., Li, Y., 2011. Reduced thymic output is a major mechanism of immune reconstitution failure in HIV-infected patients after long-term antiretroviral therapy. Clin. Infect. Dis. 53, 944–951. https://doi.org/10.1093/cid/cir552.
Man, S.M., Karki, R., Kanneganti, T.D., 2017. Molecular mechanisms and functions of pyroptosis, inflammatory caspases and inflammasomes in infectious diseases. Immunol. Rev. 277, 61–75. https://doi.org/10.1111/imr.12534.
Miao, E.A., 2011. Caspase-1-induced pyroptotic cell death. Immunol. Rev. 243, 206–214.
Miller, S.A., Dykes, D.D., Polesky, H.F., 1988. A simple salting out procedure for extracting DNA from human nucleated cells. Nucleic Acids Res. 16, 1215.
Pontillo, A., Guimarães, R.L., Branda, L.A., Segat, L., Athanasakis, E., Crovella, S., 2010. A 3'UTR SNP in NLRP3 gene is associated with susceptibility to HIV-1 infection. J. Acquir. Immune Defic. Syndr. 54, 236–240.
Pontillo, A., Carvalho, M.S., Kamada, A.J., Moura, R., Schindler, H.C., Duarte, A.J.S., Crovella, S., 2013. Susceptibility to mycobacterium tuberculosis infection in HIV-positive patients is associated with CARD8 genetic variant. JAIDS J. Acquir. Immune Defic. Syndr. 63, 147–151. https://doi.org/10.1097/QAI.0b013e31828f93bb.
Rodriguez-Galan, M.C., Bream, J.H., Farr, A., Young, H.A., 2005. Synergistic effect of IL-2, IL-12, and IL-18 on Thymocyte apoptosis and Th1/Th2 cytokine expression. J. Immunol. 174, 2796–2804. https://doi.org/10.4049/jimmunol.174.5.2796.

Supplementary Table. Sociodemographic and clinical characteristics of the HIV-positive patients under antiretroviral therapy enrolled in our study.

Variables		INR n=126 (%)	IR n=122 (%)	*P*
Sex	Male	62 (49.2)	45 (36.9)	0.055[a]
	Female	64 (50.8)	77 (63.1)	
Age (years old) at ART start date, median (IQR)		32.0 (29.0–39.0)	32.0 (28.0–36.0)	0.212[b]
Body mass (kg) at ART start date, median (IQR)		61.5 (55.0–70.5)	64.0 (54.0–75.0)	0.293[b]
Time (months) until ART starting post-diagnosis, median (IQR)		5.0 (1.0–30.0)	11.0 (3.0–29.0)	0.101[b]
PI/r-containing ART (n=219/248)	Yes	38 (34.2)	52 (48.2)	0.040[a]
	No (NNRTI use instead)	73 (65.8)	56 (51.8)	
AZT-containing ART (n=212/248)	Yes	92 (86.8)	91 (85.8)	1.00[a]
	No (TDF use instead)	14 (13.2)	15 (14.2)	
ART regimen change (n=229/248)	Yes	12 (10.1)	4 (3.6)	0.070[a]
	No	107 (89.9)	106 (96.4)	
Pre-treatment CD4+ T-cell count		267 ± 192	332 ± 170	0.024[c]
Post-treatment CD4+ T-cell count		402 ± 167	723 ± 261	<0.001[c]
Pre-treatment PVL (log_{10} RNA copies/mL)		4.51 (3.25 – 5.07)	4.43 (3.71 – 5.10)	0.648[b]
Coinfections Serology Status				
Syphilis (VDRL test)		13 (10.3)	10 (8.2)	0.663[a]
Toxoplasmosis* (IgG test)		10 (7.9)	15 (12.3)	0.295[a]
CMV* (IgG test)		14 (11.1)	19 (15.6)	0.352[a]

[a]Fisher exact test. [b]Wilcoxon-Mann-Whitney test (Shapiro Wilk: <0.05). [c]*t*-test (Shapiro Wilk: >0.05).

* Chronic infection – Immunoglobulin G tests.

ART: antiretroviral therapy; AZT: zidovudine; CMV: cytomegalovirus; INR: immunological non-responders; IR: immunological responders; IQR: interquartile range; NNRTI: non–nucleoside reverse transcriptase inhibitor; PI/r: ritonavir-"boosted" protease inhibitor; PVL: plasma viral load; TDF: Tenofovir; VDRL: venereal disease research laboratory.

APÊNDICE B – ARTIGO PUBLICADO NA *IMMUNOGENETICS*: DIFERENÇAS NOS NÍVEIS DE PIROPTOSE INFLUENCIADAS PELO SEXO

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SHORT COMMUNICATION

Differences in pyroptosis of recent thymic emigrants CD4+T Lymphocytes in ART-treated HIV-positive patients are influenced by sex

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Abstract
Pyroptosis cell death in recent thymus emigrants (RTE) CD4+T lymphocytes plays an important role on HIV-1 infection as a cause of CD4+T cell depletion, being influenced by several factors, among them, the sex. Thus, the aim of this study was evaluated pyroptosis levels in RTE CD4+T lymphocytes of individuals under antiretroviral therapy (ART) stratified by sex. Thirty-seven ART-treated HIV-positive patients (22 females and 15 males) and 12 (seven females and five males) clinically health subjects were recruited. Analysis by flow-cytometry of RTE CD4+cells (CD4+CD31+/fluorescent-labeled inhibitors of caspases-Caspase-1+) were performed. Clinical and sociodemographic aspects were also evaluated from medical records. We observed statistically higher levels of pyroptosis RTE CD4+T cells in male individuals (69.3%) compared with female group (39.1%) ($P = 0.0356$). Pre- and post-treatment CD4+T cell counts were also higher in women than men ($P = 0.004$ and $P = 0.012$, respectively). Our data provides important evidence of the sex as a potential predictor of immunological reconstitution in ART-treated individuals.

Keywords Antiretroviral therapy · Caspase-1 activity · Cell death · Immunological recovery

Despite the advances provided to human immunodeficiency virus (HIV)-infected individuals under antiretroviral therapy (ART), there are some clinical features that still influence the therapeutic responses even though suppressed viral concentration, such as deficiency of immune reconstitution. Immunological non-recovery (INR) is characterized by decreased plasma viral load but incomplete recovery of CD4+T lymphocytes, affecting between 10 and 40% of ART-treated patients (Gaardbo et al. 2012; Yang et al. 2020).

The exact mechanisms that lead to impaired CD4+T cell recovery are still unclear. However, several studies have demonstrated that INR condition is a consequence of two main processes: reduced production and exacerbated death of CD4+T cells, mainly by pyroptosis, a highly inflammatory cell death pathway mediated by caspase-1 (Doitsh and Greene 2016). In HIV-1 infection, caspase-1 activation is increased in the lymphoid organs generating high levels of pyroptotic lymphocyte populations (Vidya Vijayan et al. 2017).

Diverse factors have already been associated with poor immunological recovery, considered a multifactorial condition (Massanella et al. 2013). Sex has been shown as an important factor in maintaining the immune response during HIV-1 infection. Although sometimes contradictory, there are immune parameters that demonstrate female advantages compared with male, such as lower plasma viral load and higher CD4+T cell count during ART (Castilho et al. 2014). Additionally, supporting the hypothesis that women has a better thymic function, higher numbers of recent thymic emigrant (RTE) CD4+T cells have been observed in female individuals compared with males, resulting in better immune performance mainly under treatments, besides to effects related to steroids hormones of female individuals. Moreover, it is widely shown the importance of RTE CD4+T lymphocytes in

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INR individuals during ART (Corbeau and Reynes 2011; Carvalho-Silva et al. 2020).

Based on this context, differences between males and females in HIV-infection and the importance of cell death for disease progression, this study aimed to evaluate cell death levels mediated by caspase-1 in RTE CD4+ T lymphocytes according to sex, evaluating the possible relationship with CD4+ T cells count.

The study consisted of 49 individuals (37 ART-treated HIV-positive patients and 12 uninfected healthy controls). HIV-positive patients came from different regions of Pernambuco (Northeast Brazil) and were recruited at Instituto of Medicina Integral Professor Fernando Figueira—IMIP, between 2016 and 2018. Inclusion criteria were age over 18 years old, being under ART for at least 18 months, good adherence to therapy, and achieved prolonged undetectable viral load (< 40 RNA copies/mL). Exclusion criteria were pregnancy, autoimmune diseases, and history of injecting drug use. Clinical, laboratory and sociodemographic data were collected from medical records. All study subjects answered standard questionnaires and gave written informed consent authorizing the collection of data and blood samples for further analysis. This study was approved by the IMIP Research Ethics Committee under protocol no. 3629-13.

Peripheral blood samples (4 mL) were collected in tubes containing EDTA. Peripheral blood mononuclear cells (PBMCs) were obtained using the Ficoll-Paque Plus gradient technique with consecutive washes in phosphate-buffered saline (PBS 1x) following the manufacturer's recommendations (Healthcare GE, 2007). Cellular viability (> 90% in average) was determined by Trypan Blue staining (0.4%) exclusion test. FAM-fluorescent-labeled inhibitors of caspases (FLICA) Caspase-1 Kit (FAM-YVAD-FMK) following the manufacturer's instructions (ImmunoChemistry Technologies) was used for pyroptosis detection. Then, immunophenotypic analysis for detection of CD8+ and RTE CD4+ T cells was also carried out by staining with monoclonal antibodies PECy7-CD8, APC-CD4, and PE-CD31 (BD Biosciences). Flow cytometry analyses were performed by BD Accuri C6 cytometer (BD Biosciences). A total of 50,000 events were acquired in each analysis and gated to detect dead RTE CD4+ T cells by caspase-1 activation (CD4+/CD31+ FLICA-Caspase1+). Acquired data were analyzed using FCS Express 6 Plus software.

The obtained data were evaluated by Shapiro–Wilk test to determine the normality distribution. For variables in normal distribution, the Student *t* test was used; data that did not present a normal distribution were assessed by Wilcoxon-Mann-Whitney test. Fisher's exact test was performed for categorical variables. The significance level (α) was set at 0.05. All statistical analyses were performed using software R version 3.5.0

HIV-positive group consisted of 37 individuals, 15 males and 22 females. The mean age at the start of ART was lower in the female group (29.2 ± 8.41) compared with the male group (34.9 ± 7.95); however, the difference was not statistically significant but showed a trend ($P = 0.046$). Pre-ART viral load was similar between the groups. However, the number of pre-treatment CD4+ cells were higher in the female group (352 cells/μL) as well as the post-ART CD4+ cell count, mean 792 cells/μL, while the male group showed 228 and 529 cells/μL in pre- and post-treatment CD+ cell count, respectively, both with significant statistical differences ($P = 0.004$ and $P = 0.012$, respectively; Table 1). Regarding CD8+ lymphocytes, there was no statistical difference for pre- and post-ART CD8+ T cell counts as well as for CD4/CD8 ratio though slightly higher in female compared with male individuals. Moreover, there was no significant differences for therapeutic regimens: NNRTI versus PI/r-based ART, or zidovudine- versus tenofovir-containing ART. The HIV-positive patients did not present any active coinfection during ART. The healthy control group consisted of 12 individuals, seven males and five females; average age was 28.4 ± 8.04 and 24.1 ± 4.5, respectively. Population characterization data grouped by sex are shown in Supplementary Table 1 (HIV-infected patients) and Supplementary Table 2 (healthy control).

