Plasma Cytokine Expression as a Marker of Immunoadjuvant Strategy in HIV-infected Patients Undergoing Early or Delayed Antiretroviral Treatment

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Research Article

Keywords: cytokine expression, acute or chronic HIV infection, early or delayed anti-HIV treatment, immunoadjuvant strategy, immune reconstitution

Posted Date: May 5th, 2022

DOI: https://doi.org/10.21203/rs.3.rs-1545927/v1

Abstract

Background: Currently, there is insufficient information on the impact of anti-HIV drugs on immune-inflammatory conditions at various stages of infection. Early initiation of antiretroviral treatment has been approved for better therapeutic effects. Understanding the effects of antiretroviral therapy on the immune function of HIV patients plays a crucial role in the selection and optimization of treatment regimens. Therefore, we analyzed multiple HIV-context situations to examine the levels of different cytokines under these conditions to determine the need for immunoadjuvant therapy.

Methods: Luminex was used to measure variations in the levels of 45 cytokines during antiretroviral treatment of HIV. Plasma samples were collected from 15 patients in the early treatment group and 18 patients in the delayed treatment group (CD4 < 350 cell/mm^3). Samples were drawn at 0, 24, 48, and 96 weeks, and the Mann-Whitney U test was used to evaluate the natural course of infection under normal conditions. A generalized estimating equation (GEE) was used to describe the changes at each time point during antiretroviral therapy. The changes in the early and delayed treatments were verified using the Wilcoxon test. The results obtained after 96 weeks of treatment were compared to those obtained under normal conditions to confirm the curative effect. Spearman's test was used to build cytokine networks for the identification of crucial cytokines that manipulate the HIV-related immune environment.

Results: In our study, a few cytokines, such as TRAIL, fractalkine, and Fit-3 ligand, in the plasma were significantly reduced ($P < 0.05$), which finally returned to normal. Chronically high or low levels (consisting of 40 cytokines) were dominant, even after long-term antiretroviral therapy. Cytokine networks provide further insights into the activities and correlations of cytokines with each other. Th1/Th2 cells showed considerably reduced expression, whereas chemotactic factors or CSF exhibited high expression.

Conclusions: The levels of various cytokines could not be restored to normal even after long-term antiretroviral therapy. Under exceptional circumstances, efficient measures should be implemented to establish a better immune environment. Precise regulation of the levels of these crucial cytokines may help further establish a benign prognosis and favorable therapeutic effects.

1 Introduction

By effectively reducing the levels of HIV RNA and intracellular pre-DNA, early initiation of antiretroviral therapy can reduce the stress associated with immune deficiency caused by HIV infection. Lymphocytes can regularly differentiate into cells. HIV-related immune functions can be protected by altering the immune trajectory, which can effectively reduce the complications caused by immune decompensation. However, the extent to which antiretroviral therapy may assist the recovery of the immune environment requires further investigation. In addition, an understanding of the crucial immune family members that form unconventional immune systems may help in developing long-term optimized treatment strategies [1].

The results of early therapy and chronic infection studies in non-human primates indicate that the timing of antiretroviral therapy can result in a significant difference in the dynamics and quality of the immune response or differentiation trajectory during HIV infection [2]. Early antiretroviral therapy is not only effective in reducing overall HIV DNA quantity but can also significantly alleviate the activation of CD8 + T cells and accelerate the transformation of HIV-specific CD8 + T cells into effector or memory T cells, which effectively reduces immune disuse due to excessive activation or apoptosis of cytotoxic T cells (CTL)[3]. Furthermore, the Fiebig I/II treatment is more effective in maintaining normal lymphocyte levels. For example, Th17 function is not impaired before Fiebig III function [4]. Hellmuth et al. demonstrated that, although the levels of some cytokines present in the cerebrospinal fluid were significantly elevated, early ART effectively reduced the levels of these cytokines to almost normal levels in HIV-infected patients [5]. Meanwhile, a cytokine analysis by Muema et al. demonstrated that plasma pro-inflammatory cytokine and type Ⅰ FN cytokine levels were near baseline during the first month after ETI testing in the Fiebig I/II stages [6]. In this study, we analyzed and compared the levels of 45 cytokines in the plasma of HIV patients. By studying the expression of cytokines during early or delayed anti-HIV treatment, we hope to develop a more effective and accurate strategy for regulating abnormal immune environments.

The immune condition during HIV infection is closely related to the viral load or therapeutic time point, which exerts pressure on the differentiation, proliferation, and apoptosis of crucial lymphocytes. Furthermore, the presence of cytokines as an immediate cause is important for the immune environment as it may further exacerbate complex and changeable immune conditions [5, 7]. In view of this, we chose different immune conditions for the 45 cytokines in the plasma samples of HIV patients to examine their behavior. Our findings suggest that, irrespective of the timing of antiretroviral therapy, cytokine levels will most likely not return to normal. Adjuvant therapy may be helpful if more delicate and favorable curative effects are required.

2 Materials And Methods

Study Subjects

Cryopreserved whole blood samples from 33 patients in the early or delayed stages of infection were collected for analysis. The isolated peripheral blood mononuclear cell PBMC samples were immediately sent for analysis to the Beijing Key Laboratory for HIV/AIDS Research, Clinical and Research Center for Infectious Diseases, Beijing Youan Hospital. Plasma samples were collected at 0, 24, 48, and 96 weeks from 15 patients in the early treatment stage and 18 patients in the delayed treatment stage (CD4 counts below 350 cell/mm^3). The research protocol and all related experiments were approved by the Research Ethics Committee of the Beijing Youan Hospital. All study participants provided written informed consent upon admission for blood sample storage and for subsequent studies. These methods were carried out in accordance with approved guidelines and regulations (National Science and Technology Major Project of China during the 13th Five-Year Plan Period, grant no. 2017ZX10201101).

Peripheral Blood Processing

PBMC were isolated from whole blood collected in EDTA tubes containing Ficoll-Hypaque and analyzed immediately (BD FACS Melody). The plasma was separated from whole blood and stored at -20°C until use. All paired plasma and PBMC samples used in this study were obtained from the same blood sample.

Viral Load Determination

Plasma HIV RNA levels were measured using an automated real-time PCR M2000 system (Abbott Molecular Inc., Des Plaines, IL, USA) with a lower limit of 40 copies/mL.

