Influence of the HIV GWG variant in the HIV infection progression in mono and HCV coinfected patients

Flavia Hebeler-Barbosa, PhD\textsuperscript{a,b}, Viviam Milanez Massolini, MSc\textsuperscript{b}, Thais Watanabe\textsuperscript{c}, Giovanni Faria Silva, MD, PhD\textsuperscript{d}, Alexandre Naime Barbosa, MD, PhD\textsuperscript{d}, Rafael Plana Simões, PhD\textsuperscript{c}, Adriana Camargo Ferrasi, PhD\textsuperscript{a,b}, Paolo Marinho de Andrade Zanotto, PhD\textsuperscript{d}, Maria Inês de Moura Campos Pardini, PhD\textsuperscript{a,b}, Rejane Maria Tommasini Grotto, PhD\textsuperscript{b,c,*}

Abstract

The HIV subtype B is the most frequent in Brazil. The HIV subtype B' codes the amino acids glicine-tryptophan-glicine (GWG) instead of glicine-proline-glicine on the tip of gp120 V3 loop. This variant was associated to a slower HIV progression in mono-infected patients; however, there is no information in coinfected patients. This study evaluated the infection progression of HIV variant B' on the hepatitis C virus presence. RNA isolated from plasma of the 601 infected patients were used to human immunodeficiency virus (HIV) subtyping and to classify the virus according their syncytium-inducing ability. The HIV infection progression was evaluated by clinical and laboratorial data. The results showed a significant association between HIV B' variant and CD4 count and time of AIDS in HIV mono-infected patients. Notwithstanding the fact that we did not find a direct association between GWG variant and AIDS and in HIV coinfected patients no mitigating effect due to GWG presence was found. We did observe that the association between GWG variant and CD4 counts is lost in coinfected patients. This is first work showing influence of the HIV GWG variant in coinfected patients. Nevertheless, the presence of the GWG variant can indicate a better prognostic in the mono-infected patients.

Abbreviations: GPG = glicine-proline-glicine, GWG = glicine-tryptophan-glicine, HCV = hepatitis C virus, HIV = human immunodeficiency virus, NSI = non-syncytium-inducing, PSSM = position-specific scoring matrix, SI = syncytium-inducing.

Keywords: glicine-tryptophan-glicine variant, hepatitis C virus, human immunodeficiency virus sequence variability., human immunodeficiency virus subtype B, human immunodeficiency virus

1. Introduction

1.1. Background

The human immunodeficiency virus (HIV) shows high genetic variability throughout its entire genome but the \textit{env} gene is the most variable.\textsuperscript{[1]} This variability becomes particularly important in the HIV-1 gp120 V3 loop, in which the sequence variations have been associated with HIV antigenicity, immune dominance,\textsuperscript{[2]} and cytopathogenicity modifications.\textsuperscript{[3]}

Using algorithms based on the position-specific scoring matrix (PSSM),\textsuperscript{[4,5]} the HIV gp120 V3 loop has been described as an important motif associated with HIV cytopathic activity. Non-syncytium-inducing (NSI) HIV has been described as less cytopathic and to be present in the asymptomatic phase. On the other hand, the syncytium-inducing (SI) variants appear during AIDS and present more cytopathic activity.\textsuperscript{[6–9]}

The HIV major immunogenic region is the gp120 V3 loop,\textsuperscript{[10,11]} which has the sequence CTRPNNTGRKHGPGGRAFYTTGEII GDIRQAHC in most of the cases\textsuperscript{[12]} and exhibits a loop conformation due to presence of 2 cysteine residues at the end of sequence that interact by a disulfide bond. The tip of the V3 loop presents the conserved motif, a glicine-proline-glicine (GPG), in the HIV subtype B, which is the most frequent in Brazil. Nevertheless, a HIV-1 subtype B variant (named B') has been described in Brazil, in which the proline is substituted for a tryptophan in the V3 loop tip, creating a glicine-tryptophan-glicine (GWG) motif.\textsuperscript{[13–15]}