The CD4+ T lymphocyte percentage determined by flow cytometry in HIV-positive patients was higher in female group (30.0 ± 9.93%) compared with male individuals (23.1 ± 6.14%) with significant statistical difference $P = 0{,}02$; Table 1. On the other hand, CD8+ T cell percentage was lower in female than male individuals, 16.9% and 22.4%, respectively, but with no statistical difference. Furthermore, the male group demonstrated 69.3% (43.7–96.7) of dead recent thymic emigrants CD4+ T cell by caspase-1 activity (CD4+/CD31+ FLICA-Caspase1+) compared with 39.1% (10.1–75.0) in female individuals. This difference was statistically significant $P = 0.0356$ (Fig. 1a). Cell death levels of RTE CD4+ T lymphocytes was quite low and similar between male (1.04%) and female (0.81%) healthy controls.

Several parameters have already been correlated with differences in ART outcomes and immune response of HIV-infected patients comparing male and female sex, such as viral load, body mass, CD4+ T cell count, and survival rate of these individuals. Most of these studies indicated the female advantage over males (Scully 2018). In agreement with some studies (Jarrin et al. 2008; Maskew et al. 2013; Klein and Flanagan 2016), which evaluated the T cell profile in female and male individuals, our results demonstrated significant differences regarding pre- and post-treatment CD4+ T lymphocyte counts as well as CD4+ percentage among sexes, being higher in female patients. This trend was also observed in the study developed by Qiu et al. (2017), which demonstrated that there is a higher susceptibility of

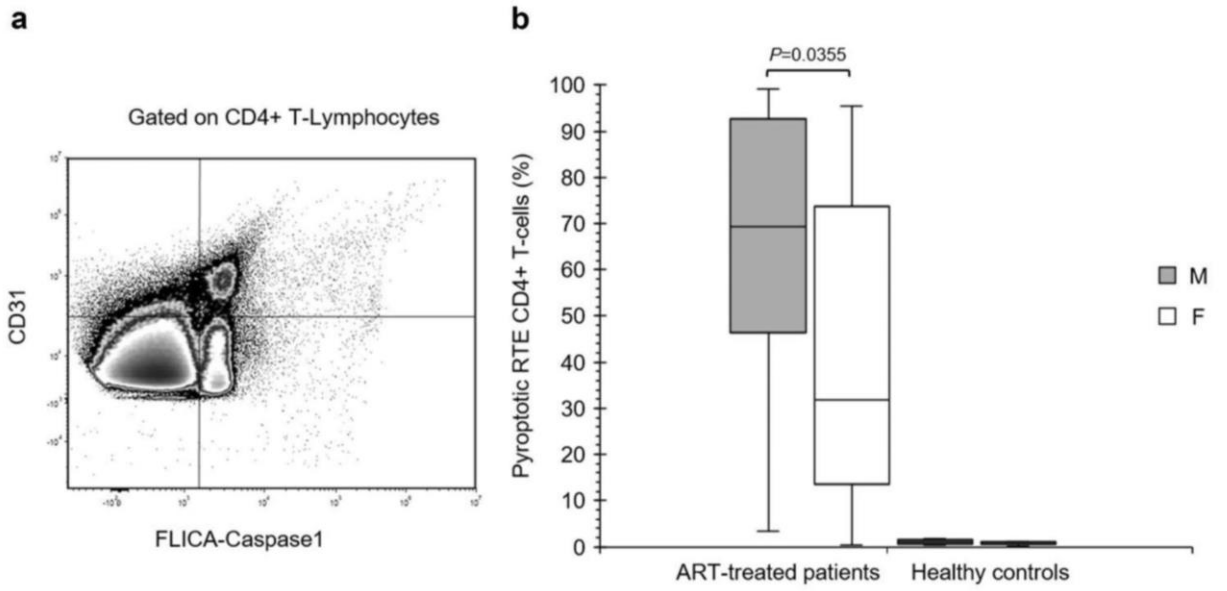

Fig. 1 Pyroptosis levels in recent thymic emigrants (RTE) CD4+T lymphocytes of ART-treated individuals stratified by sex. **a** Representative flow cytometry plot showing gating strategy for pyroptotic RTE CD4+T cells (CD4+/CD31+FLICA-Caspase1+) from lymphocytes gated on CD4+. **b** Pyroptosis levels of RTE among HIV-positive individuals on ART by sex; data from control subjects. Medians, IQR, and *P* value (according Student's *t* test) are shown. ART antiretroviral therapy, FLICA fluorescent-labeled inhibitors of caspases, IQR interquartile range

male individuals to exhibit poor immune reconstitution during ART being, among other factors, a consequence of low pre-treatment CD4+T cell count.

A study by our group has shown that HIV-positive patients under ART have high cell death levels of RTE CD4+T lymphocytes mainly by pyroptosis, and female sex as a protective factor for immunological recovery but no correlation with cell death (Carvalho-Silva et al. 2020). Comparing with these data in the context of incomplete immunological recovery, our findings suggested that there is a considerable difference between the sex' outcome, mainly in terms of cell death. ART-treated males showed elevated dead RTE CD4+T cell by pyroptosis when compared with ART-treated females. These highlight the immune vulnerability of males under ART and evidence the increased loss of CD4+ cells in ART-treated patients during treatment. Previous analyses of cell death in HIV-1 infection showed apoptosis as the main pathway for massive cell loss. However, other studies have demonstrated that this cell death pathway acts as adjuvant, being pyroptosis the main cause of CD4+T cell depression during HIV-1 infection (Doitsh et al. 2014; Cai et al. 2017). RTE T cell population has a crucial role in immune responses and, consequently, in inflammatory mechanisms that are evidenced by HIV-positive individuals. Cell death process by pyroptosis is exacerbated in lymphoid organs to induce cell death of neighboring cells since proximity between cells from adhesion molecules such as ICAM-1 and LFA-1 triggers a mechanism of virological synapse, which induces persistent inflammation in these tissues (Galloway et al. 2015).

In addition, RTE cells have structures called T cell receptor excision circle (TRECs), which is formed during gene rearrangement process for TCR production and to ensure the variability of immune responses (Corbeau and Reynes 2011). According to Pido-Lopez et al. (2001) there is a meaningful variance in the amount of RTE CD4+T cells, being higher in females. Corroborating with these data, a study developed by De Voeght et al. (2017) demonstrated that males have fewer TRECs than female individuals, resulting in decreased immune activity since they show reduced diversity in CD4+T cell populations.

Investigations, comparing immunological mechanisms based on sex, demonstrated that steroid hormones in women are strong protective factors for immune responses due to their physiological functions (Klein and Flanagan 2016). Chen et al. (2020) observed in knockout mice that removing estrogen and progesterone could increase the activation of NLRP3 inflammasome, culminating in caspase-1 activation and thus increasing inflammatory process in atherosclerotic plaques of female mice. In addition, estrogen hormones may influence the release of pro-inflammatory interleukin IL-1β during pyroptosis pathway; therefore, high cell death levels are correlated with low estrogens concentration (Straub 2007). Accordingly, protective character previously associated with

anti-apoptotic aspects (Morrissy et al. 2010; Chen et al. 2013) may also be related to cell death by pyroptosis.

Variations in immune protein production may be linked to the triggering of cell death by pyroptosis depending on sex. Studies have shown that accumulation of reactive oxygen species (ROS) is able to induce response mediated by inflammasome complex activation (Abais et al. 2015). Comparative proteomic analysis among sexes carried out by Zhang et al. (2015) demonstrated a decreased production of SOD2, an important mitochondrial antioxidant that is active in response to cellular damage and high production of α-Synuclein (SNCA), in males (Zhang et al. 2015). Accumulation of SNCA can induce mitochondrial damage and proteasome dysfunction, which may lead cell death by pyroptosis (Yang et al. 2019). In addition, CD4-cell surface CCR5 density is a suggestive factor in susceptibility of males to cell loss. This occurs since these individuals present higher CCR5 distribution in the lymphocyte surface, increasing immune activation with consequent inflammatory response mediated by caspase-1 activation (Corbeau and Reynes 2011). Furthermore, CCR5 expression on cell surface seems to be influenced by synergy exerted by interleukin-18, a product of caspase-1 activation, with other pro-inflammatory cytokines (Rodriguez-Galan et al. 2005).

Based on recent data focusing on pyroptosis as the main mechanism of CD4+ T cell depletion during ART, our results demonstrate male sex as a predisposing factor to immunological non-recovery. This study, being aware of the limitation due to the analyses performed on a low number of patients, provides new subsidies for a better understanding of mechanisms involved in poor immune reconstitution of HIV-positive individuals undergoing ART.