Luminex

We used Luminex (R&D Systems, Inc., 614 McKinley Place NE, Minneapolis, MN 55413) to examine plasma cytokine levels. The following 45 cytokines were analyzed according to the manufacturer's instructions: CD40 ligand, epidermal growth factor (EGF), eotaxin, fibroblast growth factor (FGF) basic, FMS-like tyrosine kinase (Flt)-3 ligand, fractalkine, granulocyte colony-stimulating factor (G-CSF), granulocyte macrophage colony-stimulating factor GM-CSF, granzyme B, growth-related oncogene(GRO)a, GROb, interferon (IFN)-a, IFN-b, IFN-g, interleukin (IL)-1a, IL-1b, IL-1ra, IL-2, IL-3, IL-4, IL-5, IL-6, IL-7, IL-8, IL-10, IL-12p70, IL-13, IL-15, IL-17A, IL-17E, IL-33, IFN-g induced protein 10 kDa (IP-10), monocyte chemoattractant protein (MCP)-1, macrophage inflammatory protein (MIP)-1a, MIP-1b, MIP-3a, MIP-3b, platelet-derived growth factor (PDGF)-AA, PDGF-AB/BB, programmed death ligand (PD-L)1/B7-H1, regulated on activation, normal T cell expressed and secreted (RANTES), transforming growth factor (TGF)-a, tumor necrosis factor TNF-a, TNF-related apoptosis-inducing ligand (TRAIL), and vascular endothelial growth factor (VEGF). The cryopreserved plasma samples, stored at -80℃, were centrifuged at 16000 x g for 4 min and analyzed using the Bioplex-200 system according to the manufacturer's instructions. A 75 µL sample was diluted with twofold RD6-65 and centrifuged (16000 x g, 4 min). Then, 0.5 mL magnetic beads were introduced into 5 mL magnetic bead buffer and mixed properly. Subsequently, 55 µL of RANTES and 55 µL of IL-17E were added to magnetic bead buffer. A standard or sample of 50 µL per well was added to 96-well plates containing a diluted microparticle cocktail of 50 mL. The plates were then incubated for 2 h at room temperature (RT) in a shaker at 800 rpm. The 96-well plates were then washed with wash buffer. Then, 50 µL of diluted biotin-antibody cocktail was added to each well. After incubation for 1 h, the cells were washed and 50 µL of streptavidin-PE was added to each well. The wells were washed again after incubation for 30 minutes. The readings were recorded within 90 min using a Luminex-200. The detection limit of the kit was 3.16 pg/ml.

Data Analysis

The Mann-Whitney *U* test was used to compare the baseline characteristics at the early and delayed treatment stages at 0 weeks (UTx). The variations in cytokine levels during the early and delayed stages of treatment were compared using the Mann-Whitney *U* test with healthy conditions (HC). To further explore the impact of antiretroviral therapy on HIV inflammation, the variables of cytokine quantity from 0 to 96 weeks were determined using the generalized estimation equation (GEE). The Wilcoxon test was used in the comparative analysis of HIV infection in the early or delayed treatment stages. The results

were presented as a box diagram (GraphPad Prism). The cytokine levels after 96 weeks of treatment were compared specifically via the Mann-Whitney *U* test using HC. Spearman's test was used to draw heat maps (R programming language). Differences were considered statistically significant at $P < 0.05$.

3 Results

Basic Characteristics of HIV-infected Individuals

In this study, plasma samples from 15 individuals in the early treatment stage and 18 individuals in the delayed treatment stage were selected for analysis and comparison. The median age of the patients in the early treatment group was 28 years (interquartile range (IQR], 26–36 years), and that of the delayed treatment group was 32 years (interquartile range [IQR], 28–38.5 years]. All selected patients were male, except for one candidate. Men engaging in same-sex relations (MSM) accounted for the highest transmission rate. There was no significant difference in the viral load or lymphocyte counts before and after early or delayed treatment (Table 1).

Table 1
Basic characteristics of HIV-infected individuals.

Characteristic	AHI n = 15	CHI N = 18	*P*-value
Age, years	28(26,36)	32(28,38.5)	0.319
Sex, n (%)			
Male	14 (93)	18 (100)	NA
Female	1 (7)	0 (0)	NA
Baseline: plasma viral load, (HIV RNA copies/ml)	24559 (2788,79646)	22500 (6675,75591)	0.857
ART: plasma viral load, (HIV RNA copies/ml)	TND	TND	1.000
Co-morbidities (%)			
Syphilis	4(33)	5(33)	NA
HBV	0(0)	0(0)	NA
Syphilis + HBV	0(0)	3(20)	NA
Transmission category, n (%)			
Hetero	2 (17)	2 (13)	NA
MSM	9 (75)	11 (73)	NA
PWID	1 (8)	0 (0)	NA
Unknown	0 (0)	2 (13)	NA
Baseline: CD4 + T cell count (cells/μL)	358.29 (263.4,497.3)	296 (225,366.4)	0.148
cART (96 w): CD4 + T cell count (cells/μL)	637.76 (499.14,954.43)	563.72 (466.52,687.68)	0.357
Baseline: CD8 + T cell count (cells/μL)	997.51 (659.5,1628)	735.37 (515.8,1002)	0.075
cART (96 w): CD8 + T cell count (cells/μL)	801.43 (544.59,855.84)	906.41 (675.38,1223.20)	0.139
cART Treatment, n (%)			
cART (AZT/3TC/NVP)	0 (0)	9 (60)	NA

Notes: Data are presented as medians with IQRs. Participant characteristics were analyzed using the Mann-Whitney U test. Statistical significance was set at P < 0.05. Abbreviations: AHI, acute HIV infection; CHI, chronic HIV infection.

Characteristic	AHI n = 15	CHI N = 18	*P*-value
cART (TDF/3TC/EFV)	100 (0)	5 (33)	NA
Regimen adjustment*	0 (0)	1 (6)	NA

Notes: Data are presented as medians with IQRs. Participant characteristics were analyzed using the Mann-Whitney U test. Statistical significance was set at P < 0.05. Abbreviations: AHI, acute HIV infection; CHI, chronic HIV infection.

To determine the patterns of inflammatory cytokine levels under natural conditions (before treatment), we first examined those with significantly altered levels (Table S1). We found that the levels of PD-L1, IL-8, EGF, G-CSF, granzyme B, IFN-b, IL-12p70, IL-15, IL-17A, IL-17E, IL-1b, IL-33, IL-5, TGF-a, VEGF, and FGF basic were significantly lower than the normal levels. As expected, some cytokines with significantly elevated levels may be constitutional components of the cytokine storm ($P < 0.05$). Higher levels of eotaxin, MIP-3b, MCP-1, MIP-1a, IP-10, Fit-3 ligand, GM-CSF, IFN-a, IL-10, IL-1ra, IL-3, IL-4, PDGF-AB/BB, TNF-a, TRAIL, and fractalkine are shown in Table S1.

Levels of Few Cytokines Decreased Significantly through 96 weeks of Anti-HIV Therapy

First, we aimed to directly observe the changes in cytokine levels before and after antiretroviral therapy. Upon comparing the results of the natural infection group with those of the long-term (96 weeks) anti-HIV treatment group in early and delayed treatment stages, we found that MIP-3b, fractalkine, IL-10, IP-10, GM-CSF, Fit-3 ligand, and TRAIL were significantly reduced after long-term highly active antiretroviral therapy (HAART) (Fig. 1, A-G). Early treatment could be effective in alleviating the risk of inflammatory cytokine storms [1]. MIP-3b, fractalkine, IL-10, GM-CSF, and Fit-3 ligand showed significantly reduced expression during the early treatment stages (Fig. 1A–C, E, F). IP-10 levels decreased more significantly in the delayed treatment group ($P < 0.001$). TRAIL expression was significantly reduced only in the delayed treatment group ($P < 0.05$). Although early treatment can be effective in reducing the levels of some cytokines, the results are not the same for all cytokines. Moreover, early antiretroviral therapy (ART) is ineffective in eliminating HIV-induced immune responses [6].