Previous studies have demonstrated the association between the GWG variant and progression of HIV infection in mono-infected patients.\textsuperscript{[16–20]} Importantly, the patients with the GWG variant present slower HIV infection progression and better prognosis. However, so far there are no reports concerning the association of the GWG HIV variant and infection evolution in HIV patients coinfected with hepatitis C virus (HCV).
On the other hand, particular sets of human genetic polymorphisms have been found to differ in patients coinfected with HIV and HCV when compared to patients mono-infected with HIV or HCV.[21,22] Likewise, the association of the host genetic polymorphisms with the progression of hepatic fibrosis is different in HCV mono-infected patients compared to HIV/HCV coinfected patients.[23]

The coinfection of HIV/HCV has been described in 5% to 25% of patients with HIV.[24,25] and HIV/HBV coinfection in approximately 10% of HIV patients.[26,27] The coinfection of HIV/HCV leads to a faster immunological debilitation.[28]

Considering that the tip of V3 loop is the major immunodominant region of HIV gp120,[29] the presence of the GWG motif could modify the immunological status of HIV mono-infected or HIV/HCV coinfected patients.

Immune debilitation in HIV/HCV coinfection has been described as a consequence of the hepatic cells signaling pathway that occurs when HIV gp120 interacts with CD4 and C-C chemokine receptor type 5 (CCR5) or CXCR4 receptors and coreceptors.[30] One way of the immune debilitation that occurs in HIV/HCV coinfection could be mediated by the inhibition in interferon function. Type I interferon have been described as essential molecules with antiviral action[31,32] and the presence of HCV could alter cell signaling controlling interferon secretion.[33]

1.2. Objectives

Considering the association of the HIV variant B’ (GWG) and a slower progression of HIV infection in mono-infected patients, the goal of this study was to evaluate the presence of HIV variant B’ and its association with infection progression in patients’ coinfected with HIV/HCV.

2. Methods

2.1. Study design, setting, and participants

Aliquots of Ethylenediamine tetraacetic acid-anticoagulated peripheral blood were collected from 601 HIV patients from the health service of the Botucatu region in Sao Paulo, Brazil. Inclusion criteria were that patients must be 18 years old or more and be infected by HIV subtype B confirmed by molecular assays. Exclusion criteria included the presence of other hepatic diseases, patients coinfected with more than 2 virus and pregnant women.

The patients were divided into 2 groups: group 1 (G1) included the HIV mono-infected patients and group 2 (G2) included the HIV/HCV coinfected patients. The patients included in study are followed in health service after diagnosis of the HIV or HCV infection. HIV/HCV coinfection was defined as individuals with positive serology for both viruses and criteria and HIV risk factors were obtained from medical records.

The Kaplan–Meier and Cox proportional survival analyses were performed to evaluate the influence of the GWG and GPG motifs on the patient’s immunological status (endpoint: CD4 count more than 200 cell/mm³).[34–36] The level of significance for all statistical tests was set at 0.05.

2.4. Ethics approval and consent to participate

This study was approved by the Research Ethics Committee of Botucatu Medical School, UNESP (document number 1.258.824). The written informed consent was obtained from...
all patients. The study including clinical data collection questionnaires obtained from physician.

3. Results

3.1. Participants

From the 601 patients included in this study, 87 were excluded because they were infected by an HIV subtype other than B (exclusion criteria).

3.2. Descriptive data, outcome data, and main results

The characteristics of the 514 patients with HIV subtype B that were included in the data analysis were shown in Table 1. The statistical analysis done within groups had no significant difference (P > .05) in frequencies of the GWG or GPG motif according to cytopathic ability, AIDS presence and HIV risk factors for both HIV mono-infected and patients coinfected with HIV/HCV (Table 2). Nevertheless, there were significant associations between GWG variant and the CD4 count (P < .05) and time of AIDS (P < .05) in HIV mono-infected patients. Interestingly, there was no difference among these variables in the HIV/HCV coinfected groups (Table 3). The Kaplan–Meier and Cox proportional survival analyses indicated a higher CD4 level over time for the HIV mono-infected patients with the GWG variant (Fig. 1A). This behavior was not observed in HCV/HIV coinfection (Fig. 1B).