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References

Abais JM, Xia M, Zhang Y et al (2015) Redox regulation of NLRP3 inflammasomes: ROS as trigger or effector? 22:1111–1129. https://doi.org/10.1089/ars.2014.5994

Cai R, Liu L, Luo B et al (2017) Caspase-1 activity in CD4 T cells is downregulated following antiretroviral therapy for HIV-1 infection. AIDS Res Hum Retroviruses 33:164–171. https://doi.org/10.1089/aid.2016.0234

Carvalho-Silva WHV, Andrade-Santos JL, Souto FO et al (2020) Immunological recovery failure in cART-treated HIV-positive patients is associated with reduced thymic output and RTE CD4+ T cell death by pyroptosis. J Leukoc Biol 107:85–94. https://doi.org/10.1002/JLB.4A0919-235R

Castilho JL, Melekhin VV, Sterling TR (2014) Sex differences in HIV outcomes in the highly active antiretroviral therapy era: a systematic review. AIDS Res Hum Retroviruses 5:446–456. https://doi.org/10.1089/aid.2013.0208

Chen FP, Chien MH, Chen HY et al (2013) Effects of estradiol and progestogens on human breast cells: regulation ofsex steroid receptors. Taiwan J Obstet Gynecol 52:365–373. https://doi.org/10.1016/j.tjog.2012.09.038

Chen S, Markman JL, Shimada K et al (2020) Sex-specific effects of the Nlrp3 inflammasome on atherogenesis in LDL receptor-deficient mice. JACC Basic to Transl Sci. https://doi.org/10.1016/j.jacbts.2020.03.016

Corbeau P, Reynes J (2011) Review article Immune reconstitution under antiretroviral therapy: the new challenge in HIV-1 infection. Therapy 117:5582–5590. https://doi.org/10.1182/blood-2010-12-322453

De Voeght A, Martens H, Renard C et al (2017) Exploring the link between innate immune activation and thymic function by measuring sCD14 and TRECs in HIV patients living in Belgium. PLoS ONE 12:1–11. https://doi.org/10.1371/journal.pone.0185761

Doitsh G, Galloway NLK, Geng X et al (2014) Cell death by pyroptosis drives CD4 T-cell depletion in HIV-1 infection. Nature 505:509–514. https://doi.org/10.1038/nature12940

Doitsh G, Greene WC (2016) Dissecting how CD4 T cells are lost during HIV infection. Cell Host Microbe 19:280–291. https://doi.org/10.1016/j.chom.2016.02.012

Gaardbo JC, Hartling HJ, Gerstoft J, Nielsen SD (2012) Incomplete immune recovery in HIV infection: mechanisms, relevance for clinical care, and possible solutions. Clin Dev Immunol 2012 https://doi.org/10.1155/2012/670957

Galloway NLK, Doitsh G, Monroe KM et al (2015) Cell-to-cell transmission of HIV-1 is required to trigger pyroptotic death of lymphoid-tissue-derived CD4 T cells. Cell Rep 12:1555–1563. https://doi.org/10.1016/j.celrep.2015.08.011

Healthcare, G. E. (2007). Ficoll-Paque Plus intended use for in vitro isolation of lymphocytes. *GE Healthcare, Uppsala, Sweden*.

Jarrin I, Geskus R, Bhaskaran K et al (2008) Gender differences in HIV progression to AIDS and death in industrialized countries: slower disease progression following HIV seroconversion in women. Am J Epidemiol 168:532–540. https://doi.org/10.1093/aje/kwn179

Klein SL, Flanagan KL (2016) Sex differences in immune responses. Nat Rev Immunol 16:626–638. https://doi.org/10.1038/nri.2016.90

Maskew M, Brennan AT, Westreich D et al (2013) Gender differences in mortality and CD4 count response among virally suppressed HIV-positive patients. J Women's Heal 22:113–120. https://doi.org/10.1089/jwh.2012.3585

Massanella M, Negredo E, Clotet B, Blanco J (2013) Immunodiscordant responses to HAART-mechanisms and consequences. Expert Rev Clin Immunol 9:1135–1149. https://doi.org/10.1586/1744666X.2013.842897

Morrissy S, Xu B, Aguilar D et al (2010) Inhibition of apoptosis by progesterone in cardiomyocytes. Aging Cell 9:799–809. https://doi.org/10.1111/j.1474-9726.2010.00619.x

Pido-Lopez J, Imami N, Aspinall R (2001) Both age and gender affect thymic output: more recent thymic migrants in females than males as they age. Clin Exp Immunol 125:409–413

Qiu T, Ding P, Fu G et al (2017) Immunologic treatment failure among HIV-infected adult patients in Jiangsu province, China. Sci Rep 7:1–6. https://doi.org/10.1038/srep42381

Rodriguez-Galan MC, Bream JH, Farr A, Young HA (2005) Synergistic effect of IL-2, IL-12, and IL-18 on thymocyte apoptosis and Th1/Th2 cytokine expression. J Immunol 174:2796–2804. https://doi.org/10.4049/jimmunol.174.5.2796

Scully EP (2018) Sex Differences in HIV Infection. Curr HIV/AIDS Rep 15:136–146. https://doi.org/10.1007/s11904-018-0383-2

Straub RH (2007) The complex role of estrogens in inflammation. Endocr Rev 28:521–574. https://doi.org/10.1210/er.2007-0001

VidyaVijayan KK, Karthigeyan KP, Tripathi SP, Hanna LE (2017) Pathophysiology of CD4+ T-cell depletion in HIV-1 and HIV-2 infections. Front Immunol 8:580. https://doi.org/10.3389/fimmu.2017.00580

Yang, X, Su, B, Zhang, X, Liu, Y, Wu, H, Zhang, T (2020) Incomplete immune reconstitution in HIV/AIDS patients on antiretroviral therapy: Challenges of immunological non-responders. J Leukoc Biol 107:597–612. https://doi.org/10.1002/JLB.4MR1019-189R

Yang Y, Wang H, Kouadir M et al (2019) Recent advances in the mechanisms of NLRP3 in flammasome activation and its inhibitors. Cell Death Dis. https://doi.org/10.1038/s41419-019-1413-8

Zhang L, Wang Z, Chen Y et al (2015) Label-free proteomic analysis of PBMCs reveals gender differences in response to long-term antiretroviral therapy of HIV. J Proteomics 126:46–53. https://doi.org/10.1016/j.jprot.2015.05.033

Differences in Pyroptosis of Recent Thymic Emigrants Lymphocytes in ART-Treated HIV-Positive Patients are influenced by sex

Supplementary **Table 2.** Sociodemographic, clinical and laboratory parameters of HIV-positive patients under ART according to sex.

Variables		ART-treated HIV-positive patients		
		Male n= 15	Female n=22	*P*
Age (years old) at ART start date, mean ± SD		34.9 ± 7.95	29.2 ± 8.41	0.046
Body mass (kg) at ART start date, mean ± SD		67.1 ± 12.7	67.1 ± 13.3	1.00
PI/r-containing ART n= 34/37 (%)	Yes	8 (23.5)	12 (35.3)	1.00
	No (NNRTI instead)	6 (17.7)	8 (23.5)	
AZT-containing ART n=34/37 (%)	Yes	13 (38.2)	20 (58.9)	0.41
	No (TDF instead)	1 (2.9)	0 (0)	
ART regimen change[a] n=2/37 (%)		01 (50)	01 (50)	-
Therapy Time (years), mean ± SD		10.4 ± 3.0	9.3 ± 2.7	0.232
Pre-treatment PVL (log_{10} RNA copies/mL) (IQR)		4.14 (3.19 – 5.01)	4.52 (3.70 – 4.81)	0.82
Pre-treatment CD4+ T-cell count (IQR)		228 (58 – 316)	352 (295 – 450)	**0.004****
Post-treatment CD4+ T- cell count, mean ± SD		529 ± 261	792 ± 285	**0.012****
Pre-treatment CD8+ T-cell count (IQR)		772 (569 – 987)	862 (719 – 1368)	0.14
Post-treatment CD8+ T- cell count, mean ± SD		916 ± 227	994 ± 408	0.57
CD4/CD8 ratio (IQR)		0.63 (0.28 – 0.82)	0.76 (0.60 – 0.90)	0.16
Most recent CD4+ (%), mean ± SD		23.1 ± 6.14	30.0 ± 9.93	**0.02****
Most recent CD8+ (%) (IQR)		22.4 (15.8 – 26.9)	16.9 (13.2 – 25.6)	0.16
CD4+/CD31+FLICA+ (%) (IQR)		69.3 (43.7 – 96.7)	31.9 (10.1 – 75.0)	**0.035****
Coinfections Serology Status*	Syphilis n=3/37 (%)	2 (67)	1 (33)	-
	Toxoplasmosis n= 6/37 (%)	3 (50)	3 (50)	
	CMV n= 3/37 (%)	3 (50)	3 (50)	

*Chronic infection – Immunoglobulin G tests.
**Significant statistical difference
[a]Change from a NNRTI-containing cART to a PI/r-containing cART regime

ART: antiretroviral therapy; AZT: zidovudine; CMV: cytomegalovirus; IQR: interquartile range; NNRTI: non–nucleoside reverse transcriptase inhibitor; PI/r: ritonavir- “boosted” protease inhibitor; PVL: plasma viral load; SD: standard deviation; TDF: tenofovir.

Supplementary Table 3. Sociodemographic and clinical dates of healthy controls according to sex.

Variables	**Controls**	
	Male (n=05)	Female (n=07)
Age (years old) mean ± SD*	28.4 ± 8.04	24.1 ± 4.5
Body mass (kg) mean ± SD*	78.8 ± 8.6	62.7 ± 13.3
CD4+ count (%)*	26.2 ± 10.9	17.8 ± 7.81
CD8+ count (%)*	14.6 ± 3.8	16.4 ± 5.8
CD4+CD31+/FLICA+	1.04 ± 0.66	0.81 ± 0.60

SD: standard deviation;

APÊNDICE C – ARTIGO PUBLICADO NO *JOURNAL OF PHARMACY AND PHARMACOLOGY*: TONTURA ASSOCIADA COM COMBINAÇÃO AZT–EFZ.

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Research Paper

Increased risk of dizziness in human immunodeficiency virus-infected patients taking zidovudine and efavirenz combination: a Brazilian cohort study

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antiretroviral therapy; dizziness; HIV-1; neuropsychiatric adverse effects

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Abstract

Objectives Neuropsychiatric adverse effects (NPAE) related to efavirenz, mainly dizziness, is detrimental to human immunodeficiency virus (HIV) treatment. Our study aims at evaluating if zidovudine use potentiates the risk of dizziness related to efavirenz when used together and whether there are significant differences in over time distribution of this NPAE and others relatively frequents regarding efavirenz regimen without zidovudine.

Methods Human immunodeficiency virus-infected patients under efavirenz-containing different therapy were enrolled. A retrospective analysis of official medical records was accomplished to collect clinical data regarding NPAE occurrence and severity. Univariate statistic and statistical model based on survival analyses were performed.

Key findings One hundred sixty-two patients were included, of these seventy-seven (47.5%) had NPAE reported, such as dizziness (more frequent), depression and insomnia. Univariate statistical analysis demonstrated that the combined use of efavirenz with zidovudine increased the NPAE risk (OR: 2.5; *P*-value: 0.008), mainly dizziness risk (OR: 3.5; *P*-value: 0.009) and survival analysis showed that such combination is associated with dizziness occurrence faster (HR: 2.9; *P*-value: 0.02).

Conclusions The results may contribute to clarify the dizziness occurrence dynamics in therapy with efavirenz and zidovudine by identifying susceptibilities and assisting in the choice of combined antiretroviral therapy.