To determine changes in the levels of different cytokines during this process, we collected samples at four time points (0, 24, 48, and 96 weeks). First, we used GEE to analyze all 33 samples, both acute and chronic. After 96 weeks of antiretroviral therapy, the levels of a few cytokines, including IL-1ra, TRAIL, eotaxin, MIP-3b, fractalkine, IP-10, Fit-3 ligand, GM-CSF, granzyme B, IL-10, TNF-α, and IL-1a, changed significantly ($P < 0.05$). Eotaxin levels increased dramatically after treatment, whereas those of the other cytokines decreased significantly ($P < 0.05$) (Table S2). To further examine the performance of the cytokines, we conducted an analysis (Wilcoxon test) for the early or delayed treatment separately to examine the changes from 0 to 96 weeks. Notably, the levels of all cytokines mentioned above decreased significantly within 24 weeks (Fiebig III) in the early treatment group (Fig. 2A–J). Some cytokines

rebounded after 24 weeks (Figure D-J). However, MIP-3b, IP-10, IL-10, GM-CSF, fractalkine, IL-1a, and Fit-3 ligand still showed reduced levels after 96 weeks of treatment compared to those at the beginning of the treatment (Fig. 2A–G). In the delayed treatment group, MIP-3b, IP-10, and IL-10 levels continuously decreased (Fig. 2A–C). In addition, GM-CSF and TRAIL levels decreased significantly after 96 weeks of treatment (Fig. 2D, I). Despite these changes, most cytokines did not exhibit any obvious variations during the entire process.

Few Cytokines could be Restored to Normal with Anti-HIV Therapy

As antiretroviral therapy could significantly reduce the levels of some cytokines, we sought to determine the cytokines whose levels returned to normal. We found that TRAIL, fractalkine, Fit-3 ligand, IL-10, and IL-1a levels recovered to normal levels compared to those under normal conditions at 96 weeks (Fig. 3, A-E).The levels of granzyme B were significantly lower than normal levels after 96 weeks of treatment (Fig. 3, J), although these levels were low from the onset. The levels of MIP-3b, GM-CSF, IL-1ra, and IP-10 were significantly higher than normal levels (Fig. 3F–I). However, we found that variations during the early and delayed treatment stages did not correspond. The GM-CSF levels returned to normal after 96 weeks of treatment in the chronic group and remained high in the early treatment group ($P < 0.001$).

Classification of Cytokines associated with HIV Infection Demonstrated using Cytokine Networks

As antiretroviral therapy alone cannot alter cytokine levels, we sought to examine how cytokines function together during HIV infection. Using the information collected earlier, we separated cytokines into two groups: those with higher-than-normal levels (Fig. 4) and those with lower-than-normal levels (Fig. 5). In this process, we selected cytokines whose levels were higher or lower than normal but did not return to normal after 96 weeks, to fit into these networks. This was performed to determine how these crucial cytokines interact with each other to maintain the immune condition from recovering to normal. Each group was further separated into four specific circumstances: acute _UTx, acute _Tx, chronic _UTx, and chronic _Tx. The selected cytokines were closely associated with each other, creating a significant HIV-related immune environment. To clarify this, we further grouped cytokines according to their functionality (Table 2). Deciphering how these cytokines function may aid in developing a more detailed anti-HIV therapy for better recovery.

Table 2
Classification and description of cytokines.

Classification	Cytokine Level		Presentation of Cytokine Functions	
Th1/Th2	E	IL-4	Promotes Th2 lymphocyte proliferation and differentiation.	[30]
	E	IL-10	Mainly Th1 proliferation, also with Th2 in specific condition; IL-6/IL-10 can lead to B-cell lymphoma.	
	D	Granzyme B	Produced by NK cells through ADCC function.	
	D	IL-12P70	IL-2/IL-12 significantly activates Th1 reaction.	[31]
	D	IL-15	Potent testing Th1 activator.	[24]
	D	IL-17E(IL-25)	Lead to Th2 reaction.	
	D	IL-5	Promotes B cell growth; Also called “Eo-CSF”.	
Chemokine	E	Eotaxin (CCL11)	Natural antagonist of CCR2; Natural activator of CCR5.	[32]
	E	MIP-3b	CCL19; C-C chemokine subfamily has potent antiviral ability.	
	E	MIP-1a	CCL3;	
	E	IP-10	CXCL10; extreme rise in HIV infection.	
	E	MCP-1	CCL2; targeting CCR1, CCR2, CCR5.	
	D	IL-8	CXCL8; specifically attracts neutrophil granulocytes.	
CSF	E	FLT-3Ligand	Activates granulocyte with GM-CSF; AML deteriorate.	[33]
	E	GM-CSF	Granulocytes and macrophages.	[34]
	E	IL-3	Also called “Multi-CSF”.	
	D	G-CSF	Granulocyte stimulating factor.	
IFN	E	IFN-a	Poor effect on HIV; promotes significant CD4 + T decrease.	
	D	IFN-b	Has shown some efficacy in treating COVID-19.	
Inhibitor	E	IL-1ra	Can be induced by GM-CSF, IL-3, IL-5.	

Abbreviations: E means “Elevated”; D means “Deficiency”.

Classification	Cytokine Level		Presentation of Cytokine Functions	
	D	PD-L1	Immune checkpoint; proven crucial roles in both tumor immunity and viral immunity.	[35]
Pro-inflammatory	D	IL-17A	Primarily activating neutrophils.	
	D	IL-1b	IL-1 family member; Large damage of normal issue.	
	D	IL-33	IL-1 family member; IL-33 antibody can effectively reduce neutrophil amount.	
TNF	E	TNF-a	TNFi can significantly reducing inflammatory syndrome.	
	E	TRAIL	TNF family has not shown strong killing effect on HIV-infected cells.	
Growth Factor	E	PDGF-AB/BB	Imatinib, Sorafinib, Sunitinib play perfect antagonism effect.	[36]
	D	TGF-a	Potent immune response suppressing factor; Promote regeneration of thymus epithelium after injury.	
	D	FGF Basic	Fibroblast growth factor; activates epithelium transcription factor.	
	D	EGF	Epithelial growth factor; cooperate with TGF and FGF.	
	D	VEGF	Vascular endothelial growth factor; also impacts on lymphatic vessel.	
Abbreviations: E means “Elevated”; D means “Deficiency”.				

4 Discussion

Our results show that the immune environment was severely compromised in each scenario. The recovery of CD4 + T cells does not represent the entire immune system. This shows that relying solely on antiretroviral therapy for natural immune recovery cannot produce satisfactory results. Long-term poor immune reconstitution may lead to serious complications, especially in elderly patients with critical complications, pregnant women, and patients with multiple drug resistance [8]. Based on this, we hope to establish a better immune regulation system to augment the existing antiretroviral therapy and improve the medical experience of patients in various situations.

Among the 15 cytokines that were maintained at high levels, we found that the C-C antiviral chemokine subfamily, including Eotaxin (CCL11), MIP-3b (CCL19), MIP-1a (CCL3), MCP-1 (CCL2), and IP-10 (C-X-C motif chemokine ligand 10 - CXCL10), occupied an important position. However, it may cause serious side effects [9]. All other cytokines promote inflammatory reactions, except for IL-1ra. IL-4 and IL-10 lead

to a Th2 immune response in terms of immune trajectory. IL-15, IL-17E(IL-25), and IL-5, which are strong enhancers of the Th2 response, exhibited extremely low levels throughout the study. High levels of TNF-α, TRAIL, IFN-α, IP-10, and Flt3L, which are powerful proinflammatory cytokines, lead to a Th1 immune reaction with acute inflammatory syndrome. In contrast, granzyme B, IL-12p70, and PD-L1(also known as “immune checkpoint”, as it effectively inhibits cytotoxic reactions) remained at extremely low levels, making no contribution to the regulation of the HIV-related immune environment. Meanwhile, we observed that except for the cytokines mentioned above, GM-CSF and IL-3 (Multi-CSF) remained at extremely high levels, which can be harmful to HIV patients with co-infections. Interestingly, the members of the cytokine groups remained approximately equal, whether before or after treatment, indicating that the impact of these cytokines can be persistent.

