Abstract

Background: Despite the high rate of virological success of combined antiretroviral therapy (cART), HIV infected individuals continue to fail. In this contest, it is unclear whether having previously experienced virological failure (VF) of cART remains an important predictor of future risk of VF in people receiving cART in modern times. We investigated the rate of VF and factors potentially associated with this event in 9,220 HIV-1 infected patients enrolled in the Icona Cohort who showed a stable viral suppression on modern cART regimens after January 1, 2006.

Methods: We investigated two main exposure factors: current calendar period (2006-2009; 2010-2013; 2014-2017) and number of VFs (0; 1-3; >3) prior to baseline. Relative rates of VF were estimated from fitting a Poisson regression model.

Results: Seven-hundred-seventy-nine patients experienced VF over follow-up for an overall rate of 2.08 per 100 person years of follow-up (PYFU, 95%CI: 1.93-2.22). The rate of VF increased with higher numbers of previous VFs: patients with >3 previous VFs had a rate of 4.87 (4.10-5.78), 2.75-fold higher than that observed in patients without any previous VF (p<0.001). The rate of VF was lower in recent years: 3.81 (3.36, 4.32) in 2006-2009; 1.36 (1.20-1.53) in 2014-2017 (p<0.001). Other factors independently associated with lower risk of VF were Italian origin, longer history of virological suppression, and university education level.

Conclusions: In HIV-infected patients virologically suppressed after January 2006, the rate of VF continues to show a decline even in the most recent years. Previous VFs should be carefully considered.

1. Background

Despite huge advances in terms of the impact of combined antiretroviral therapy (cART) on HIV-related morbidity and mortality, patients continue to fail therapy [1-4]. A history of prior virological failure (VF) has been shown to be associated with the risk of subsequent VF, emergence of resistance, and death [5-7]. However, the dynamics of VF in HIV-infected population receiving modern cART and factors associated with a greater risk of VF in this population have been not thoroughly investigated [8,9]. Specifically, in people whose viral load is currently suppressed, it is unclear whether having previously experienced VF of cART remains an important predictor of future risk of VF in people receiving cART in modern times. There are several factors that could lead to the development of VF, such as lack of adherence, which can impact on the efficacy of different drug regimens [10,11], high levels of pre-cART viremia, which are associated with a lower probability of achieving virological suppression, and drug resistance [12-18].

In the present work, we aimed at identifying the rate and the presence of independent predictors of VF in HIV-1 infected individuals with plasma viral load ≤50 copies/mL on modern cART regimens after January 1, 2006.

2. Study design

2.1. Study population

All patients analyzed were from the ICONA Foundation Study (ICONA), a multi-centre prospective observational study (http://www.fondazioneicona.org/). Each patient included in the present analysis had a record of two consecutive plasma HIV-RNA ≤50 copies/mL after January 1, 2006: baseline was defined at the date of the second of these values. A patient was classified as having experienced VF to a regimen before baseline if, after at least 4 months from starting the regimen

and while he/she was receiving the same regimen, plasma HIV-RNA was still >400 copies/mL. A counter for the number of regimens to which a participant had experienced VF prior to baseline was constructed. Plasma HIV-RNA cut-off was chosen by considering the different limits of quantification of assays used to quantify this parameter before 2006.

2.1. Estimation of rates of virological failure during prospective follow-up by Poisson regression model

VF over prospective follow-up was defined at the time of the first of two consecutive values of HIV-RNA >50 copies/mL while the person was still receiving cART after baseline. Participants' follow-up accrued from baseline until the date of estimated VF or the date of their last available HIV-RNA value. Rates of current VF were calculated as number of VF divided by person years of follow-up (PYFU) and unadjusted and adjusted relative rates (RR) were estimated from fitting a Poisson regression model. Main exposure factors investigated were: i) current calendar year periods of baseline (stratified as: 2006-2009, 2010-2013, 2014-2017); ii) number of VFs experienced before baseline (stratified as: 0, 1-3, >3). Whether the risk associated with previous number of VFs varied according to current calendar period of viral suppression was formally investigated by fitting an interaction term in the Poisson regression model. The association between other socio-demographic, viro-immunological and clinical factors measured at baseline and the risk of VF were also investigated. Analyses were repeated after using a plasma HIV-RNA value of 200 copies/mL as the threshold to define VF.