Box plots of the significant data were plotted to illustrate the differences between the means. The HIV GWG variant was associated with higher CD4 levels and time of AIDS (Fig. 2).

4. Discussion

Although several HIV subtypes have been described in the world,[41] subtype B is the major subtype circulating in America,[42] including Brazil.[15] This HIV subtype contains the GPG motif in the tip of the V3 loop. The HIV subtype B variant B′ has been reported in Brazil, which shows the GWG motif in the tip of the V3 loop.[17]

In this study, the GWG (B′ variant) was found in 30.0% and 35.8% of the HIV mono-infected patients and HIV/HCV coinfected patients, respectively. Despite the fact that the presence of this variant in the coinfected patients is poorly understood, the studies in Brazil with HIV mono-infected patients have shown different proportions of the GWG variant. In this study, the frequency of the GWG variant (Table 1) is in agreement with recent reports from southeast Brazil.[15,43]

There is evidence in the literature concerning the association between HIV GWG variant and slower disease progression in HIV mono-infected patients.[17,26,44] In this work, nevertheless we did not establish the direct association between the GWG variant and AIDS presence, cytopathic ability, or HIV risk factors in the mono-infected group (Table 2). However, it may be only a

| Table 1 |
| --- |
| Demographical, virological, immunological, and clinical characteristics of the patients included in this study (n = 514). |

| Characteristics | HIV mono-infected patients (N = 447) | HCV/HIV coinfected patients (N = 67) |
| --- | --- | --- |
| Age, years (median [IQR]) | 36 (29.0–44.0) | 35 (35.0–42.0) |
| Sex, male [N [%]] | 228 (51.0) | 46 (68.7) |
| HIV-1 risk factors [N [%]] | | |
| Heterosexual | 332 (74.27) | 25 (37.31) |
| MSM | 18 (4.13) | 03 (46.48) |
| IDU | 31 (6.94) | 21 (31.34) |
| No information | 00 | 18 (26.87) |
| Time of AIDS, days (median [IQR]) | 223.0 (34.5–1016.5) | 439.0 (70.5–1902.5) |
| CD4 count, cells/mm³ (N [%]) | | |
| ≤ 200 | 110 (24.61) | 16 (23.89) |
| 200 < CD4 < 350 | 125 (27.96) | 12 (17.91) |
| ≥ 350 | 212 (47.43) | 39 (58.20) |
| HIV plasma viral load (PVL) RNA copies/mL (N [%]) | | |
| Undetectable* | 136 (30.42) | 37 (55.22) |
| Undetectable < PVL < 1000 | 75 (16.78) | 10 (14.93) |
| 1000 < PVL ≤ 10000 | 102 (22.82) | 07 (10.45) |
| PVL > 100000 | 134 (29.98) | 13 (19.40) |
| AIDS [N [%]] | | |
| Yes | 390 (87.25) | 62 (92.54) |
| No | 57 (12.75) | 05 (07.46) |
| Cytopathic activity [N [%]] | | |
| NSI | 317 (70.02) | 49 (73.14) |
| SI | 130 (29.08) | 18 (26.86) |
| HIV gp120 tip of V3 loop [N [%]] | | |
| GP | 190 (42.51) | 34 (50.75) |
| NS | 119 (26.62) | 20 (29.85) |
| Others | 138 (30.87) | 13 (19.40) |

GPG = glicine-proline-glicine, GWG = glicine-tryptophan-glicine, HCV = hepatitis C virus, HIV = human immunodeficiency virus, IDU = injection drug use, IQR = interquartile range, MSM = men who have sex with men, NSI = non-syncytium-inducing, SI = syncytium-inducing.

* Undetectable = Plasma viral load lower than 40 RNA copies/mL.

Hebeler-Barbosa et al. Medicine (2019) 98:29 www.md-journal.com
consequence of characteristics of the patients included in this study, in which aids presence was predominant (Table 1).

Crucially, as the most important result of this work we show here that the GWG variant was associated with higher levels of CD4 in HIV mono-infected patients (Table 3 and Fig. 1). The CD4 count has been already described as the most important prognostic marker of progression during HIV infection.\textsuperscript{[17]} Furthermore, the time of AIDS was longer in the HIV mono-infected patients with the GWG motif at the tip of the V3 loop (Fig. 2), suggesting that the GWG motif can be a marker to more time living with the disease.