Our results support current research on the design of anti-HIV immune therapies. First,CD8 + T cells can be precisely regulated in different ways to cooperate with antiretroviral therapy, avoiding the long-term activation of cytotoxic cells, leading to functional obsolescence [10]. Given the low response of IL-12/IL-15, PD-L1, or granzyme B, this movement aims to manipulate the body’s immune preserves for the recall response of PD-1^{lo} resources [11, 12]. In our case, both low expression of IL-12/IL-15 or PD-L1 with potent CD8 + T can be a good signal for benign immune conditions. Second, Limiting Th2 response or large activation of ineffective B cells through approaches including high levels of IL-6/IL-10, which pose a risk of tumor in the later stages of treatment, especially for elderly patients [13–15]; Third, avoiding excessive recruitment of granulocytes or lymphocytes while being ineffective against viruses can alleviate the damage caused by inflammatory storms or neutrophil extracellular traps (NET) to normal tissues [16–18]. In this study, chemotactic cytokines with large amounts of stimulating factors were produced. Fourth, existing challenges, such as low efficiency by naturally produced cytokines, primary regulatory mechanisms, and difficult internal problems (e.g. receptor cells can be activated while snatching viral targets, which could exacerbate viral transmission) represent limitations, which emphasize that artificial immunoadjuvant therapy is urgently needed [19, 20].

At present, studies on HIV-related complications/cytokines and their related targets are urgently needed. First, studies on elite controllers of HIV suggest that cytotoxic cells play crucial roles in the control of the immune environment; however, B cell-based research has been disappointing [21]. The reservoir of HIV, which is composed of resting CD4 + T cells, occupies a major part of all reservoirs. Co-inhibitory receptors, such as CTLA4+, PD-1+, and cell immunoglobulin and mucin domain- containing protein 3 (TIM-3) + are highly transient [22]. In addition to the elimination of HIV reservoirs, rebuilding the immune system from the beginning is undoubtedly the best approach [23]. The IL-2/IL-15 super agonist is in phase of clinical trials [24]. Keratinocyte growth factor (KGF) has been shown to promote thymic epithelial proliferation. A large number of CCR5 + CD4 + T cells are activated in the intestine, causing irreversible damage to the gastrointestinal tract, which has a serious impact on elderly patients. Artificially modified CCR5 + CD4 + T cells have obvious selection advantages and do not exhibit excessive immunogenicity [25]. In terms of complications, HIV-1 infection occurs in a large number of macrophages, which strengthens the destruction of Th1 type response, promoting synergy with

Mycobacterium tuberculosis [26]. In the alveolar environment of tuberculosis infection, C-X-C motif chemokine ligand 4 (CXCR4) + strains are specifically selected due to the high production of MIP-1b and RANTES (CCL5), which poses a challenge to treatment [27]. HIV prevalence has been shown to significantly increase the risk of transmission of acquired drug resistance [28]. IL-6, IL-10, and CD40 ligands lead to increased morbidity of advanced B-cell lymphoma, whereas HIV combined with HBV or Kaposi sarcoma human virus KSHV is more likely to directly lead to the occurrence of tumors [29].

There were limitations in the design of this study. For example, there was no precise time control for the natural infection stage before treatment beyond the CD4 + T cell counts or viral load with an epidemiological history, which we believe is insufficient because cytokine levels may be affected by the duration of natural infection. Otherwise, the ability to control HIV in each patient can be very different. In addition, the sample size should be increased.

Declarations

Acknowledgments

This study was supported by the Beijing Key Laboratory for HIV/AIDS Research, Clinical and Research Center for Infectious Diseases, Beijing Youan Hospital. Our study received assistance from the Biomedical Information Center, Beijing Youan Hospital, and the Biomedical Informatics Laboratory, Capital Medical University. We are grateful to the patients and their families who would like to participate in this trial and make all this possible.

Author Contributions

CL, NW, ZL, and XH contributed to the conceptualization and clinical information; JS, ZL, and CL performed the experiments, provided samples, and generated data; NW and CL analyzed the data; CL wrote the original draft; CL, NW, PY, and ZL reviewed and edited the manuscript. All authors have contributed to the manuscript and approved the submitted version.

Funding

This work was supported by the Fund for Capital Health Development Research (2022-2-2185; 2022-1G-3011), Beijing Talent Project in the New Millennium (2020A35), and National Science and Technology Major Project of China during the 13th Five-year Plan Period (Grant No. 2017ZX10201101).

Availability of Data and Materials

The datasets used and/or analysed during the current study available from the corresponding author on reasonable request.

Ethics approval and consent to participate

The research protocol and all related experiments were approved by the Research Ethics Committee of the Beijing Youan Hospital. All study participants provided written informed consent upon admission for blood sample storage and for subsequent studies. These methods were carried out in accordance with approved guidelines and regulations (National Science and Technology Major Project of China during the 13th Five-Year Plan Period, grant no. 2017ZX10201101).

Consent for publication

Not applicable

Competing Interests

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as potential conflicts of interest.

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References

1. Kazer SW, Walker BD, Shalek AK: **Evolution and Diversity of Immune Responses during Acute HIV Infection**. Immunity 2020, **53**(5):908–924.
2. Crowell TA, Fletcher JL, Sereti I, Pinyakorn S, Dewar R, Krebs SJ, Chomchey N, Rerknimitr R, Schuetz A, Michael NL *et al*: **Initiation of antiretroviral therapy before detection of colonic infiltration by HIV reduces viral reservoirs, inflammation and immune activation**. J Int AIDS Soc 2016, **19**(1):21163.
3. Zaza M.Ndhlovu SWK, Thandeka Nkosi, Funsho Ogunshola, Daniel M. Muema, Gursev Anmole, et al.: *Sci Transl Med* 2019, **eaau0528**.
4. Schuetz A, Deleage C, Sereti I, Rerknimitr R, Phanuphak N, Phuang-Ngern Y, Estes JD, Sandler NG, Sukhumvittaya S, Marovich M *et al*: **Initiation of ART during early acute HIV infection preserves mucosal Th17 function and reverses HIV-related immune activation**. PLoS Pathog 2014, **10**(12):e1004543.
5. Hellmuth J, Slike BM, Sacdalan C, Best J, Kroon E, Phanuphak N, Fletcher JLK, Prueksakaew P, Jagodzinski LL, Valcour V *et al*: **Very Early Initiation of Antiretroviral Therapy During Acute HIV**

Infection Is Associated With Normalized Levels of Immune Activation Markers in Cerebrospinal Fluid but Not in Plasma. J Infect Dis 2019, **220**(12):1885–1891.