All the analyses were performed using SAS version 9.4 (SAS Institute Cary NC, U). In all the analyses a p-value <0.05 was considered as statistically significant.

3. Results

3.1. Patients' characteristics

Nine-thousand and two-hundred-twenty HIV-infected patients, who had been under viral suppression after January 1, 2006 were included. The median (interquartile range, IQR) calendar year of baseline was 2012 (2009, 2015). At baseline, patients have been virologically suppressed for a median (IQR) of 20 (9, 36) months and have been subsequently followed for a total of 37,499 PYFU. Baseline characteristics, stratified by calendar year periods at baseline, and previous history of VFs are shown in Table 1, Table 2 and Figure 1.

3.2. Rates of virological failures during follow-up and independent predictors

Seven-hundred and seventy-nine patients showed a current VF (cut-off 50 copies/mL) with a rate of 2.08 per 100 PYFU (95%CI: 1.93-2.22). The median value of HIV-RNA (log_{10}) at VF did not differ among the 3 current time periods: 4.10 (2.99-4.88) in 2006-2009, 3.82 (2.95-4.72) in 2010-2013, 3.91 (2.91-4.22) in 2014-2017 (p=0.320). In contrast, the risk of the current VF was higher with larger number of VFs experienced before baseline. Specifically, patients with >3 previous VFs had a rate (95%CI) of 4.87 (4.10-5.78) per 100 PYFU, 2.87-fold higher than that observed in patients without any previous VF (Table 3). This result was confirmed after controlling for potential confounding factors (adjusted RR=2.75; 95% CI: 2.13-3.56, p<0.001).

In addition, the rate of VF was lower with more recent calendar years, ranging from 3.81 (3.36, 4.32) in 2006-2009 to 1.36 (1.20-1.53) in 2014-2017. This association remained significant after adjustment for a number of potential confounding factors: adjusted RR=0.36 (95%CI: 0.29-0.44, p<0.001: comparison 2014-2017 vs. 2006-2009). The association between number of previous failures and the risk of subsequent VF was also confirmed in different multivariable models after controlling for several socio-demographic, viro-immunological and clinical variables

(Supplementary Table 1), and did not vary by current calendar period. This was documented by the lack of evidence for an interaction between previous VFs and calendar years (p=0.18, Supplementary Figure 1).

Other independent predictors negatively associated with the risk of VF were Italian origins, a longer duration of time with virological suppression, declaring University as the highest level of education achieved, and use of efavirenz (Table 3). The GSS at baseline showed no association with the risk of VF.

We obtained similar results after repeating the analyses with a plasma HIV-RNA value of 200 copies/mL as the threshold to define VF (Supplementary Table 2).

4. Discussion

In our large cohort of virologically suppressed HIV-1 infected patients, we found that, after controlling for a number of demographic, health-related, viro-immunologic and therapeutic factors, the rate of VF continues to show a decline even in the most recent study period, *i.e.* 2014-2017. Although the association between history of previous VFs on cART and subsequent risk of VF has been previously documented [5-7], our data extend this finding to more recent years for people exposed to modern cART. In addition, our data show that, despite the introduction of new drugs/drug classes with reduced cross-resistance and the increased tolerability of modern compounds, the impact of having virologically failed in the past remains unchanged on the current risk of VF.

Other factors were identified as independent predictors of higher risk of VF over prospective follow-up and should be considered to identify people at greater risk of treatment failure: foreign nationality, shorter duration of viral suppression, and having the highest level of education lower than University. We understand that our large cohort study is observational, so that issues such a

confounding and collision are less likely to be adequately controlled as it is in randomized trial. On the other hand, not many trials have sample sizes or duration of follow-up similar to that of our study, which extends over several decades and has allowed the time-periods comparison.