The V3 loop is the major HIV immunogenic region.\textsuperscript{[43]} The GPG motif has been associated with the $\beta$-hairpin conformation of the V3 loop,\textsuperscript{[46]} which is essential for the interaction between HIV and CCR5 and/or CXCR4, which are HIV entry coreceptors.\textsuperscript{[47]} In this way, the change of a proline for a tryptophan in the tip of the V3 loop (GPG GWG) may result in conformational modifications that could influence the immune response to the virus,\textsuperscript{[13]} leading to an immunological protective effect for HIV patients who harbor the $B'$ variant (GWG motif).

In addition, the presence of the GWG motif could modify the interaction with the HIV coreceptor, leading to lower progression of HIV infection and occurrence of opportunistic infections. On the other hand, the levels of CD4 and the time of AIDS were the same in coinfected patients, independent of the presence of the GPG or GWG motif (Fig. 1). These results represent the first report about the GWG variant in HIV/HCV coinfected patients.

We did observe that in HIV/HCV coinfected patients the protective effect provided by the GWG variant is lost in the presence of HCV. Despite the fact that the mechanism involved in

Table 3

| Tip of V3 loop | GWG | GPG | P   |
|---------------|-----|-----|-----|
| HIV mono-infected |     |     |     |
| Time of AIDS (days) | 354 | 178 | .01378* |
| CD4 count (cells/mm$^3$) | 360.5 | 327.0 | .04844* |
| HIV plasma viral load (RNA copies/mL) | 1606.5 | 1500.0 | .8072 |
| HIV/HCV coinfected |     |     |     |
| Time of AIDS (days) | 343.5 | 537.0 | .3915 |
| CD4 count (cells/mm$^3$) | 365.5 | 401.0 | .7320 |
| HIV plasma viral load (RNA copies/mL) | 2034 | 7913 | .6111 |

GPG = glicine-proline-glicine, GWG = glicine-tryptophan-glicine, HCV = hepatitis C virus, HIV = human immunodeficiency virus, NSI = non-syncytium-inducing, SI = syncytium-inducing. * Significance difference.

Figure 1. The Kaplan–Meier and Cox proportional analyses of the GWG and GPG motifs influence on patient immunological status for: (A) HIV mono-infected and (B) HIV/HCV coinfected patients. The HIV mono-infected patients with GWG motif in tip of V3 loop showed higher CD4 levels by the time according both: Kaplan–Meier and Cox proportional analyses. GPG = glicine-proline-glicine, GWG = glicine-tryptophan-glicine, HCV = hepatitis C virus, HIV = human immunodeficiency virus.
this process is still unknown, we could suppose that the immunological response changed due to the presence of HCV.

Some mechanisms may agree with this hypothesis. It was shown that HIV/HCV coinfection results in immune depletion due to hepatic cell signaling pathways that are triggered when the HIV gp120 interacts with CD4.[30] Moreover, the presence of HCV interferes in signaling pathways involved in type I interferon release,[33] which leads to antiviral effects.[31,32] The presence of HCV also enhances HIV replication.[48] The CD4 T cell depletion has shown to have a profibrogenic role, leading the fibrosis progression in HCV infection.[49]

One could argue that, the HCV coinfection could increase HIV replication,[48] leading to increased CD4 depletion and a weakened immunological response.[49] This effect could also be associated with HCV-dependent type I interferon that would impair infection control by also, intensifying the immunological depletion.[133]

In this way, the HCV presence can explain the lack of protective effect of the GWG variant in coinfected patients. New studies should be conducted to investigate the mechanisms contributing to this process.

This is first study that evaluated the presence of the HIV GWG variant in coinfected patients. The presence of the GWG variant in HIV mono-infected patients can indicate a better prognostic in the clinical practice. Nevertheless, the HIV GWG variant did not influence the decline of the CD4 counts in coinfected patients.

Acknowledgments

We thank all patients that participated of this study.