6. Muema DM, Akilimali NA, Ndumnego OC, Rasehlo SS, Durgiah R, Ojwach DBA, Ismail N, Dong M, Moodley A, Dong KL *et al*: **Association between the cytokine storm, immune cell dynamics, and viral replicative capacity in hyperacute HIV infection**. BMC Med 2020, **18**(1):81.
7. Mabuka JM, Dugast A-S, Muema DM, Reddy T, Ramlakhan Y, Euler Z, Ismail N, Moodley A, Dong KL, Morris L *et al*: **Plasma CXCL13 but Not B Cell Frequencies in Acute HIV Infection Predicts Emergence of Cross-Neutralizing Antibodies**. Frontiers in Immunology 2017, **8**.
8. Lucas S, Nelson AM: **HIV and the spectrum of human disease**. J Pathol 2015, **235**(2):229–241.
9. Anja Schmall HMA-t, Susanne Herold, Marian Kampschulte, Andreas Weigert, Astrid Wietelmann,etc.: **Macrophage and cancer cell crosstalk via CCR2 and CX3CR1 is a fundamental mechanism driving lung cancer**. *AJRCCM* 2014.
10. Dolina JS, Van Braeckel-Budimir N, Thomas GD, Salek-Ardakani S: **CD8(+) T Cell Exhaustion in Cancer**. Front Immunol 2021, **12**:715234.
11. Hashimoto M, Kamphorst AO, Im SJ, Kissick HT, Pillai RN, Ramalingam SS, Araki K, Ahmed R: **CD8 T Cell Exhaustion in Chronic Infection and Cancer: Opportunities for Interventions**. Annu Rev Med 2018, **69**:301–318.
12. Beltra JC, Manne S, Abdel-Hakeem MS, Kurachi M, Giles JR, Chen Z, Casella V, Ngiow SF, Khan O, Huang YJ *et al*: **Developmental Relationships of Four Exhausted CD8(+) T Cell Subsets Reveals Underlying Transcriptional and Epigenetic Landscape Control Mechanisms**. Immunity 2020, **52**(5):825–841 e828.
13. Dunleavy K, Wilson WH: **How I treat HIV-associated lymphoma**. Blood 2012, **119**(14):3245–3255.
14. Moffitt AB, Dave SS: **Clinical Applications of the Genomic Landscape of Aggressive Non-Hodgkin Lymphoma**. J Clin Oncol 2017, **35**(9):955–962.
15. Shingleton J, Wang J, Baloh C, Dave T, Davis N, Happ L, Jadi O, Kositsky R, Li X, Love C *et al*: **Non-Hodgkin Lymphomas: Malignancies Arising from Mature B Cells**. Cold Spring Harb Perspect Med 2021, **11**(3).
16. Castanheira FVS, Kubes P: **Neutrophils and NETs in modulating acute and chronic inflammation**. Blood 2019, **133**(20):2178–2185.
17. Gigon L, Yousefi S, Karaulov A, Simon HU: **Mechanisms of toxicity mediated by neutrophil and eosinophil granule proteins**. Allergol Int 2021, **70**(1):30–38.
18. Yipp BG, Kubes P: **NETosis: how vital is it?** Blood 2013, **122**(16):2784–2794.
19. Eggink D, Bontjer I, de Taeye SW, Langedijk JPM, Berkhout B, Sanders RW: **HIV-1 anchor inhibitors and membrane fusion inhibitors target distinct but overlapping steps in virus entry**. J Biol Chem 2019, **294**(15):5736–5746.
20. Venanzi Rullo E, Ceccarelli M, Condorelli F, Facciola A, Visalli G, D'Aleo F, Paolucci I, Cacopardo B, Pinzone MR, Di Rosa M *et al*: **Investigational drugs in HIV: Pros and cons of entry and fusion**

inhibitors (Review). Mol Med Rep 2019, **19**(3):1987–1995.

21. Collins DR, Gaiha GD, Walker BD: **CD8(+) T cells in HIV control, cure and prevention**. Nat Rev Immunol 2020, **20**(8):471–482.
22. McGary CS, Deleage C, Harper J, Micci L, Ribeiro SP, Paganini S, Kuri-Cervantes L, Benne C, Ryan ES, Balderas R *et al*: **CTLA-4(+)PD-1(-) Memory CD4(+) T Cells Critically Contribute to Viral Persistence in Antiretroviral Therapy-Suppressed, SIV-Infected Rhesus Macaques**. Immunity 2017, **47**(4):776–788 e775.
23. Corbeau P, Reynes J: **Immune reconstitution under antiretroviral therapy: the new challenge in HIV-1 infection**. Blood 2011, **117**(21):5582–5590.
24. Miller JS, Davis ZB, Helgeson E, Reilly C, Thorkelson A, Anderson J, Lima NS, Jorstad S, Hart GT, Lee JH *et al*: **Safety and virologic impact of the IL-15 superagonist N-803 in people living with HIV: a phase 1 trial**. Nat Med 2022.
25. Tebas P, Stein D, Tang WW, Frank I, Wang SQ, Lee G, Spratt SK, Surosky RT, Giedlin MA, Nichol G *et al*: **Gene editing of CCR5 in autologous CD4 T cells of persons infected with HIV**. N Engl J Med 2014, **370**(10):901–910.
26. Bell LCK, Noursadeghi M: **Pathogenesis of HIV-1 and Mycobacterium tuberculosis co-infection**. Nature Reviews Microbiology 2017, **16**(2):80–90.
27. Esmail H, Riou C, Bruyn ED, Lai RP, Harley YXR, Meintjes G, Wilkinson KA, Wilkinson RJ: **The Immune Response to Mycobacterium tuberculosis in HIV-1-Coinfected Persons**. Annu Rev Immunol 2018, **36**:603–638.
28. Khan PY, Yates TA, Osman M, Warren RM, van der Heijden Y, Padayatchi N, Nardell EA, Moore D, Mathema B, Gandhi N *et al*: **Transmission of drug-resistant tuberculosis in HIV-endemic settings**. The Lancet Infectious Diseases 2019, **19**(3):e77-e88.
29. Dolcetti R, Gloghini A, Caruso A, Carbone A: **A lymphomagenic role for HIV beyond immune suppression?** Blood 2016, **127**(11):1403–1409.
30. Raches Ella SR, Harsh Jogdand, Vamshi Sarangi, Brunda Ganneru, Sai Prasad, et al.: **Safety and immunogenicity of an inactivated SARS-CoV-2 vaccine,BBV-52: interim results from a double-blind, randomised, multicentre, phase 2 trial, and 3-month follow-up of a double-blind, randomised phase 1 trial**. *Lancet Infect Dis* 2021, **21**:**950 – 61**.
31. Ngcobo S, Molatlhegi RP, Osman F, Ngcapu S, Samsunder N, Garrett NJ, Abdool Karim SS, Abdool Karim Q, McKinnon LR, Sivro A: **Pre-infection plasma cytokines and chemokines as predictors of HIV disease progression**. Sci Rep 2022, **12**(1):2437.
32. Bekele Feyissa Y, Chiodi F, Sui Y, Berzofsky JA: **The Role of CXCL13 in Antibody Responses to HIV-1 Infection and Vaccination**. Front Immunol 2021, **12**:638872.
33. Zelalem Temesgen CDB, Jason Baker, Christopher Polk, Claudia R Libertin, Colleen F Kelley, et al.: **Lenzilumab in hospitalised patients with COVID-19 pneumonia (LIVE-AIR): a phase 3, randomised, placebocontrolled trial**. Lancet Respir Med 2022, **10**: 237–46.