In conclusion, our data supports the concept that people with a current suppressed plasma viral load but a history of extensive VF (>3 regimens) are still a fragile population for whom careful monitoring of viral load should be maintained. Similarly, even in our ART modern era, a history of virological failure should be carefully examined and accounted for when a treatment switch is needed.

Figure legends

Figure 1. Antivirals included in the suppressive regimen received at baseline among the overall patients analyzed in study (panel A), according to calendar year (panel B) and to previous virological failures (panel C). Among the antiretrovirals used at baseline, tenofovir was the nucleoside reverse transcriptase inhibitor (NRTI) mostly used (71.8%) together with emtricitabine (66.4%) or lamivudine (3.7%). As far as the third drug used in the current regimen, 3,494 (37.9%) patients were receiving a non-NRTI (NNRTI), 3,745 (40.6%) were treated with a ritonavir-boosted protease inhibitor, while 1,941 (21.0%) were treated with an integrase inhibitor.

Supplementary figure 1. Rates of VF >50 copies/mL according to current calendar period (3 time periods examined) and number of VF >400 copies/mL experienced before baseline. PYFU: person years of follow-up.

Funding

ICONA Foundation is supported by unrestricted grants from Gilead Sciences, Janssen, Merck Sharp and Dohme and ViiV Healthcare.

Conflict of interest

The authors declare no conflict of interest.

Acknowledgements

This manuscript is dedicated to the memory of Professor Andrea De Luca: a great friend, physician, mentor, and scientist.

The authors wish to thanks Miss A. Rodano' for excellent technical assistance and data management.

Icona Foundation Study Group

BOARD OF DIRECTORS: A d'Arminio Monforte (President), A Antinori (Vice-President), M Andreoni, A Castagna, F Castelli, R Cauda, G Di Perri, M Galli, R Iardino, G Ippolito, A Lazzarin, GC Marchetti, G Rezza, F von Schloesser, P Viale.

SCIENTIFIC SECRETARY: A d'Arminio Monforte, A Antinori, A Castagna, F Ceccherini-Silberstein, A Cozzi-Lepri, E Girardi, S Lo Caputo, C Mussini, M Puoti, CF Perno.

STEERING COMMITTEE: A Antinori, F Bai, C Balotta, A Bandera, S Bonora, M Borderi, A Calcagno, A Capetti, MR Capobianchi, A Castagna, F Ceccherini-Silberstein, S Cicalini, A Cingolani, P Cinque, A Cozzi-Lepri, A d'Arminio Monforte, A De Luca, A Di Biagio, E Girardi, N Gianotti, A Gori, G Guaraldi, G Lapadula, M Lichtner, S Lo Caputo, G Madeddu, F Maggiolo, G Marchetti, L Monno, C Mussini, S Nozza, CF Perno, C Pinnetti, M Puoti, E Quiros Roldan, R Rossotti, S Rusconi, MM Santoro, A Saracino, L Sarmati.

STATISTICAL AND MONITORING TEAM: A Cozzi-Lepri, I Fanti, L Galli, P Lorenzini, A Rodano', M Macchia, A Tavelli.

BIOLOGICAL BANK INMI: F Carletti, S Carrara, A Di Caro, S Graziano, F Petroni, G Prota, S Truffa.

PARTICIPATING PHYSICIANS AND CENTERS: Italy A Giacometti, A Costantini, V Barocci (Ancona); G Angarano, L Monno, C Fabrizio (Bari); F Maggiolo, C Suardi (Bergamo); P Viale, V Donati, G Verucchi (Bologna); F Castelnuovo, C Minardi, E Quiros Roldan (Brescia); B Menzaghi, C Abeli (Busto Arsizio); B Cacopardo, B Celesia (Catania); J Vecchiet, K Falasca (Chieti); A Pan, S Lorenzotti (Cremona); L Sighinolfi, D Segala (Ferrara); P Blanc, F Vichi (Firenze); G Cassola, C Viscoli, A Alessandrini, N Bobbio, G Mazzarello (Genova); M Lichtner, S Vita, (Latina); P Bonfanti, C Molteni (Lecco); A Chiodera, P Milini (Macerata); G Nunnari, G Pellicanò (Messina); A d'Arminio Monforte, M Galli, A Lazzarin, G Rizzardini, M Puoti, A Castagna, S Cannizzo, MC Moioli, R Piolini, D Bernacchia, S Salpietro, C Tincati, (Milano); C Mussini, C Puzzolante (Modena); C Migliorino, G