Author contributions

Conceptualization: Maria Inês de M.C. Pardini, Rejane Maria Tommasini Grotto.

Data curation: Flavia Hebeler-Barbosa, Viviam Milanez Massolini, Thais Watanabe, Giovanni Faria Silva, Alexandre Naime Barbosa, Adriana Camargo Ferrasi.

Formal analysis: Rafael Plana Simões.

Funding acquisition: Paolo Marinho de Andrade Zanotto, Rejane Maria Tommasini Grotto.

Investigation: Flavia Hebeler-Barbosa, Viviam Milanez Massolini, Giovanni Faria Silva, Alexandre Naime Barbosa, Rafael Plana Simões, Paolo Marinho de Andrade Zanotto, Rejane Maria Tommasini Grotto.

Methodology: Flavia Hebeler-Barbosa, Viviam Milanez Massolini, Thais Watanabe, Giovanni Faria Silva, Alexandre Naime Barbosa, Adriana Camargo Ferrasi.

Project administration: Rejane Maria Tommasini Grotto.

Resources: Rejane Maria Tommasini Grotto, Maria Inês de M.C. Pardini, Paolo Marinho de Andrade Zanotto.

Supervision: Paolo Marinho de Andrade Zanotto, Rejane Maria Tommasini Grotto.

Writing – original draft: Flavia Hebeler-Barbosa, Rafael Plana Simões, Maria Inês de M.C. Pardini, Rejane Maria Tommasini Grotto.

Writing – review & editing: Flavia Hebeler-Barbosa, Rafael Plana Simões, Maria Inês de M.C. Pardini, Paolo Marinho de Andrade Zanotto.

Flavia Hebeler-Barbosa orcid: 0000-0001-7355-5179.

References

[1] Freed EO, Martin MA. HIVs and their replication. In: Fields Virology. 4th ed. Philadelphia: Wolters Kluwer Health/Lippincott Williams & Wilkins; 2007. p. 1601-54

[2] Luo L, Li Y, Chang J-S, Cho S-Y, Kim T-Y, Choi M-J, et al. Induction of V3-Specific Cytotoxic T Lymphocyte Responses by HIVgagParticles Carrying Multiple Immunodominant V3 Epitopes of gp120. Virology [Internet]. 1998 Jan 20;240(2):316-25. Available at: http://linkinghub.elsevier.com/retrieve/pii/S0042682297989224. Accessed April 19, 2018

[3] Hartley O, Klasse PJ, Sattentau QJ, et al. V3: HIV’s switch-hitter. AIDS Res Hum Retroviruses 2005;21:771-89.

[4] Brunme ZL, Dong WW, Yip B, et al. Clinical and immunological impact of HIV envelope V3 sequence variation after starting initial triple antiretroviral therapy. AIDS 2004;18:F1–9.

[5] Jensen MA, Coetzer M, van ’t Wout AB, et al. A reliable phenotype predictor for human immunodeficiency virus type 1 subtype c based on envelope v3 sequences. J Virol 2006;80:4698-704.

[6] Berger EA, Murphy PM, Farber JM. Chemokine receptors as HIV-1 coreceptors: roles in viral entry, tropism, and disease. Annu Rev Immunol [Internet] 1999;17:657–700.

[7] Callaway DX, Ribeiro RM, Nowak MA. Virus phenotype switching and disease progression in HIV-1 infection. Proceedings Biol Sci [Internet] 1999;266:2523–30.

[8] Koont M, van Leeuwen R, de Goede RE, et al. Conversion rate towards a syncytium-inducing (SI) phenotype during different stages of human immunodeficiency virus type 1 infection and prognostic value of SI phenotype for survival after AIDS diagnosis. J Infect Dis 1999;179:254–8.
[9] Kuper B, Kaiser R, Rockstroh JK, et al. Role of HIV-1 phenotype in viral pathogenesis and its relation to viral load and CD4+ T-cell count. J Med Virol 1998;56:259–63.

[10] Goudsmid J, Deboeck C, Meloen RH, et al. Human immunodeficiency virus type 1 neutralization epitope with conserved architecture elicits early type-specific antibodies in experimentally infected chimpanzees. Proc Natl Acad Sci U S A [Internet] 1988;85:4478–82.