34. Leth S, Schleimann MH, Nissen SK, Højen JF, Olesen R, Graversen ME, Jørgensen S, Kjær AS, Denton PW, Mørk A *et al*: **Combined effect of Vacc-4x, recombinant human granulocyte macrophage colony-stimulating factor vaccination, and romidepsin on the HIV-1 reservoir (REDUC): a single-arm, phase 1B/2A trial**. The Lancet HIV 2016, **3**(10):e463-e472.
35. Varchetta S, Mele D, Oliviero B, Mantovani S, Ludovisi S, Cerino A, Bruno R, Castelli A, Mosconi M, Vecchia M *et al*: **Unique immunological profile in patients with COVID-19**. Cell Mol Immunol 2021, **18**(3):604–612.
36. Abramson J, Anderson G: **Thymic Epithelial Cells**. Annu Rev Immunol 2017, **35**:85–118.

Figures

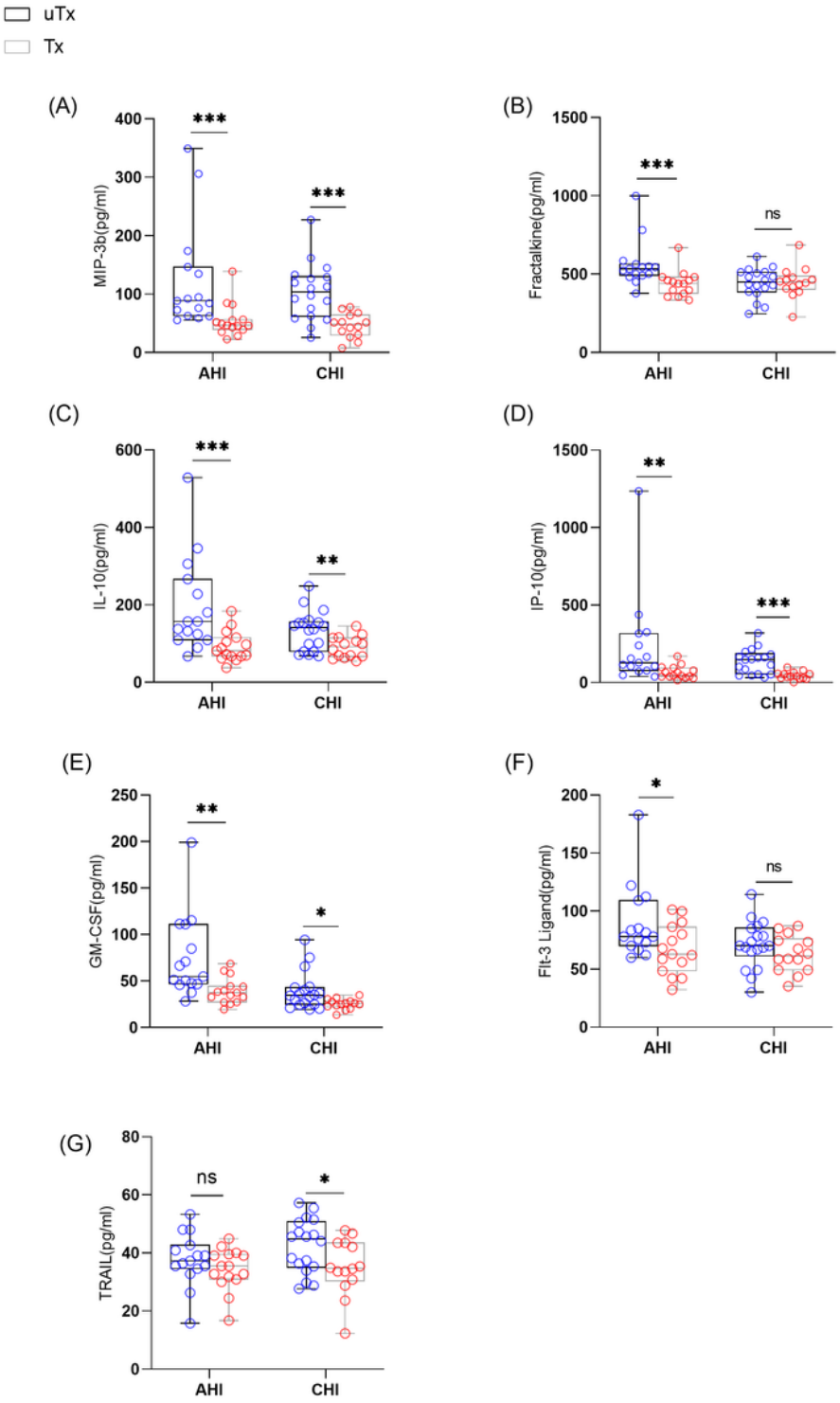

Figure 1

Significant variations in few cytokines before and after anti-HIV treatment.

Abbreviations: uTx, untreated; Tx, treated; AHI, acute HIV infection; CHI, chronic HIV infection. Untreated samples were collected at 0 weeks (initiation point). The treated samples were collected after 96 weeks

(therapeutic endpoint). The Mann-Whitney U test was used. *** means P < 0.001; ** means P < 0.01; * means P < 0.05.AHI (n = 15); CHI (n = 18).