Lapadula (Monza); V Sangiovanni, G Borgia, V Esposito, F Di Martino, I Gentile, L Maddaloni (Napoli); AM Cattelan, S Marinello (Padova); A Cascio, C Colomba (Palermo); F Baldelli, E Schiaroli (Perugia); G Parruti, F Sozio (Pescara); G Magnani, MA Ursitti (Reggio Emilia); M Andreoni, A Antinori, R Cauda, A Cristaudo, V Vullo, R Acinapura, G Baldin, M Capozzi, A Mondi, A Cingolani, M Rivano Capparucia, G Iaiani, A Latini, R Gagliardini, MM Plazzi, S Savinelli, A Vergori (Roma); M Cecchetto, F Viviani (Rovigo); G Madeddu, P Bagella (Sassari); A De Luca, B Rossetti (Siena); A Franco, R Fontana Del Vecchio (Siracusa); D Francisci, C Di Giuli (Terni); P Caramello, G Di Perri, S Bonora, GC Orofino, M Sciandra (Torino); M Bassetti, A Londero (Udine); G Pellizzer, V Manfrin (Vicenza); G Starnini, A Ialungo (Viterbo).

Contributors

SR and MMS conceived the study and coordinated the manuscript. AA, SB, AC, NG, FCS collected patients' data and gave their input on data analysis. AT managed all data from the ICONA cohort. AdAM coordinated all clinical activities. AC-L performed all statistical evaluations. SR, MMS and AC-L wrote and circulated the final version of the manuscript.

Ethical approval

This study was conducted on data collected for clinical purposes. All data used in the study were previously anonymized, according to the requirements set by Italian Data Protection Code (leg. decree 196/2003) and by the General authorizations issued by the Data Protection Authority. The ICONA Foundation study has been separately approved by IRB of all the participating centers; sensitive data from patients are seen only in aggregate form. Written informed consent for medical procedures/interventions performed for routine treatment purposes was collected for each patient included in the Icona Foundation Study or from other clinical centers involved in the study, in accordance with the ethics standards of the committee on human experimentation and

the Helsinki Declaration (1983 revision).

References

[1] J.S. Montaner, V.D. Lima, P.R. Harrigan, L. Lourenço, B. Yip, B. Nosyk, E. Wood, T. Kerr, K. Shannon, D. Moore, R.S. Hogg, R. Barrios, M. Gilbert, M. Krajden, R. Gustafson, P. Daly, P. Kendall, Expansion of HAART coverage is associated with sustained decreases in HIV/AIDS morbidity, mortality and HIV transmission: the "HIV Treatment as Prevention" experience in a Canadian setting, PLoS One. 9 (2014) e87872. doi: 10.1371/journal.pone.0087872.

[2] M.S. Cohen, Y.Q. Chen, M. McCauley, T. Gamble, M.C. Hosseinipour, N. Kumarasamy, J.G. Hakim, J. Kumwenda, B. Grinsztejn, J.H. Pilotto, S.V. Godbole, S. Mehendale, S. Chariyalertsak, B.R. Santos, K.H. Mayer, I.F. Hoffman, S.H. Eshleman, E. Piwowar-Manning, L. Wang, J. Makhema, L.A. Mills, G. de Bruyn, I. Sanne, J. Eron, J. Gallant, D. Havlir, S. Swindells, H. Ribaudo, V. Elharrar, D. Burns, T.E. Taha, K. Nielsen-Saines, D. Celentano, M. Essex, T.R. Fleming; HPTN 052 Study Team, Prevention of HIV-1 infection with early antiretroviral therapy, N Engl. J Med. 365 (2011) 493-505. doi: 10.1056/NEJMoa1105243.