[11] Kenealy WR, Matthews TJ, Ganf MC, et al. Antibodies from human immunodeficiency virus-infected individuals bind to a short amino acid sequence that elicits neutralizing antibodies in animals. AIDS Res Hum Retrovirology [Internet] 1989;5:173–82.

[12] Stanfield R, Cabezas E, Satterthwaite A, et al. Dual conformations for the HIV-1 gp120 V3 loop in complexes with different neutralizing fabs. Structure [Internet] 1999;7:131–42.

[13] Gerosa F, Baldani-Guerra B, Nisii C, et al. Reciprocal activating interaction between natural killer cells and dendritic cells. J Exp Med [Internet] 2002;195:327–33.

[14] Puoti M, Moioli M, Travi G, et al. The burden of liver disease in human immunodeficiency virus infection in urban populations: low eligibility for interferon treatment. Clin Infect Dis [Internet] 2003;36:97–100.

[15] Zolla-Pazner S, Zhong P, Revesz K, et al. The cross-clade neutralizing activity of a human monoclonal antibody is determined by the GPGR V3 motif of HIV type 1. AIDS Res Hum Retrovirology [Internet] 2004;20:1254–8.

[16] Araujo AF, Brito C, Monteiro-Cunha J, et al. Lower prevalence of human T-cell lymphotropic virus type 1 Brazilian strains. Mem Inst Oswaldo Cruz 1996;91:339–42.

[17] Kenealy WR, Matthews TJ, Ganf MC, et al. Antibodies from human immunodeficiency virus-infected individuals bind to a short amino acid sequence that elicits neutralizing antibodies in animals. AIDS Res Hum Retrovirology [Internet] 1989;5:173–82.

[18] de Brito A, Komninakis SC, Novoa P, et al. Women infected with HIV type 1 Brazilian subtype B found in northeastern Brazil with slower progression to AIDS. AIDS Res Hum Retrovirology [Internet] 2010;26:1249–54.

[19] Casseb J, Konminakis S, Abdalla L, et al. HIV disease progression: is the Brazilian variant subtype B (‘GWGR motif’) less pathogenic than US/European subtype B (‘GPGR’)? Int J Infect Dis 2002;6:164–9.

[20] de Brito A, Konminakis SC, Novoa P, et al. Women infected with HIV type 1 Brazilian variant, subtype B (‘GWGR’ Motif) have slower progression to AIDS, compared with patients infected with subtype B (‘GPGR’ motif). Clin Infect Dis 2006;43:1476–81.

[21] Santoro-Lopes G, Harrison LH, Tavares MD, et al. Human disease progression and V3 serotypes in Brazil: is B different from B BR? AIDS Res Hum Retrovirology 2000;16:953–8.

[22] Tommasini-Grotto RM, Montes B, Triglia D, et al. Variability of the conserved V3 loop tip motif in HIV-1 subtype B isolates collected from Brazilian and French patients. Brazilian J Microbiol 2010;41:720–6.

[23] de Araújo ES, Daha H, Cotler SJ, et al. Pharmacodynamics of PEG-IFN-α2b and HCV response as a function of IL28B polymorphism in HIV/HCV-coinfected patients. J Acquir Immune Defic Syndr 2013;63:56–9.

[24] Grotto RM, Picelli N, de Souza LR, et al. Human platelet polymorphism can be a genetic marker associated with HIV/HCV coinfection. J Med Virol [Internet] 2015;87:1677–81.

[25] Picelli N, Tanikawa AA, Grotto RM, et al. The absence of the human platelet antigen polymorphism effect on fibrosis progression in human immunodeficiency virus type 1-infected patients. Rev Soc Bras Med Trop [Internet] 2015;48:406–9. http://www.scielo.br/scielo.php?script=sci_arttext&pid=S0037-86822015000400406&lng=en&tlng=en.

[26] Puoti M, Moioli M, Travi G, et al. The burden of liver disease in human immunodeficiency virus-infected patients. Semin Liver Dis [Internet] 2012;32:103–13.