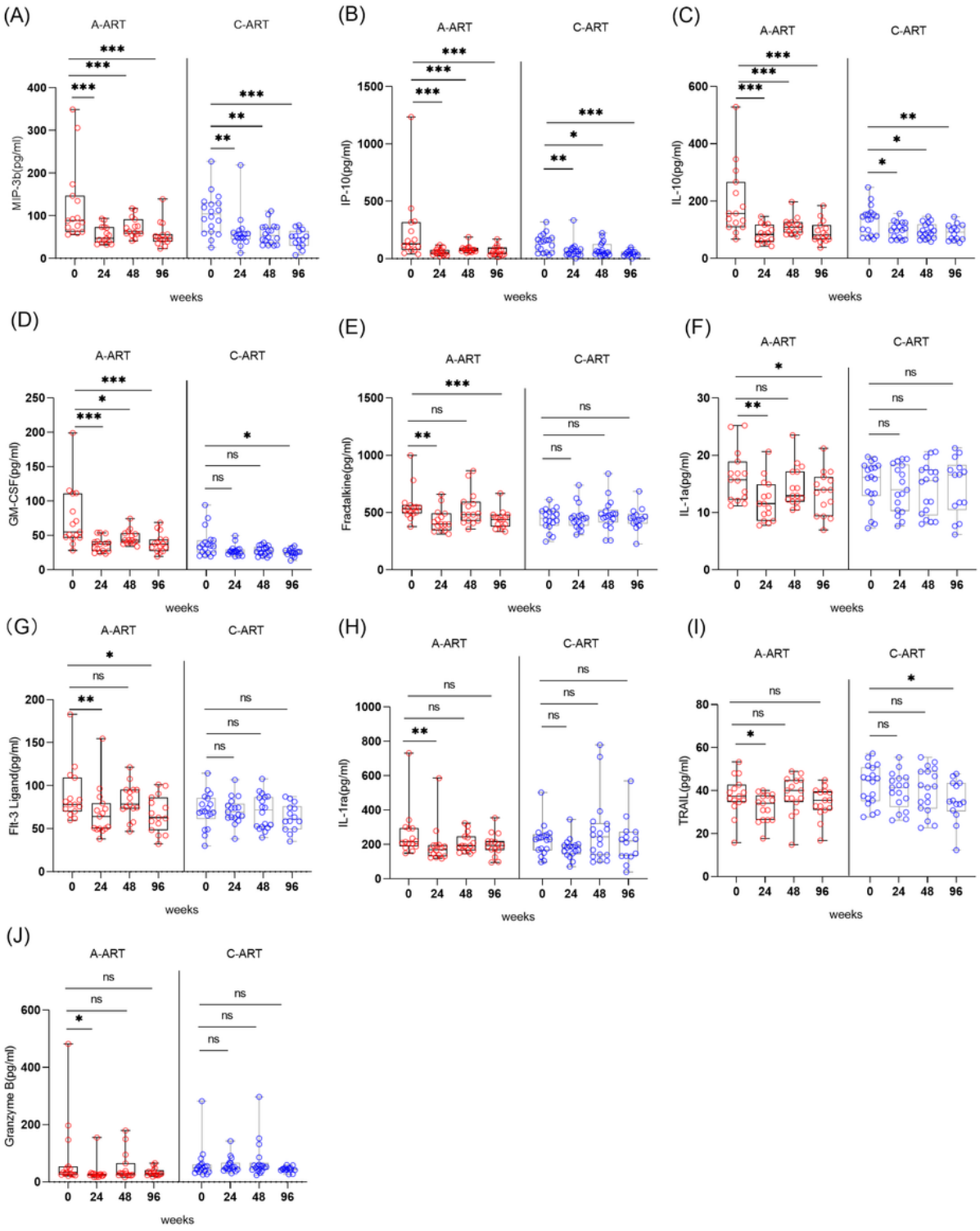

Figure 2

Cytokine levels during anti-HIV process in early or delayed treatment stages.

Abbreviations: A-ART, ART initiated in early (acute infection) treatment; C-ART, ART initiated in delayed (chronic infection) treatment. Each time point was compared with 0 weeks (initiation point). Wilcoxon test was used. This analysis of long-term ART was based on the generalized estimating equation (GEE) for the gross effect on HIV infection (Table S2). On this basis, selected cytokines were further analyzed in the early (acute infection) and delayed (chronic infection) treatment stages using the Mann-Whitney test.

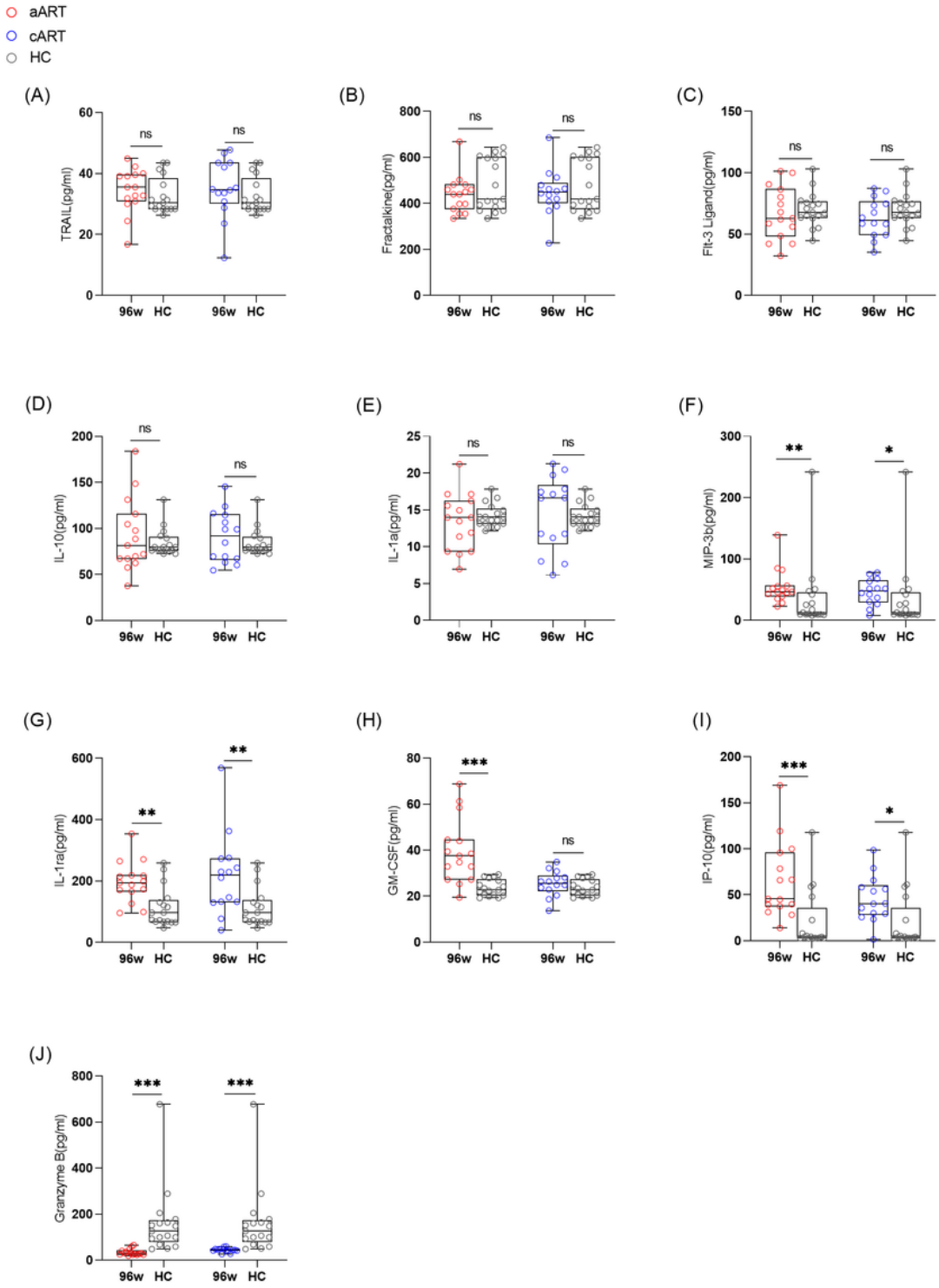

Figure 3

Therapeutic effect after 96 weeks of ART compared with that under healthy conditions (HC).

Based on the significant reduction in cytokine levels with long-term ART treatment, a therapeutic evaluation was performed by comparing the treatment endpoint (96 weeks) with healthy conditions (HC). The Mann-Whitney U test was used. aART, acute ART treatment; cART, chronic ART treatment; HC, healthy condition.