[3] J. Troya, J. Bascuñana, Safety and Tolerability: Current Challenges to Antiretroviral Therapy for the Long-Term Management of HIV Infection, AIDS Rev. 18 (2016) 127-137.

[4] A.D. Castel, M.M. Kalmin, R.L. Hart, H.A. Young, H. Hays, D. Benator, P. Kumar, R. Elion, D. Parenti, M.E. Ruiz, A. Wood, L. D'Angelo, N. Rakhmanina, S. Rana, M. Bryant, A. Hebou, R. Fernández, S. Abbott, J. Peterson, K. Wood, T. Subramanian, J. Binkley, L.P. Happ, M. Kharfen, H. Masur, A.E. Greenberg, Disparities in achieving and sustaining viral suppression among a large cohort of HIV-infected persons in care - Washington, DC, AIDS Care. 28 (2016) 1355-1364. doi: 10.1080/09540121.2016.1189496.

[5] J. Reekie, A. Mocroft, B. Ledergerber, M. Beniowski, B. Clotet, J. van Lunzen, A. Chiesi, C. Pradier, L. Machala, J.D. Lundgren; EuroSIDA Study Group, History of viral suppression on

combination antiretroviral therapy as a predictor of virological failure after a treatment change, HIV Med. 11 (2010) 469-478. doi: 10.1111/j.1468-1293.2009.00816.x.

[6] C. Laprise, A. de Pokomandy, J.G. Baril, S. Dufresne, H. Trottier, Virologic failure following persistent low-level viremia in a cohort of HIV-positive patients: results from 12 years of observation, Clin. Infect. Dis. 57 (2013) 1489-1496. doi: 10.1093/cid/cit529.

[7] S. Bonora, A. Calcagno, O. Vigano, P. Bigliano, L. Marinaro, E. Colella, G. Orofino, L. Trentini, M.C. Tettoni, A. D'Avolio, S. Mercadante, M. Galli, G. Di Perri, S. Rusconi, Efficacy, tolerability and virological consequences of long-term use of unboosted atazanavir plus 2 NRTIs in HIV-infected patients, Curr. HIV Res. 12 (2014) 339-346.

[8] G.K. Robbins, K.L. Johnson, Y. Chang, K.E. Jackson, P.E. Sax, J.B. Meigs, K.A. Freedberg, Predicting virologic failure in an HIV clinic, Clin. Infect. Dis. 50 (2010) 779-786. doi: 10.1086/650537.

[9] G.K. Robbins, S.E. Cohn, L.J. Harrison, L. Smeaton, L. Moran, D. Rusin, M. Dehlinger, T. Flynn, S. Lammert, A.W. Wu, S.A. Safren, N.R. Reynolds, Characteristics associated with virologic failure in high-risk HIV-positive participants with prior failure: a post hoc analysis of ACTG 5251, HIV Clin. Trials. 17 (2016) 165-172. doi: 10.1080/15284336.2016.1189754.

[10] L.L. Gordon, D. Gharibian, K. Chong, H. Chun, Comparison of HIV virologic failure rates between patients with variable adherence to three antiretroviral regimen types, AIDS Patient Care STDS. 2015. 29 (2015) 384-388. doi: 10.1089/apc.2014.0165.

[11] W.M. Bezabhe, L. Chalmers, L.R. Bereznicki, G.M. Peterson, Adherence to antiretroviral therapy and virologic failure: a meta-analysis, Medicine (Baltimore). 95 (2016) e3361. doi: 10.1097/MD.0000000000003361.

[12] M.M. Santoro, D. Armenia, C. Alteri, P. Flandre, A. Calcagno, M. Santoro, C. Gori, L. Fabeni, R. Bellagamba, V. Borghi, F. Forbici, A. Latini, G. Palamara, R. Libertone, V. Tozzi, E. Boumis, C.