Introduction

Efavirenz is a non-nucleoside reverse transcriptase inhibitor (NNRTI), an antiretroviral widely used in many countries in antiretroviral therapy (ART) against human immunodeficiency virus (HIV). Although frequently prescribed together with zidovudine and lamivudine because of the availability of formulations and its effectiveness, it is no longer included at the first-line ART regimen.[1–3] However, the efavirenz is naturally related to higher rate of neuropsychiatric adverse effects (NPAE) and minimizing these effects is a necessity because they undermine patients' health and adherence to therapy.[4–7]

The low adherence to HIV therapy, because of adverse events, is the main cause of treatment failure (inability to suppress HIV viral load to undetectable levels), usually resulting in ART interruption.[8–10] Since antiretroviral treatment is a lifelong one, it is difficult to measure the damage of long-term adverse effects.[4–6,11] NPAE due to efavirenz is related to elevated plasma concentrations of this drug, being the effects most common: dizziness, insomnia, somnolence, irritability, tremors and hyperhidrosis, with some patients coming to develop more serious effects such as depression, psychosis, mania, suicidal thoughts, paranoia and cognitive impairment.[10,12–15]

Does zidovudine use increase the risk of any NPAE related to efavirenz when used together? This question is one of the objectives of this study besides to evaluate the frequency of reported neuropsychiatric effects during efavirenz-containing treatment.

Methods

Study design

HIV-infected subjects under efavirenz-containing ART regimens, followed at Institute of Integral Medicine Professor Fernando Figueira (IMIP) in Recife, Pernambuco state (Northeast Brazil), were enrolled. The IMIP research ethics committee approved the study (protocol number 3629-13), in accordance with the Declaration of Helsinki, and all patients consented to their participation through interviews and signed a written informed consent form. A retrospective analysis of official medical records of these patients was performed to collect clinical data regarding efavirenz-related NPAE occurrence and severity. Medical records are clinical and laboratory data annotated in standard document of medical assessments of treatment every two months for each patient, where the doctor evaluated the clinical status, effectiveness of anti-HIV treatment, including the occurrence of adverse effects. All patients underwent the same clinical evaluation procedure. Inclusion criteria were as follows: older than 18 years; received ART regimen at standard dose according to guideline (for efavirenz an oral dose 600 mg once a day)[2]; optimal therapy adherence (estimated indirectly by medication possession ratio (MPR),[16] where optimal adherence was defined as MPR ≥95%); and no reported history of neurological diseases or neuropsychiatric treatment. Exclusion criteria were as follows: virological treatment failure and comedication with drugs known to be inducers or inhibitors of antiretroviral metabolism.[5,7]

The patients were characterized according to efavirenz-containing ART regimens used during treatment. Then, they were classified according to efavirenz-related adverse effects occurrence: individuals who discontinued efavirenz-containing regimens due to NPAE versus those who did not. Another classification was performed according to adverse effect period: patients that reported NPAE until the fourth week of therapy versus who that reported these effects after such period since the NPAE reported in therapy with efavirenz occur for two up to four weeks, then disappear.[8,10,12,17]

Demographical and clinical evaluations

In medical record analysis, information from queries in the period of ART containing efavirenz was collected, including the date of each event. Demographic (sex and age at the beginning of treatment with efavirenz) and clinical information (viral load and CD4+ T lymphocyte counts during the treatment period with efavirenz) were also assessed.

Statistical analysis

Statistical analyses were performed by R software, version 3.5.1 for Windows. Fisher exact test was used to test neuropsychiatric effects occurrence risk regarding sex and zidovudine and efavirenz combination use. Odds ratios (OR) and their respective 95% confidence intervals (95% CI) were calculated.

Additionally, a survival analysis was used to evaluate if zidovudine and non-zidovudine in efavirenz-containing ART regimens differ significantly regarding the NPAE occurrence as time-dependent exposure. In summary, it means assessing whether a variable influence in adverse effects occurrence in less time or if there are no significant differences. The time (therapy duration) was registered in weeks and analysis was done retrospectively through patient's medical record, measuring exposure time until the report of NPAE. Endpoint primary: occurrence of any NPAE. Second endpoint: dizziness, depression or insomnia occurrence.

The log-rank test was used for univariate survival analyses and Cox proportional hazards regression demonstrated the contribution of each variable in modulation the efavirenz-related NPAE risk. Hazards ratios (HR) and their 95% CI were calculated. The level of statistical significance for all analysis was set at $\alpha = 0.05$.

Results

Study population

One hundred and sixty-two patients (87 female and 75 male) treated with efavirenz-containing backbone met all the criteria and were included in the analysis. Demographical and clinical characteristics of the patients are summarized in Table 1. The follow-up time of therapy varied between 1.4 and 723 weeks (approximately 14 years), with a mean follow-up time of 143 weeks (approximately 3 years). In general, the patients presented an efficient clinical response during the treatment. The mean CD4+ lymphocyte cell count before treatment was about 395 cells/μl and after efavirenz-containing therapy was about 499 cells/μl, in addition over 90% of patients reduced viral load to an undetectable (<40 copies/ml) plasma HIV load. However, 22% of the patients that presented NPAE had also detectable viral load peaks interspersed with undetectable viral load periods during ART. Sixty-three per cent of patients ($n = 102$) were therapy naïve at the beginning of efavirenz treatment.

Neuropsychiatric adverse effects clinically diagnosed during quarterly medical appointments were dizziness, headache, hallucinations, insomnia, somnolence, abnormal dreams (nightmares), sleep disturbances, photophobia, phonophobia, memory ailments, sadness, depression and suicidal thoughts, anxiety, irritability, hyperactivity, convulsions, hyperhidrosis, paresthesia and fatigue. Others non-specific NPAE that caused efavirenz intolerance were described in official medical records as 'intolerance to efavirenz'.

Table 1 Demographic and clinical data of study population, (n = 162)

Characteristics	Value, mean ± SD (range) or n (%)
Age at efavirenz-containing ART start (years)	32.3 ± 8.4 (18–62)
Sex – n (% female)	87 (53.7%)
AZT/3TC/EFV regimen[a]	127 (69.4%)
TDF/3TC/EFV regimen[a]	50 (27.3%)
Other/EFV regimens[a]	6 (3.3%)
Presence of NPAE	77 (47.5%)
Discontinued efavirenz-containing regimens due to NPAE	23 (29.9%)
NPAE frequency in AZT/3TC/EFV regimen[a]	65 (51.2%)
NPAE frequency in non-AZT/3TC/EFV regimens[a]	16 (29.6%)
Therapy naive at efavirenz-containing ART start	102 (63%)
Duration of efavirenz-containing ART (weeks)	143.5 ± 147.3 (1.4–723.4)
Duration of AZT/3TC/EFV therapy (weeks)	145.6 ± 139.2 (1.4–705.1)
Duration of TDF/3TC/EFV therapy (weeks)	73 ± 84 (1.6–398.4)
Duration of Other/EFV therapy (weeks)	197.6 ± 267 (9.4–723.4)
Duration of efavirenz-containing ART until discontinuation due to NPAE (weeks)	51.8 ± 60 (1.4–219)
CD4+ T cell counts (cells/μl) before starting efavirenz-containing ART	395 ± 237.5 (36–1008)
CD4+ T cell counts (cells/μl) during efavirenz-containing ART	499 ± 236.7 (35–1259)
Undetectable plasma viral load	149 (92%)
Patients who reduced viral load after efavirenz-containing ART	152 (94%)
Patients who had NPAE and detectable viral load peaks interspersed with undetectable viral load periods	17 (22%)

3TC, lamivudine; ART, antiretroviral therapy; AZT, zidovudine; EFV, efavirenz; Non-AZT, other nucleoside reverse transcriptase inhibitors except zidovudine; NPAE, neuropsychiatric adverse effects; TDF, tenofovir. [a]Nineteen patients used two different efavirenz-based regimens, while one patient used three different efavirenz-based regimens during the therapy.

Neuropsychiatric adverse effects occurrence

One hundred twenty-seven patients used zidovudine/lamivudine/efavirenz regimen, fifty patients received tenofovir/lamivudine/efavirenz combination and six patients were submitted to other efavirenz-containing regimens. It is noteworthy that nineteen patients used two different efavirenz-containing regimens and one patient was submitted to three efavirenz-containing regimens. In our study, 47.5% (n = 77) of patients reported NPAE during efavirenz therapy. Specifically, 51.2% (n = 65) of exposure periods to zidovudine/lamivudine/efavirenz regimen resulted in efavirenz-related NPAE, while that exposure periods to efavirenz-containing regimens without zidovudine (non-zidovudine/lamivudine/efavirenz) had NPAE occurrences in 29.6% (n = 16; Table 1). Among patients who used zidovudine/lamivudine/efavirenz and non-zidovudine/lamivudine/efavirenz regimens in different moments of treatment, 40% (n = 8) reported NPAE only when zidovudine was present, 10% (n = 2) only when zidovudine was not present, and 15% (n = 3) in both cases.

The most common reported NPAE was dizziness, present in 24.1% (n = 39) of the patients, followed by depression 10.5% (n = 17), insomnia 8.6% (n = 14) and asthenia 7.4% (n = 12). Other less-frequent ($n \leq 10$) effects were also reported, such as sadness, anxiety, sleeps disturbance, paresthesia, abnormal dreams, somnolence, irritability, hallucinations and photophobia. Unspecific NPAE reported as 'intolerance to efavirenz', occurred in 6.2% of patients (n = 10). Phonophobia, memory loss, suicidal thoughts, hyperactivity, convulsion and cognitive impairment were also observed but with low frequency ($n \leq 3$), and hyperhidrosis was not reported in our cohort. Headache was reported in 13.6% (n = 22) of patients, but this symptom is commonly attributed to zidovudine alone, therefore, was not considered.[7,10] The NPAE frequencies are summarized in Figure 1.

Among the seventy-seven patients who presented efavirenz-related NPAE, 29.9% (n = 23) had the efavirenz-containing regimen discontinued after the occurrence of these events (Table 1). They represent 14.2% of all HIV-positive patients treated with efavirenz. Zidovudine/lamivudine/efavirenz combination was the most discontinued ART regimen with 14.2% of the cases (n = 18) whereas the discontinuation of non-zidovudine/lamivudine/efavirenz occurred in 9% (n = 5).

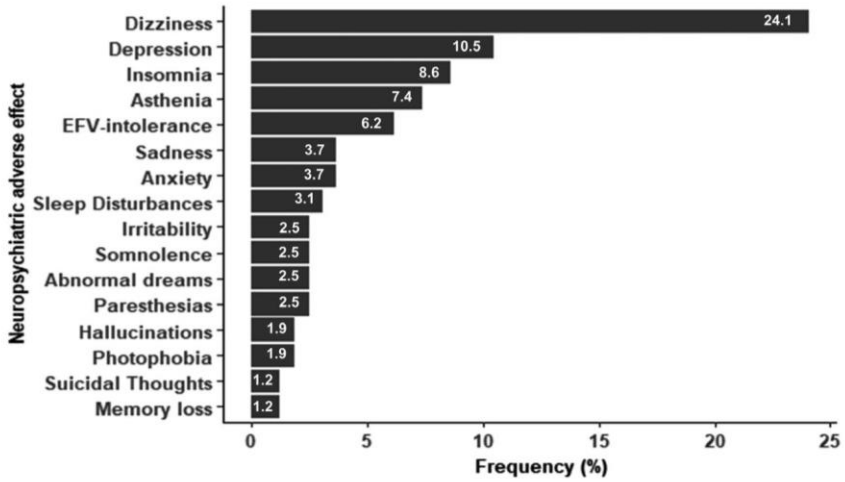

Figure 1 Neuropsychiatric adverse effects frequency in human immunodeficiency virus-positive patients under efavirenz-containing antiretroviral therapy. The data were extracted through a retrospective analysis in the official medical records of patients.