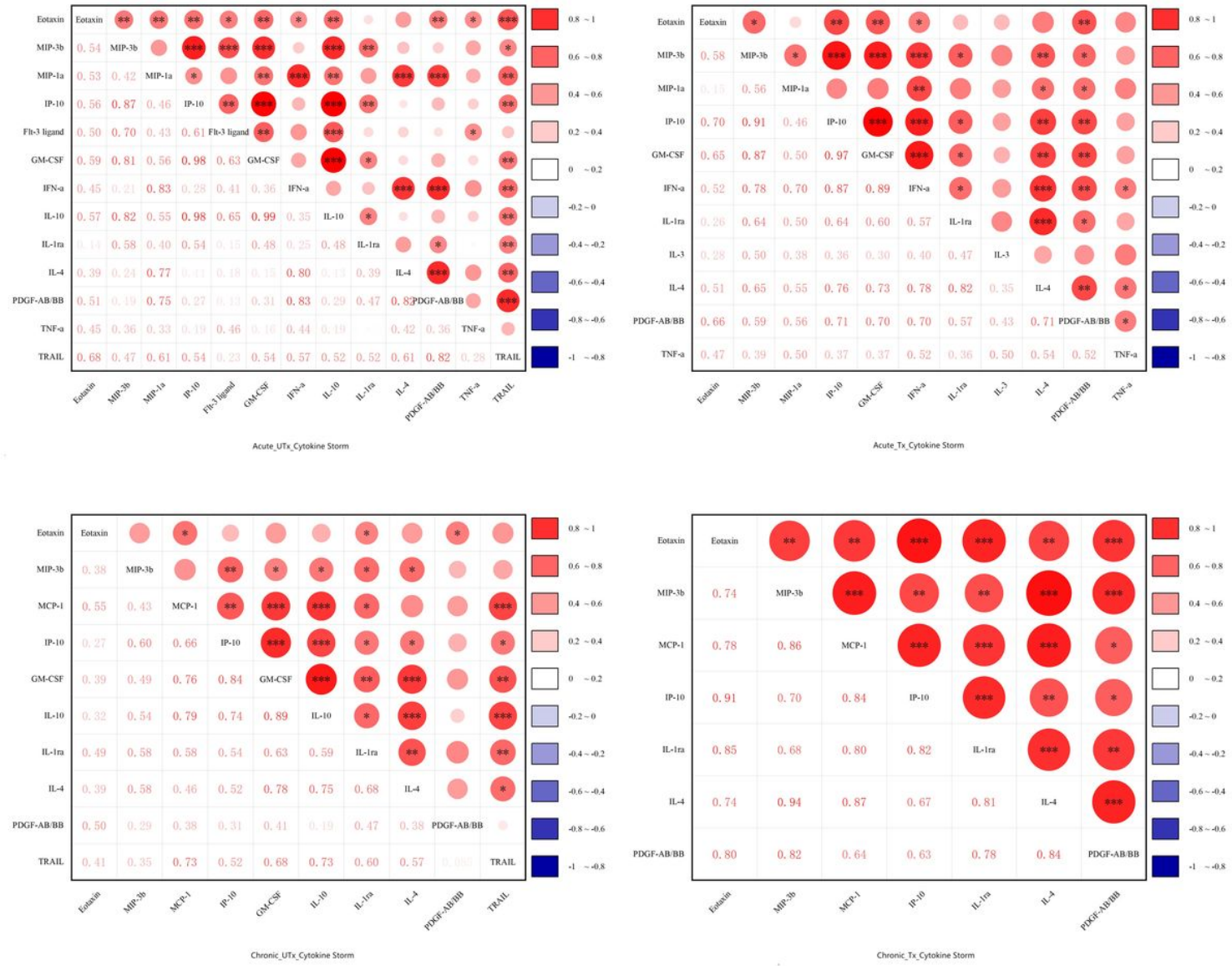

Figure 4

High levels of cytokine before and after ART treatment.

Abbreviations: Acute_ means early (acute infection) ART treatment; Chronic_ means delayed (chronic infection) ART treatment; UTx_ means untreated HIV-related condition; Tx_ means treated HIV-related

condition. Cytokine storm refers to cytokine levels higher than normal, which did not return to normal even after antiretroviral therapy.

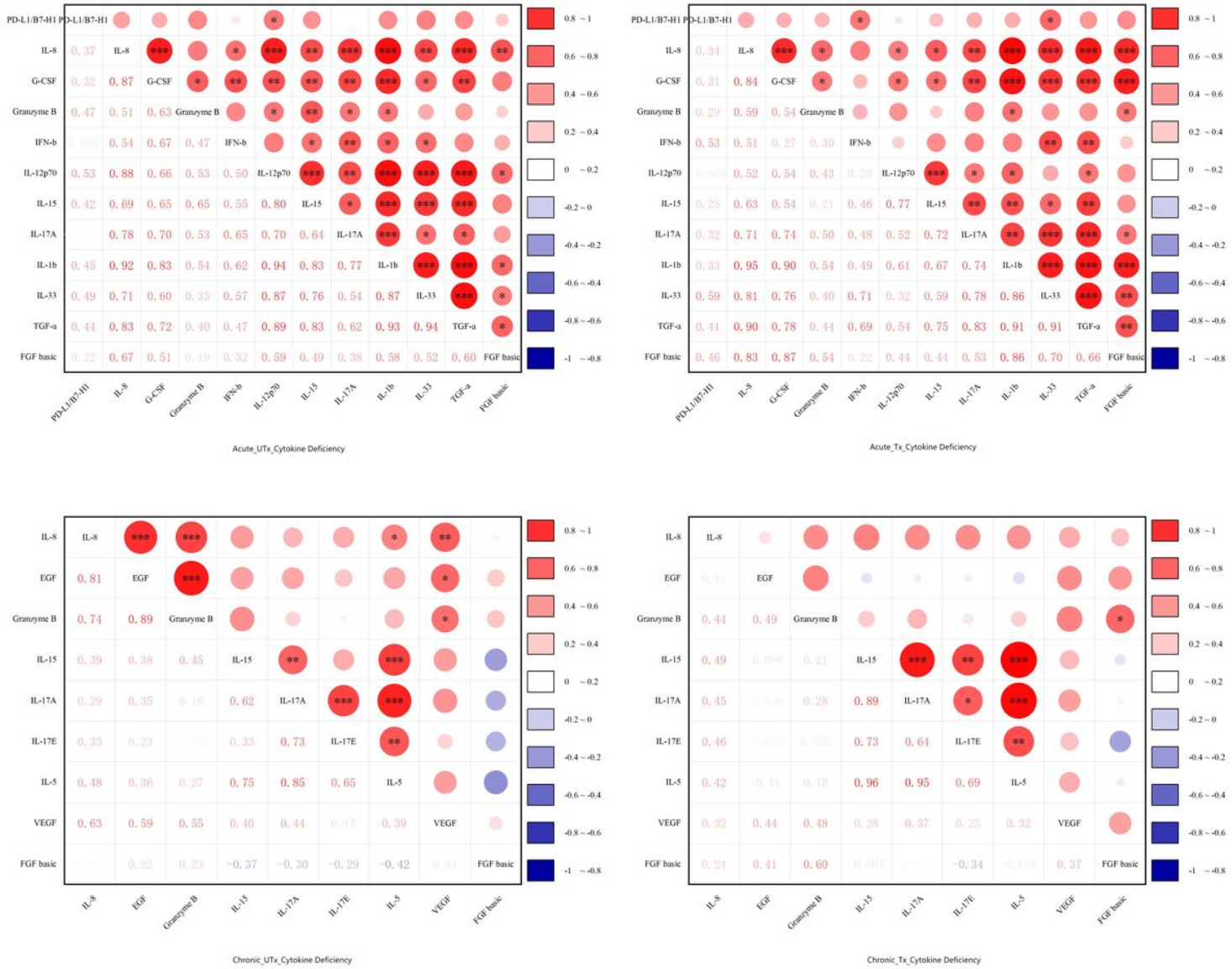

Figure 5

Low levels of cytokine before and after ART treatment.

Abbreviations: Acute_ means early (acute infection) ART treatment; Chronic_ means delayed (chronic infection) ART treatment; UTx_ means untreated HIV-related condition; Tx_ means treated HIV-related condition. Cytokine deficiency refers to cytokine levels lower than normal before treatment, which did not return to normal even after long-term antiretroviral therapy.

Supplementary Files

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- SupplementaryMaterials.docx