Tommasi, C. Pinnetti, A. Ammassari, E. Nicastri, A. Buonomini, V. Svicher, M. Andreoni, P. Narciso, C. Mussini, A. Antinori, F. Ceccherini-Silberstein, G. Di Perri, C.F. Perno, Impact of pre-therapy viral load on virological response to modern first-line HAART, Antivir. Ther. 18 (2013) 867-876. doi: 10.3851/IMP2531.

[13] M.M. Santoro, D. Di Carlo, D. Armenia, M. Zaccarelli, C. Pinnetti, M. Colafigli, F. Prati, A. Boschi, A.M. Degli Antoni, F. Lagi, L. Sighinolfi, C. Gervasoni, M. Andreoni, A. Antinori, C. Mussini, C.F. Perno, V. Borghi, G. Sterrantino, VIRO-immunological response of drug-naive HIV-1-infected patients starting a first-line regimen with viraemia >500,000 copies/ml in clinical practice, Antivir. Ther. 23 (2018) 249–257. doi:10.3851/IMP319.

[14] A. Di Biagio, S. Rusconi, A. Marzocchetti, A. Signori, I. Schiavetti, B. Bruzzone, L. Monno, G. Punzi, M.G. Colao, G. Penco, M. Zazzi, A. De Luca, ARCA Collaborative Group, The role of baseline HIV-1 RNA, drug resistance, and regimen type as determinants of response to first-line antiretroviral therapy, J. Med. Virol. 86 (2014) 1648–1655. doi:10.1002/jmv.24017.

[15] L. Wittkop, H.F. Günthard, F. de Wolf, D. Dunn, A. Cozzi-Lepri, A. de Luca, C. Kücherer, N. Obel, V. von Wyl, B. Masquelier, C. Stephan, C. Torti, A. Antinori, F. García, A. Judd, K. Porter, R. Thiébaut, H. Castro, A.I. van Sighem, C. Colin, J. Kjaer, J.D. Lundgren, R. Paredes, A. Pozniak, B. Clotet, A. Phillips, D. Pillay, G. Chêne; EuroCoord-CHAIN study group, Effect of transmitted drug resistance on virological and immunological response to initial combination antiretroviral therapy for HIV (EuroCoord-CHAIN joint project): a European multicohort study, Lancet Infect Dis. 11 (2011) 363-371. doi: 10.1016/S1473-3099(11)70032-9.

[16] D.S. Clutter, W.J. Fessel, S.Y. Rhee, L.B. Hurley, D.B. Klein, J.P. Ioannidis, M.I. Silverberg, R.W. Shafer. Response to Therapy in Antiretroviral Therapy-Naive Patients With Isolated Nonnucleoside Reverse Transcriptase Inhibitor-Associated Transmitted Drug Resistance, J. Acquir. Immune Defic Syndr. 72 (2016) 171-176. doi: 10.1097/QAI.0000000000000942.

[17] D. Armenia, D. Di Carlo, A. Calcagno, G. Vendemiati, F. Forbici, A. Bertoli, G. Berno, S. Carta, F. Continenza, V. Fedele, R. Bellagamba, S. Cicalini, A. Ammassari, R. Libertone, M. Zaccarelli, V. Ghisetti, M. Andreoni, F. Ceccherini-Silberstein, S. Bonora, G. Di Perri, A. Antinori, C.F. Perno, M.M. Santoro, Pre-existent NRTI and NNRTI resistance impacts on maintenance of virological suppression in HIV-1-infected patients who switch to a tenofovir/emtricitabine/rilpivirine single-tablet regimen, J. Antimicrob. Chemother. 72 (2017) 855-865. doi: 10.1093/jac/dkw512.

[18] H.F. Günthard, V. Calvez, R. Paredes, D. Pillay, R.W. Shafer, A.M. Wensing, D.M. Jacobsen, D.D. Richman. Human Immunodeficiency Virus Drug Resistance: 2018 Recommendations of the International Antiviral Society-USA Panel, Clin. Infect. Dis 2018 Jul 20. doi: 10.1093/cid/ciy463.