Regarding the NPAE occurrence distribution before or after the fourth week of efavirenz therapy, dizziness was the unique effect that had similar occurrence distribution in these two periods, averaging 67.0 weeks among those who reported it after the fourth week of treatment. Despite that, insomnia, 78.5% (n = 11, mean = 61.3 weeks) and depression, 100% (mean = 78.6 weeks), were more frequent after the fourth week of treatment. Dizziness and insomnia persisted in about 45% (n = 9; n = 5, respectively) of the patients who reported such effects after the fourth week, with respective persistence mean duration of 36.5 and 43.6 weeks. Depression persisted in 6% (n = 1) of patients; however, 41% (n = 7) had medical intervention to prevent persistence. These data are summarized in Table 2.

Univariate statistical analysis results

Neuropsychiatric adverse effects in patients who received regular doses of efavirenz was twice more common in women than men (OR = 2.1; 95% CI = 1.1 to 4.3; P-value = 0.01). Moreover, when comparing such effects in exposure periods to zidovudine/lamivudine/efavirenz regimen versus non-zidovudine/lamivudine/efavirenz combinations, we observed that the zidovudine presence increases by two-and-half-fold the risk to develop any NPAE (OR = 2.5; 95% CI = 1.2 to 5.3; P-value = 0.008). When dizziness is assessed, the risk is about three-and-half-fold higher (OR = 3.5; 95% CI = 1.3 to 12.4; P-value = 0.009). For depression (OR = 3.4; 95% CI = 0.7 to 32.3; P-value = 0.1), and insomnia (OR = 0.7; 95% CI = 0.2 to 3; P-value = 0.76) we found no statistical significant differences according to Fisher Exact test. Somnolence, sleep disturbances, abnormal dreams and insomnia were also analysed, but all together as sleep-related NPAE. However, we did not observe a significant difference when comparing sleep-related NPAE in efavirenz-containing regimen with and without zidovudine (OR = 1.7; 95%

Table 2 Occurrence distribution of dizziness, depression and insomnia before or after the fourth weeks of efavirenz-containing antiretroviral therapy

	Under EFV-related NPAE occurrence							
	T = 0–4 weeks			$T \geq 4$ weeks				
Event	Occurrence distribution n (%)	Mean (weeks)	EFV swap[a] n (%)	Occurrence distribution n (%)	Mean (weeks)	EFV swap[a] n (%)	Effect persistence[b] n (%)	Mean (weeks)[b]
Dizziness	19 (48.7)	2.4	1 (5.2)	20 (51.3)	67.0	5 (25.0)	9 (45.0)	36.5
Depression[c]	0 (0.0)	–	–	17 (100.0)	78.6	6 (35.3)	1 (5.8)	–
Insomnia	3 (21.4)	1.6	1 (33.3)	11 (78.5)	61.3	2 (18.2)	5 (45.4)	43.6

EFV, efavirenz; NPAE, neuropsychiatric adverse effects; T, duration on of EFV therapy. [a]Change for a non-efavirenz-containing therapy. [b]'Effect persistence' refers to the patients who had the NPAE for a long time. [c]Forty-one per cent (n = 7) of depression-patients had medical intervention to prevent persistence.

Table 3 Univariate analysis results for NPAE occurrence between efavirenz-containing regimens with and without zidovudine

	EFV-containing ART regimens		Fisher exact test	
Event	Non-AZT/ 3TC/EFV n = 54 (%)	AZT/3TC/ EFV n = 127 (%)	OR (95% CI)	P-value (α = 0.05)
Any NPAE	16 (29.5)	65 (51.2)	**2.5 (1.2–5.3)**	**0.008**
Dizziness	5 (9.2)	34 (26.8)	**3.5 (1.3–12.4)**	**0.009**
Depression	2 (4.0)	15 (11.8)	3.4 (0.7–32.3)	0.1
Insomnia	5 (9.2)	9 (7.0)	0.7 (0.2–3)	0.76
Sleep-related NPAE	6 (11.0)	22 (17.3)	1.7 (0.6–5.4)	0.37

3TC, lamivudine; 95% CI, 95% confidence interval; ART, antiretroviral therapy; AZT, zidovudine; EFV, efavirenz; Non-AZT, any nucleoside reverse transcriptase inhibitor, except zidovudine; NPAE, neuropsychiatric adverse effects; OR, odds ratio.

The level of statistical significance for all analysis was set at α = 0.05 (in bold).

CI = 0.6 to 5.4; P-value = 0.37). These results are summarized in Table 3.

Survival analysis results

Survival analyses were used for assessing any NPAE, as primary endpoint, and dizziness, depression and insomnia occurrence as secondary endpoint. Such outcomes were chosen because they were the most frequent effects in our cohort. Survival curves for any NPAE, dizziness, depression and insomnia are showed in Figure 2. Concerning dizziness, the log-rank test showed significant differences in treatment time until its occurrence, when comparing zidovudine/lamivudine/efavirenz to non-zidovudine/lamivudine/efavirenz use (log-rank χ^2 = 5.4; P-value = 0.02). The Cox regression showed that dizziness appeared 2.9 times faster when the patients used zidovudine and efavirenz combination (HR = 2.9; P-value = 0.02). Furthermore, the zidovudine presence or absence in efavirenz-containing regimens did not influence either on depression (log-rank χ^2 = 0.9; P-value = 0.3; HR = 2.0; P-value = 0.35), or insomnia occurrence (Log-rank χ^2 = 0.8; P-value = 0.4; HR = 0.6; P-value = 0.37) and any NPAE as primary endpoint (Log-rank χ^2 = 3.8; P-value = 0.05; HR = 1.7; P-value = 0.05; Table 4).

Discussion

The frequency of efavirenz-treated patients enrolled in our study that reported NPAE (47.5%) was like previous studies performed in different populations. Decloedt and Maartens reported that NPAE affected about 68% of efavirenz-receiving patients.[6] In fact, it has been described in other studies that the frequencies of patients with such effects range from 40% to 70%.[8,18,19] Efavirenz is a neuroactive drug being able to easily cross the blood–brain barrier.[8,18]

The high proportion of patients affected by these events, especially dizziness, shows the importance of studying the distribution of efavirenz-related NPAE and their impact on patients receiving ART. Given the effectiveness of this drug,[5,8,19] it is noteworthy that these symptoms occurred despite an efficient clinical response to treatment, undetectable viral load in 92% of patients, 94% reducing the HIV load and 499 cells/μl CD4+ lymphocyte T cell mean counts, after an average count of 395 cells/μl before efavirenz-based therapeutics.

In our cohort, 28% of patients need to change the efavirenz-containing regimens, despite the high frequency of patients who reported NPAE (47.5%), showing that the continuity of neuropsychiatric effects could favour the irregular use of therapy and, consequently, treatment failure or a transient response.[5,18] In fact, we observed that 22% of the patients who reported NPAE showed detectable viral load peaks interspersed with undetectable viral load periods, which may be consequence of the irregular use of ART because of an underlying adverse effect.[8]

The most frequent NPAE reported worldwide in therapy with efavirenz are as follows: dizziness, insomnia, abnormal dreams, irritability, somnolence, paresthesia, anxiety and depression.[5,10,20] These effects were also very frequent in our cohort, being dizziness twice more frequent compared to the second one efavirenz-related NPAE. It generally does not show a high frequency as observed in our cohort compared to other efavirenz-related NPAE.[10,12–15]

Some studies have reported that these symptoms could appear between the second and fourth week after the beginning of treatment and then disappear.[7,10,12,17] However, we observed that insomnia and depression frequently occurred after the fourth week of treatment. Dizziness and insomnia persisted for several weeks in about 45% of patients who had events after the fourth week, while in the other 55% such effects did not persist. In contrast, depression persisted among 6% of the individuals. This may be due to medical intervention to prevent the effects in the long term. Previous studies also evidenced that NPAE may last longer in some patients[5,8,12,13] and some patients could develop more serious effects such as psychosis, mania, suicidal thoughts and depression at long term.[10,14,15,21]

The incidence of NPAE in patients, followed up in our study, under the efavirenz treatment was about twice more common in women. In fact, Burger *et al.*[22] showed that the mean plasma efavirenz concentration was 30% higher in women than in men, possibly due to differences in efavirenz clearance between genders, even if these differences are not sufficient to suggest dose adjustments.[23,24]