[27] Soriano V, Puoti M, Squizzato M, et al. Care of patients coinfected with HIV and hepatitis C virus: 2007 updated recommendations from the HCV-HIV International Panel. AIDS [Internet] 2007;21:1073–89.

[28] Fleming CA, Craven DE, Thornton D, et al. Hepatitis C virus and human immunodeficiency virus coinfection in an urban population: low eligibility for interferon treatment. Clin Infect Dis [Internet] 2003;36:97–100.

[29] Zolla-Pazner S, Zhong P, Revesz K, et al. The cross-clade neutralizing activity of a human monoclonal antibody is determined by the GPGR V3 motif of HIV type 1. AIDS Res Hum Retrovirology [Internet] 2004;20:1254–8.

[30] Mastroianni C, Lichtner M, Mascia C, et al. Molecular mechanisms of liver fibrosis in HIV/HCV coinfection. Int J Mol Sci [Internet] 2014;15:9184–208.

[31] Gerosa F, Baldani-Guerra B, Nisii C, et al. Reciprocal activating interaction between natural killer cells and dendritic cells. J Exp Med [Internet] 2002;195:327–33.

[32] Megugorac NJ, Young HA, Amrute SB, et al. Virally stimulated plasmacytoid dendritic cells produce chemokines and induce migration of T and NK cells. J Leukoc Biol [Internet] 2004;75:504–14.

[33] González VD, Landay AL, Sandberg JK. In innate immunity and chronic immune activation in HIV/HCV coinfection. Clin Immunol [Internet] 2010;135:12–25.

[34] Delwart EL, Hauri H, Wendl MC, et al. Base-calling of automated sequencer traces using phred. I. Accuracy assessment. Genome Res [Internet] 1998;8:175–85.

[35] Ewing B, Hillier L, Wendl MC, et al. Base-calling of automated sequencer traces using phred. II. Error probabilities. Genome Res [Internet] 1998;8:186–94.

[36] Jensen MA, Li FS, van’t Wout AB, et al. Improved coreceptor usage prediction and genotypic monitoring of R5-to-X4 transition by motif analysis of human immunodeficiency virus type 1 env V3 loop sequences. J Virol [Internet] 2003;77:13376–88.

[37] Pedersen C, Lindhardt BD, Jensen BL, et al. Clinical course of primary HIV infection: consequences for subsequent course of infection. BMJ [Internet] 1989;299:154–7.

[38] Rich JT, Neely JG, Paniello RC, et al. A practical guide to understanding Kaplan-Meier curves. Otolaryngol Head Neck Surg [Internet] 2010;143:331–6.

[39] Therneau TM, Grambsch PM. Modeling Survival Data: Extending the Cox Model [Internet]. New York, NY: Springer New York; 2000. 287 p. [Statistics for Biology and Health]. Available at: http://link.springer.com/10.1007/978-1-4757-3294-8. Accessed May 20, 2019.

[40] Hemelaj J, Gouws E, Ghys PD, et al. Global and regional distribution of HIV-1 genetic subtypes and recombinants in. AIDS 2006;20:W13–23.

[41] Taylor BS, Sobieszczyny ME, McCatchan FE, et al. The challenge of HIV-1 subtype diversity. N Engl J Med 2008;358:1590–602.

[42] Pimentel VF, Morangado MG, Guimarães ML, et al. Human platelet polymorphism effect on fibrinogen and platelet aggregation. Platelets [Internet] 2010;21:427–30.

[43] D’Agostino RB, Sr., Vasan RS, Pencina MJ, et al. C-statistic quantifies incremental validity of multivariable risk prediction for CHD. Circulation [Internet] 2009;119:1847–51.

[44] Remontini F, Venturini P, Scattola F, et al. Virological mechanisms of the coinfection between HIV and HCV. Mediators Inflamm [Internet] 2015;2015:320532.

[45] Glassner A, Eisenhardt M, Kokordélis P, et al. Impaired CD4+ T cell stimulation of NK cell anti-fibrotic activity may contribute to accelerated liver fibrosis progression in HIV/HCV patients. J Hepatol 2013;59:427–33.