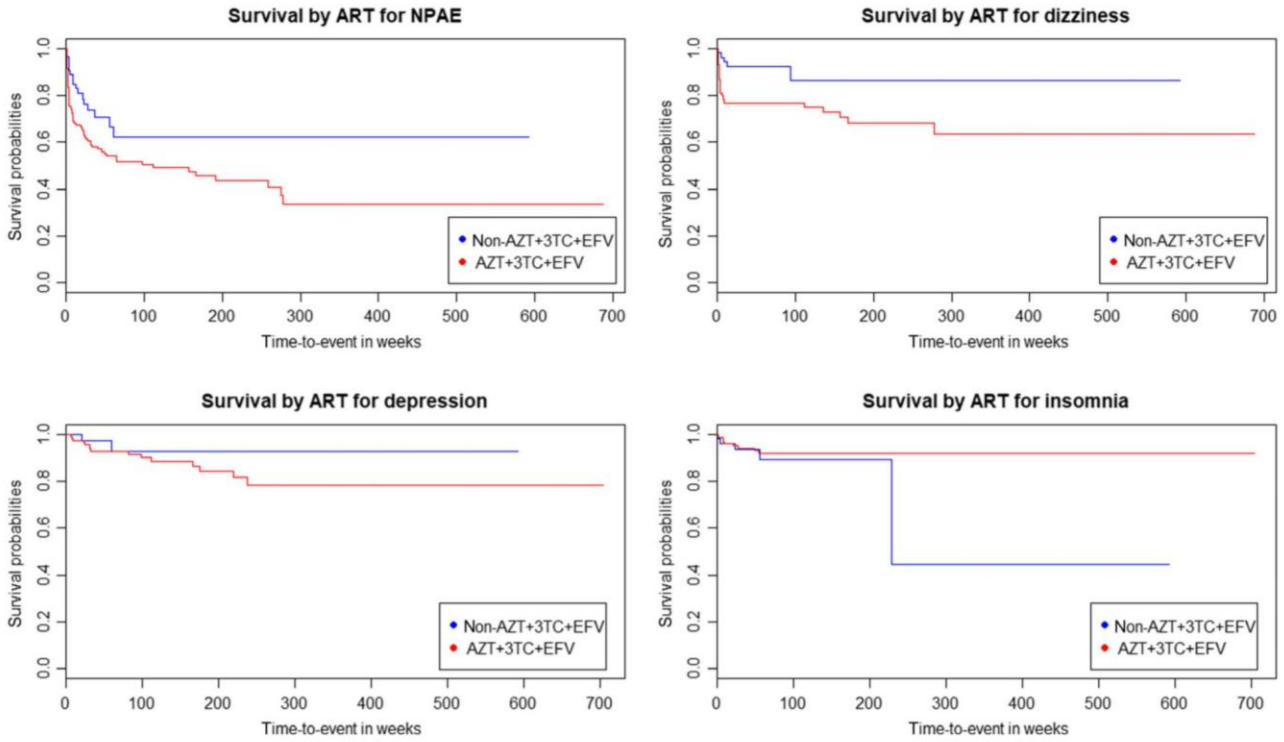

Figure 2 Survival curves generated from survival probabilities versus time-to-event in weeks regarding efavirenz-containing regimens with and without zidovudine for NPAE, dizziness, depression and insomnia. Survival probabilities decrease over time in the zidovudine and efavirenz combination presence. 3TC, lamivudine; ART, antiretroviral therapy; AZT, zidovudine; EFV, efavirenz; Non-AZT, any nucleoside reverse transcriptase inhibitor, except zidovudine; NPAE, neuropsychiatric adverse effects. [Colour figure can be viewed at wileyonlinelibrary.com]

Our results showed that the zidovudine presence in efavirenz-containing regimen significantly increases the risk of NPAE, mainly for dizziness in univariate analysis and in time-dependent exposure. Although univariate analysis showed increase to NPAE risk, it was clear that this result reflects the risk of dizziness because of its high frequency in our cohort; furthermore, the other symptoms analysed showed no significant differences in efavirenz-containing regimens with and without zidovudine. In addition, dizziness appeared faster when the patients used ART regimen with zidovudine and efavirenz. Although zidovudine has been associated with occurrence of anaemia, which dizziness is one of the symptoms, this antiretroviral by itself has never been implicated directly in dizziness regardless anaemia presence, though be involved in others NPAE occurrence.[7,25,26] Moreover, of the patients treated with zidovudine-containing ART that reported dizziness, only two individuals developed anaemia and had to change ART regimen. Even without these two patients, the statistical analyses maintained the association results, showing the increased dizziness risk in patients treated with zidovudine and efavirenz-containing regimens.

Thus, the differences observed between zidovudine/lamivudine/efavirenz and non-zidovudine/lamivudine/efavirenz combination regimens in regarding a higher risk for dizziness occurrence could exist because of a zidovudine and efavirenz synergic effect, potentiating efavirenz dizziness only when both are combined, and not an additive effect of zidovudine with efavirenz. In fact, the overall effect of these two drugs combined is greater than it could be the individual effects summing both antiretrovirals, thus, increasing efavirenz-related dizziness. If it would be merely an additive effect, zidovudine/efavirenz and non-zidovudine/efavirenz regimens should not present differences in dizziness occurrence, since both drugs without the efavirenz presence do not present differences in dizziness occurrence, as demonstrated in this work. Although no longer used in combination at the first-line ART regimen, zidovudine/efavirenz treatment is still recommended as an alternative option when the first-line regimens are contraindicated or not available.[3] However, our study shows the impact of this therapy combination choice on HIV-positive patients under ART.

Efavirenz and zidovudine have already been cited as competing for the major glucuronidation enzyme for both

Table 4 Survival analysis results for any NPAE, dizziness, depression and insomnia occurrence

Risk factor	Event	Log-rank test (*P*-value, α = 0.05)	HR [95% CI] (*P*-value, α = 0.05)
NPAE occurrence, *n* (%)			
Non-AZT + 3TC + EFV (*n* = 54)	16 (29.6)	3.8 (0.05)	Reference
AZT + 3TC + EFV (*n* = 127)	65 (51.2)		1.7 [1.0–2.98] (0.05)
Dizziness occurrence, *n* (%)			
Non-AZT + 3TC + EFV (*n* = 54)	5 (10.0)	**5.4 (0.02)**	Reference
AZT + 3TC + EFV (*n* = 127)	34 (26.7)		**2.9 [1.13–7.48] (0.02)**
Depression occurrence, *n* (%)			
Non-AZT + 3TC + EFV (*n* = 54)	2 (2.0)	0.9 (0.3)	Reference
AZT + 3TC + EFV (*n* = 127)	15 (11.8)		2.0 [0.46–9.02] (0.35)
Insomnia occurrence, *n* (%)			
Non-AZT + 3TC + EFV (*n* = 54)	5 (10.0)	0.8 (0.4)	Reference
AZT + 3TC + EFV (*n* = 127)	9 (7.0)		0.6 [0.20–1.83] (0.37)

3TC, lamivudine; 95% CI, 95% confidence interval; AZT, zidovudine; EFV, efavirenz; HR, hazard ratio (by Cox proportional model); Non-AZT, any nucleoside reverse transcriptase inhibitor, except zidovudine; NPAE, neuropsychiatric adverse effects.

The level of statistical significance for all analysis was set at α = 0.05 (in bold).

UGT2B7 when coadministered and, therefore, exhibiting inhibited glucuronidation by one another in a concentration-dependent manner.[27] This glucuronidation mechanism is responsible for the clearance of these antiretroviral drugs, and this interaction can affect the bioavailability of efavirenz in the body.[27,28]

Because of the retrospective nature, there are some limitations of this study that has to be addressed. First, the small sample number in this study may not reflect the real NPAE frequency related to efavirenz-containing ART. Another point is the occurrence of dizziness, it is known that there are many other causes of dizziness in ART-treated patients, including genetic factors, which may or may not be related to efavirenz-containing regimen or combined with zidovudine, and that could not be assessed since it was not available in medical records. However, some patient's inclusion and exclusion criteria (history of neurological diseases or neuropsychiatric treatment, for example) were established to minimize bias. Finally, it is important to note that the number of patients who used zidovudine in efavirenz-containing regimens is greater than the number of patients who used non-zidovudine/efavirenz in our cohort, which may influence the results. Faced with these, controlled studies are recommended with higher and similar numbers of individuals between groups.

Conclusions

Our study showed a high percentage of patients who presented dizziness and other NPAE related to efavirenz therapy, and these effects were not restricted to the beginning of treatment. It has been shown that the incidence of such effects was twice more common in women. In addition, the zidovudine/lamivudine/efavirenz combination was more likely to cause dizziness than the non-zidovudine combined to lamivudine and efavirenz in HIV treatment, specifically dizziness. Therefore, the study of ART adverse effects should not be limited to a single drug, but also the possible pharmacological interactions, a way to conduct better the treatment regimen choices. These susceptibility factors could be relevant to understand the dynamics of toxicity in efavirenz therapy and to direct pharmacokinetic and pharmacogenetic studies to minimize the risk of adverse effects in people living with HIV/AIDS.

Declarations

Conflicts of interest

Authors did not declare interest conflicts.

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Author's contribution statement

Valeriano is the first author, performed the collection, interpretation, analysis of the data, and wrote the paper. Carvalho-Silva made substantial contributions to the paper design and participated in data collection and drafting of the manuscript. Coelho and Moura

contributed to data collection and interpretations, statistical analysis, and study design. Arraes and Brandão made an essential contribution to the clinical study design adopted and data interpretation. Crovella and Guimarães coordinated all the study and the result analysis. All authors critically reviewed the intellectual content of the manuscript and your writing and agree to be accountable for all aspects of this work.

Data accessibility statement

Those interested in the data supporting the results of this paper should send an email to the author at jeyzon_@live.-com.

References

1. Ford N *et al.* Comparative safety and neuropsychiatric adverse events associated with efavirenz use in first-line antiretroviral therapy: a systematic review and meta-analysis of randomized trials. *J Acquir Immune Defic Syndr* 2015; 69: 422–429.
2. Ministério da Saúde. Protocolo Clínico e Diretrizes Terapêuticas para Manejo da Infecção pelo HIV em Adultos. *Ministério da Saúde-Secretaria de Vigilância em Saúde*. Brasília, published in 2013, last modification in 2018.
3. WHO. *Updated Recommendations on First-Line and Second-Line Antiretroviral Regimens and Post-Exposure Prophylaxis and Recommendations on Early Infant Diagnosis of HIV: Interim Guidance*. Geneva: World Health Organization, 2018 (WHO/CDS/HIV/18.18). License: CC BY-NC-SA 3.0 IGO.
4. Muñoz-Moreno JA *et al.* Interruptions of antiretroviral therapy in human immunodeficiency virus infection: are they detrimental to neurocognitive functioning? *J Neurovirol* 2010a; 16: 208–218.
5. Sánchez Martín A *et al.* Impact of pharmacogenetics on CNS side effects related to efavirenz. *Pharmacogenomics* 2013; 14: 1167–1178.
6. Decloedt EH, Maartens G. Neuronal toxicity of efavirenz: a systematic review. *Exp Opin Drug Saf* 2013; 12: 841–846.
7. Treisman GJ, Soudry O. Neuropsychiatric effects of HIV antiviral medications. *Drug Saf* 2016; 39: 945–957.
8. Apostolova N *et al.* Efavirenz and the CNS: what we already know and questions that need to be answered. *J Antimicrob Chemother* 2015; 70: 2693–2708.
9. Scott Sutton S *et al.* Impact of pill burden on adherence, risk of hospitalization, and viral suppression in patients with HIV infection and AIDS receiving antiretroviral therapy. *Pharmacotherapy* 2016; 36: 385–401.
10. Michaud V *et al.* The dual role of pharmacogenetics in HIV treatment: mutations and polymorphisms regulating antiretroviral drug resistance and disposition. *Pharmacol Rev* 2012; 64: 803–833.
11. Wolff MJ *et al.* A decade of HAART in Latin America: long term outcomes among the first wave of HIV patients to receive combination therapy. *PLoS ONE* 2017; 12: 1–15.
12. Clifford DB *et al.* Long-term impact of efavirenz on neuropsychological performance and symptoms in HIV-infected individuals (ACTG 5097s). *HIV Clin Trials.* 2009; 10: 343–355.
13. Abers MS *et al.* Neurological and psychiatric adverse effects of antiretroviral drugs. *CNS Drugs* 2014; 28: 131–145.
14. Ciccarelli N *et al.* Efavirenz associated with cognitive disorders in otherwise asymptomatic HIV-infected patients. *Neurology* 2011; 7: 1403–1409.
15. Ma Q *et al.* Long-term efavirenz use is associated with worse neurocognitive functioning in HIV-infected patients. *J Neurovirol* 2016; 22: 170–178.
16. Fairman K, Motheral B. Evaluating medication adherence: which measure is right for your program? *J Manag Care Pharm* 2000; 6: 499–506.
17. Sütterlin S *et al.* Neuropsychiatric complications of efavirenz therapy: suggestions for a new research paradigm. *J Neuropsy Clin Neurosci* 2010; 22: 361–369.
18. Muñoz-Moreno JA *et al.* Neuropsychiatric symptoms associated with efavirenz: prevalence, correlates, and management. A Neurobehavioral review. *Aids Rev* 2010b; 11: 103–109.
19. Kanters S *et al.* Comparative efficacy and safety of first-line antiretroviral therapy for the treatment of HIV infection: a systematic review and network meta-analysis. *The Lancet HIV* 2016; 3: e510–e520.
20. Seden K *et al.* High prevalence and long duration of nervous system and psychiatric adverse drug reactions in Ugandan patients taking efavirenz 600 mg daily. *J Antimicrob Chemother* 2018; 73: 3158–3161.
21. Mouton JP *et al.* Key toxicity issues with the WHO-recommended first-line antiretroviral therapy regimen. *Expert Rev Clin Pharmacol* 2016; 9: 1493–1503.
22. Burger D *et al.* Interpatient variability in the pharmacokinetics of the HIV non-nucleoside reverse transcriptase inhibitor efavirenz: the effect of gender, race, and CYP2B6 polymorphism. *Br J Clin Pharmacol* 2006; 61: 148–154.
23. Barrett JS *et al.* Population pharmacokinetic meta-analysis with efavirenz. *Int J Clin Pharmacol Ther* 2002; 40: 507–519.
24. Usach I *et al.* Non-nucleoside reverse transcriptase inhibitors: a review on pharmacokinetics, pharmacodynamics, safety and tolerability. *J Int AIDS Soc* 2013; 16: 18567.
25. Curkendall SM *et al.* Incidence of anaemia among HIV-infected patients treated with highly active antiretroviral therapy. *Br HIV Assoc* 2007; 8: 483–490.
26. Ikunaiye NY *et al.* Incidence of anaemia among HIV-infected patients treated with zidovudine-containing antiretroviral therapy in Northeastern

Nigeria. *Ann Ibd Pg Med* 2018;16:115–124.

27. Bélanger A *et al.* Glucuronidation of the antiretroviral drug efavirenz by UGT2B7 and an in vitro investigation of drug-drug interaction with zidovudine. *Drug Metab Dispos* 2009; 37: 1793–1796.
28. Barreiro P *et al.* Pharmacogenetics of antiretroviral therapy. *Exp Opin Drug Metab Toxicol* 2014; 10: 1–12.

ANEXO A – TERMO DE CONSENTIMENTO LIVRE E ESCLARECIDO

Título da pesquisa: Fatores Genéticos Humanos Envolvidos no Curso da Infecção pelo HIV: Transmissão Vertical, Imunidade e Resposta à Terapia Antirretroviral

Pesquisador responsável Antonio Victor Campos Coelho Local de trabalho: Laboratório de Imunopatologia Keizo Asami (LIKA) Av. Prof. Moraes Rego, 1235, Cidade Universitária, Recife, PE. CEP: 50670-901. Telefone: 2101-2542 antonio.victor@ufpe.br Médico supervisor da pesquisa Luiz Cláudio Arraes de Alencar (IMIP) lularraes@hotmail.com	Comitê de Ética em Pesquisa Rua dos Coelhos, 300, Boa Vista, Recife, PE. Diretoria de Pesquisa, Prédio Orlando Onofre, 1º Andar. Funcionamento: 2ª a 6º feira, 7h às 11h30/13h30 às 16h. Telefone:2122-4756 comitedeetica@imip.org.br

Convidamos você a participar de uma pesquisa que estamos realizando sobre diferenças genéticas envolvidas no comportamento do HIV no organismo. Algumas pessoas combatem melhor o vírus que outras durante a infecção; outras respondem melhor à terapia com os antirretrovirais. Além disso, algumas crianças adquirem o vírus durante o parto ou amamentação (transmissão vertical), enquanto outras não. Estamos fazendo essa pesquisa para tentar descobrir se diferenças genéticas expliquem essa diferença entre as pessoas convivendo com o HIV e pretendemos recrutar 400 voluntários ao todo. Essa pesquisa é importante porque poderá contribuir para que no futuro os médicos melhorem os tratamentos contra o HIV. Além disso, você poderá solicitar aos pesquisadores que comuniquem os resultados a você e a seu médico para que ele avalie se você obteria benefícios com as descobertas.

Com sua autorização, gostaríamos de realizarmos entre uma e cinco coletas de pequenas quantidades do seu sangue (no máximo 8 mL por coleta), colhidas durante os exames de rotina de acompanhamento da infecção pelo HIV. Caso sejam necessárias mais de uma coleta, elas serão feitas a cada três ou quatro meses, de acordo com a rotina de suas consultas. Solicitamos também a sua autorização para utilizar dados do prontuário, como idade que iniciou o acompanhamento médico e os resultados dos seus últimos exames de rotina para avaliar o controle do HIV. Como a Genética está em constante evolução, é possível que novas pesquisas além desta sejam realizadas com suas informações e material biológico armazenados. Caso isso

ocorra, os pesquisadores entrarão em contato com o comitê de ética e com você para solicitar nova autorização.

Informamos que o material contribuído por você será armazenado no Laboratório de Imunopatologia Keizo Asami (LIKA), que fica na Universidade Federal de Pernambuco (UFPE). Seu material não será enviado a outros pesquisadores brasileiros ou estrangeiros, permanecendo apenas no LIKA. O endereço do LIKA e as formas de contato com os pesquisadores estão no começo desse documento.

Informamos que os riscos que você corre ao participar da pesquisa são apenas sintomas provocados pela coleta do sangue como: vermelhidão e dor no braço no local da coleta e enjoo. Além disso, todas as suas informações pessoais estarão seguras. Nenhuma pessoa fora da pesquisa terá acesso a elas.

A participação na pesquisa é totalmente voluntária. Não haverá nenhum gasto pela sua participação, não recebendo cobrança com o que será realizado. Você também não receberá nenhum pagamento ou benefício financeiro pela sua participação. Da mesma forma, não haverá nenhum prejuízo a você caso não queira participar ou desistir de participar desta pesquisa.

Caso você se sinta prejudicado (a) pelo andamento da pesquisa, asseguramos que você receberá todas as assistências cabíveis neste hospital, incluindo o direito de solicitar indenização aos pesquisadores por eventuais danos. Caso você possua alguma dúvida acerca dos objetivos do estudo, por favor, entre em contato com os responsáveis pela pesquisa. Além disso, se você tiver alguma consideração ou dúvida sobre esta pesquisa, também pode entrar em contato com o comitê de Ética em Pesquisa em Seres Humanos do IMIP (CEP-IMIP), que objetiva defender os interesses dos participantes, respeitando seus direitos e contribuir para o desenvolvimento da pesquisa desde que atenda às condutas éticas.

Eu,_____

(nome completo) compreendi as informações repassadas e autorizo que seja realizada a avaliação genética da amostra de sangue coletada, e concordo que os dados obtidos sejam utilizados para pesquisa. Declaro que fui informado (a) pela equipe do pesquisador Sergio Crovella sobre os objetivos da pesquisa e estou consciente de que:

1. Concordei em participar da pesquisa sem nenhum tipo de pressão;

2. Posso a qualquer momento entrar em contato por telefone com o pesquisador se tiver qualquer dúvida sobre os procedimentos, riscos e benefícios da pesquisa;
3. Posso a qualquer momento desistir de participar da pesquisa, sem que isso prejudique meu atendimento no hospital;
4. O pesquisador poderá ter acesso ao meu prontuário e que minhas informações pessoais serão mantidas em sigilo;
5. Recebi uma cópia deste documento.

<table>
<tr><td colspan="2">Assinatura do Voluntário</td></tr>
<tr><td colspan="2"></td></tr>
<tr><td>Assinatura da Testemunha</td><td>Assinatura da Testemunha</td></tr>
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<tr><td colspan="2">Assinatura do Pesquisador Responsável</td></tr>
<tr><td colspan="2"></td></tr>
<tr><td>Número do prontuário</td><td>Código de amostra</td></tr>
<tr><td></td><td></td></tr>
<tr><td colspan="2">Inclusão no Braço (Transmissão vertical do HIV) C do estudo?</td></tr>
<tr><td>SIM ( )</td><td>NÃO ( )</td></tr>
<tr><td colspan="2">Se SIM, aplicar o termo de assentimento para a coleta do (a) filho (a) da paciente.</td></tr>
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ANEXO B – QUESTIONÁRIOS E FORMULÁRIOS DE ACOMPANHAMENTO

FORMULÁRIO A – RECRUTAMENTO: COLETA DE DADOS CLÍNICOS

1. Dados pessoais (apenas para identificação, NÃO serão divulgados)

Nome do Paciente				
Data de Nascimento	Naturalidade	Cidade de residência	Sexo	
			M ( )	F ( )

2. Informações clínicas e epidemiológicas

Etnia (segundo classificação do IBGE)		Escolaridade (anos)	Renda mensal (reais)
( ) Branca	( ) Indígena		
( ) Negra	( ) Amarela		
( ) Parda	( ) Ignorado		
Peso (kg)		Altura (m)	
Fumo		Etilismo	
SIM ( )		SIM ( )	
Se SIM, quantos cigarros por dia? _____		Se SIM, quantas un. por semana? _____	
NÃO ( )		NÃO ( )	
Se NÃO, parou de fumar? SIM ( ) NÃO ( )		Se NÃO, parou de beber? SIM ( ) NÃO ( )	

Comorbidades pré-existentes			
Desordem Psiquiátricas	SIM ( )	NÃO ( )	IGNORADO ( )
Doença autoimune	SIM ( )	NÃO ( )	IGNORADO ( )
Doença cardiovascular	SIM ( )	NÃO ( )	IGNORADO ( )
Diabetes	SIM ( )	NÃO ( )	IGNORADO ( )
Doença renal	SIM ( )	NÃO ( )	IGNORADO ( )
Doença de fígado	SIM ( )	NÃO ( )	IGNORADO ( )
Osteoporose	SIM ( )	NÃO ( )	IGNORADO ( )
Se SIM, houve fratura?	SIM ( )	NÃO ( )	IGNORADO ( )
Local da fratura:			

3. Infecção pelo HIV

Modo de transmissão (marcar todos que se apliquem)	
( ) Transmissão vertical	( ) Relação sexual heterossexual
( ) Relação sexual homossexual	( ) Transfusão sanguínea
( ) Durante tratamento para hemofilia	( ) Acidente com material biológico
( ) Uso de drogas injetáveis	( ) IGNORADO
Idade de Início da vida sexual	Data de diagnóstico de infecção pelo HIV

Possui parceiro(a) fixo(a)?		
SIM ( )	NÃO (SOLTEIRO(A)) ( )	MÚLTIPLOS PARCEIROS(AS) ( )
O paciente é usuário de drogas?	SIM ( ) Qual? _____	NÃO ( )

Se a paciente for mulher, ela está atualmente grávida?	
SIM ( ) Mês de gestação:_____	NÃO ( )

O(a) paciente tem filhos?				
Nº	Idade	Sexo	Tipo de parto	Status de HIV
1		M ( ) F ( )	normal ( ) cesariana ( )	POS ( ) NEG ( )
2		M ( ) F ( )	normal ( ) cesariana ( )	POS ( ) NEG ( )
3		M ( ) F ( )	normal ( ) cesariana ( )	POS ( ) NEG ( )
4		M ( ) F ( )	normal ( ) cesariana ( )	POS ( ) NEG ( )
5		M ( ) F ( )	normal ( ) cesariana ( )	POS ( ) NEG ( )

4. Perspectivas do paciente acerca do acompanhamento médico

O paciente concorda com a seguinte frase: “Posso ter uma vida normal se for acompanhado(a) pelo médico e seguir suas orientações”?				
SIM ( )		NÃO ( )		
Como o paciente considera as consultas médicas?				
Muito importantes ( )	Importantes ( )	Indiferente ( )	Pouca importância ( )	Sem importância ( )
O que o paciente acha do atendimento no hospital?				
Bom ( )	Regular ( )	Ruim ( )		
Com relação às consultas, o quão frequentemente o paciente falta a elas?				
Muitas vezes ( )	Algumas vezes ( )	Ocasionalmente ( )	Raramente ( )	Nunca ( )
Considerando as condições financeiras, de transporte, de trabalho, dentre outras, do paciente, como ele(ela) diria o quão fácil é chegar no hospital?				
Muito fácil ( )	Fácil ( )	Normal ( )	Difícil ( )	Muito difícil ( )
Considerando as visitas anteriores ao hospital, o(a) paciente estaria disposto(a) a continuar o tratamento no hospital, ou seja, voltaria nas próximas consultas?				
SIM ( )		NÃO ( )		

5. Coinfecções, infecções oportunistas e doenças definidoras de AIDS (ver no prontuário)

				Datas
Sorologia para Hepatite B?	POS ( )	NEG ( )	IGN ( )	
Sorologia para Hepatite C?	POS ( )	NEG ( )	IGN ( )	
Se SIM, tratamento para Hepatite C?	SIM ( )	NÃO ( )	IGN ( )	
Qual esquema?				

				Datas
Tuberculose?	POS ( )	NEG ( )	IGN ( )	
Se SIM, estado da infecção:	ATIVA ( )	LATENTE ( )	IGN ( )	
Está em tratamento?	SIM ( )	NÃO ( )	IGN ( )	
Qual esquema?				

Doenças definidora de AIDS?	
( ) Candidíase oral	( ) Candidíase do esôfago
( ) Febre ou diarreia por 1 mês ou mais	( ) Demência pela AIDS
( ) Herpes simples por 1 mês ou mais	( ) Herpes zoster
( ) Infecção pelo CMV	( ) Perda de peso acentuada
( ) Pneumonia	( ) Toxoplasmose

FORMULÁRIO B – ACOMPANHAMENTO: REVISÃO DE DADOS CLÍNICOS

NOME	CÓDIGO	PÁGINA

1. Informações clínicas e epidemiológicas

1.1 Peso e altura

	Datas	Peso (kg)	Altura (m)
1			
2			
3			
4			

1.2 Fumo e etilismo

	Datas	Fumo	Etilismo
1		SIM ( ) NÂO ( )	SIM ( ) NÂO ( )
2		SIM ( ) NÂO ( )	SIM ( ) NÂO ( )
3		SIM ( ) NÂO ( )	SIM ( ) NÂO ( )
4		SIM ( ) NÂO ( )	SIM ( ) NÂO ( )

Se **SIM**, indique quantas unidades são consumidas:

	Datas	Fumo (cigarros por dia)	Etilismo (unidades por semana)
1			
2			
3			
4			

1.3.1 Comorbidades. Se houver mudança de status, indicar a data

Comorbidade	Status	Datas
Desordem Psiquiátricas		
Doença autoimune		
Doença cardiovascular		
Diabetes		
Doença renal		
Doença de fígado		
Osteoporose		

2. Infecção pelo HIV

2.1 Parceiros sexuais

Possui parceiro(a) fixo(a)?		
SIM ( )	NÃO (SOLTEIRO(A)) ( )	MÚLTIPLOS PARCEIROS(AS) ( )

2.2 Gravidez e HIV

	Datas	Se a paciente for mulher, ela está atualmente grávida?		
1		SIM ( )	Mês de gestação:_____	NÃO ( )
2		SIM ( )	Mês de gestação:_____	NÃO ( )
3		SIM ( )	Mês de gestação:_____	NÃO ( )
4		SIM ( )	Mês de gestação:_____	NÃO ( )

Se alguma resposta for SIM durante o período de acompanhamento, qual o estado sorológico da(s) criança(s)?					Data de Nascimento
Filho1	Sexo M ( ) F ( )	POSITIVO ( )	NEGATIVO ( )	IGNORADO ( )	
Filho2	Sexo M ( ) F ( )	POSITIVO ( )	NEGATIVO ( )	IGNORADO ( )	
Filho3	Sexo M ( ) F ( )	POSITIVO ( )	NEGATIVO ( )	IGNORADO ( )	
Filho4	Sexo M ( ) F ( )	POSITIVO ( )	NEGATIVO ( )	IGNORADO ( )	

3. Se iniciou tratamento antirretroviral, indique a seguir, bem como eventuais efeitos adversos aos medicamentos

Esquema	Data de início

FORMULÁRIO C – ACOMPANHAMENTO DE EXAMES

NOME	CÓDIGO	PÁGINA

4. Coinfecções, infecções oportunistas e doenças definidoras de AIDS

4.1 Sorologia de hepatites virais

Sorologia para Hepatite B			Datas	
POSITIVO ( )	NEGATIVO ( )	IGNORADO ( )		1
POSITIVO ( )	NEGATIVO ( )	IGNORADO ( )		2
POSITIVO ( )	NEGATIVO ( )	IGNORADO ( )		3
POSITIVO ( )	NEGATIVO ( )	IGNORADO ( )		4

Sorologia para Hepatite C			Datas	
POSITIVO ( )	NEGATIVO ( )	IGNORADO ( )		1
POSITIVO ( )	NEGATIVO ( )	IGNORADO ( )		2
POSITIVO ( )	NEGATIVO ( )	IGNORADO ( )		3
POSITIVO ( )	NEGATIVO ( )	IGNORADO ( )		4

Se POSITIVO, está em tratamento contra a hepatite C?				
SIM ( )	NÃO ( )		IGNORADO ( )	
Esquema	Data de início	Adesão (%)	Data de troca	Motivo da troca
1				
2				
3				
4				

4.2 Infecção por Tuberculose

Infecção por tuberculose			Datas	
POSITIVO ( )	NEGATIVO ( )	IGNORADO ( )		1
POSITIVO ( )	NEGATIVO ( )	IGNORADO ( )		2
POSITIVO ( )	NEGATIVO ( )	IGNORADO ( )		3
POSITIVO ( )	NEGATIVO ( )	IGNORADO ( )		4

Se POSITIVO, está tratamento contra a tuberculose?				
SIM ( )	NÃO ( )		IGNORADO ( )	
Esquema	Data de início	Adesão (%)	Data de troca	Motivo da troca
1				
2				
3				

4.3 Doenças definidoras de AIDS (Se houver mudança de status, indicar a data)

Doença	Status	Data
Candidíase oral		
Febre ou diarreia por 1 mês ou mais		
Herpes simples por 1 mês ou mais		
Infecção pelo CMV		
Pneumonia		
Candidíase do esôfago		
Demência pela AIDS		
Perda de peso acentuada		
Toxoplasmose		

5. Acompanhamento de carga viral e contagem de linfócitos

Nº	DATA	CV	LOG_{10}(CV)	CD4+	CD4+ (%)	CD8+	CD8+ (%)	CD45+
1								
2								
3								
4								
5								
6								
7								
8								
9								
10								
11								
12								

6. Medição de adesão ao tratamento anti-HIV CÓDIGO DE AMOSTRA: _____

Prescrição Nº	Esquema 1	Esquema 2	Esquema 3	Esquema 4	Esquema 5	Esquema 6
1						
2						
3						
4						
5						
6						
7						
8						
9						
10						
11						
12						

7. Controle de esquemas e trocas

Esquema	Data de início	Adesão (%)	Data de troca	Motivo da troca
1				
2				
3				
4				
5				
6				

ANEXO C – CARTA DE APROVAÇÃO DO COMITÊ DE ÉTICA DO IMIP

Instituto de Medicina Integral
Prof. Fernando Figueira
Escola de Pós-graduação em Saúde Materno Infantil
Instituição Civil Filantrópica

IMIP

DECLARAÇÃO

Declaro que o projeto de pesquisa **nº 3629 - 13** intitulado **"Fatores Genéticos humanos envolvidos no curso da Infecção pelo HIV: Transmissão vertical, imunidade e resposta à terapia antirretroviral."** apresentado pelo (a) pesquisador (a) **Antonio Victor Campos Coelhos** foi APROVADO pelo Comitê de Ética em Pesquisa em Seres Humanos do Instituto de Medicina Integral Prof. Fernando Figueira – IMIP, em reunião ordinária de 13 de novembro de 2013

Recife, 18 de novembro de 2013

Dr. José Eulálio Cabral Filho
Coordenador do Comitê de Ética
em Pesquisa em Seres Humanos do
Instituto de Medicina Integral Prof. Fernando Figueira

UTILIDADE PÚBLICA MUNICIPAL - Lei. 9851 de 08/11/67
UTILIDADE PÚBLICA ESTADUAL - Lei. 5013 de 14/05/64
UTILIDADE PÚBLICA FEDERAL - Dec. 86238 de 30/07/81
INSCRIÇÃO MUNICIPAL: 05.897-1
INSCRIÇÃO ESTADUAL - Isento
CNPJ: 10.988.301/0001-29

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