U.PORTO

MESTRADO INTEGRADO EM MEDICINA

Integrated Opiate Substitution Treatment and HIV care: a cohort study in Portugal

Ricardo Miguel Mendonça Rodrigues

M

2022

Integrated Opiate Substitution Treatment and HIV care: a cohort study in Portugal

Ricardo Miguel Mendonça Rodrigues

Estudante do 6º ano do Mestrado Integrado em Medicina

Endereço de correio eletrónico: rodrigues.rmm@gmail.com

Mestrado Integrado em Medicina

Instituto de Ciências Biomédicas Abel Salazar, Universidade do Porto

Centro Hospitalar Universitário do Porto, Hospital de Santo António

---

Orientadora

Dr.ª Maria Josefina Vázquez Méndez

Docente de mobilidade externa no Instituto de Ciências Biomédicas Abel Salazar

Assistente Graduada Sénior de Doenças Infeciosas no Centro Hospitalar e Universitário do Porto

---

Coorientadora

Professora Doutora Isabel Maria Pereira Alves de Almeida

Professora Catedrática Convidada do Instituto de Ciências Biomédicas Abel Salazar

Assistente Graduada Sénior de Medicina Interna do Centro Hospitalar e Universitário do Porto

Diretora do Serviço de Urgência do Centro Hospitalar e Universitário do Porto

Junho de 2022

Junho de 2022

Assinatura do Estudante

Assinatura da Orientadora

Assinatura da coorientadora

Agradecimentos

À Dr.ª Josefina Mendez e à Professora Doutora Isabel Almeida quero agradecer a disponibilidade e orientação ao longo deste projeto de investigação.

Um agradecimento especial à Enfermeira Cristina Ferreira do Centro de Terapêutica Combinada, à senhora Dinora Sousa da Consulta Externa do Serviço de Doenças Infeciosas e à Dr.ª Rita Maciel Barbosa da Divisão de Intervenção nos Comportamentos Aditivos e Dependências da Administração Regional de Saúde do Norte, sem as quais este trabalho não teria sido possível.

À Professora Doutora Carolina Lemos e ao Professor Doutor Rui Magalhães, um agradecimento pelos conselhos para a realização da estatística deste estudo.

Resumo

Introdução: A integração da Terapêutica Antirretroviral (TARV) com a Terapêutica de Substituição de Opióides (TSO) está associada a um aumento do acesso aos cuidados de saúde, ao aumento da adesão às consultas e regimes terapêuticos e a uma maior supressão viral. Sendo assim, é importante verificar se estes benefícios se traduzem clinicamente a médio prazo, nomeadamente através da incidência de infeções oportunistas, da realização de tratamento da infeção pelo Vírus da Hepatite C (VHC), na duração dos internamentos e na mortalidade. O objetivo deste estudo é avaliar se os doentes que recebem terapêutica integrada para as diversas comorbilidades têm melhores resultados em saúde do que os doentes que recebem a terapêutica separada por especialidades médicas e por serviços.

Metodologia: Foi realizado um estudo de coorte que incluiu todos os doentes com VIH e a realizar TSO no Centro Hospitalar e Universitário do Porto (CHUPorto) entre o ano de 2015 e 2020. A informação dos doentes foi colhida através do processo clínico eletrónico.

Resultados: Numa população de 251 doentes, não foram encontradas diferenças na mortalidade, supressão viral e variação da contagem de linfócitos CD4+ entre os dois grupos em estudo. No entanto, o grupo que recebeu terapêutica separada por especialidades médicas apresentou mais eventos de doença grave (75 eventos por 1000 pessoas-ano *versus* 31 eventos por 1000 pessoas-ano), tendo em média 2.29 vezes mais doenças do que o grupo de doentes que recebeu terapêutica integrada. O número de dias de internamento foi também superior no grupo que recebeu a terapêutica separada por especialidades médicas, tanto no internamento em enfermaria (1216 dias por 1000 pessoas-ano [IC 95%: 1120-1320] *versus* 466 dias por 1000 pessoas-ano [IC 95%: 417-520]), como no internamento em Unidades de Cuidados Intensivos (47 dias por 1000 pessoas-ano [IC 95%: 31-71] *versus* 9 dias por 1000 pessoas ano [IC 95%: 4-20]). Dos doentes infetados com VHC, a percentagem que realizou tratamento durante o estudo foi superior no grupo que recebeu terapêutica integrada (94.2% *versus* 63.9%), tendo um *odds ratio* (OR) de 7.6 (IC 95%:2.9-19.9; $p<0.001$).

Conclusão: Os resultados obtidos demonstram o benefício da terapêutica integrada em termos da redução da incidência de doenças grave, da redução dos dias de internamento e da realização do tratamento para a infeção por VHC, suportando assim a recomendação da sua implementação em mais serviços do território português.

Palavras-chave: HIV; Opiate Substitution Treatment; Anti-Retroviral Agents; Hepatitis C, Chronic.

Abstract

Background: The integration of Antiretroviral Therapy (ART) with Opiate Substitution Treatment (OST) is associated with increased access to health care, increased adherence to medical appointments and treatment regimens, and greater viral suppression. Therefore, it is important to verify if these benefits have a clinical translation in the medium term, namely, through the incidence of opportunistic infections, treatment completion for Hepatitis C Virus (HCV) infection, length of hospital stays and mortality. This study aims to assess whether patients receiving integrated therapy for various comorbidities have better health outcomes than patients receiving therapy separated by medical specialities and services.

Methodology: A cohort study was conducted including all patients with HIV and enrolled on OST at the Centro Hospitalar e Universitário do Porto (CHUPorto) between 2015 and 2020. Patient information was collected through the electronic medical record.

Results: In a population of 251 patients, no differences in mortality, viral suppression, and CD4+ lymphocyte count variation were found between the two study groups. However, the group receiving separate therapy by medical specialities had more severe disease events (75 events per 1000 person-years versus 31 events per 1000 person-years) and on average 2.29 times more illness events than the group of patients receiving integrated therapy. The number of days of hospitalization was also higher in the group receiving therapy separated by medical specialities, both for ward stay (1216 days per 1000 person-years [95% CI: 1120-1320] versus 466 days per 1000 person-years [95% CI: 417-520]) and intensive care unit stay (47 days per 1000 person-years [95% CI: 31-71] versus 9 days per 1000 person-years [95% CI: 4-20]). Among the HCV-infected patients, the percentage which underwent treatment during the study was higher in the group receiving integrated therapy (94.2% versus 63.9%), with an odds ratio (OR) of 7.6 (95% CI:2.9-19.9; $p<0.001$).

Conclusion: The results obtained demonstrate the benefit of integrated therapy in terms of reducing the incidence of severe disease, reducing days of hospitalization and completion of treatment for HCV infection, thus supporting the recommendation of its implementation in more services in the Portuguese territory.

Keywords: HIV; Opiate Substitution Treatment; Anti-Retroviral Agents; Hepatitis C, Chronic.

List of abbreviations

AIDS	Acquired immunodeficiency syndrome
ART	Antiretroviral Therapy
CDC	Centers for Disease Control and Prevention
CHUPorto	Centro Hospitalar e Universitário do Porto
CRI	Centro de Resposta Integrada
CTC	Centro de Terapêutica Combinada
DOT	Directly Observed Therapy
InRs	Immunological non-Responders
HCV	Hepatitis C virus
HIV	Human Immunodeficiency Virus
IVDU	Intravenous Drug User
OC	Outpatient Clinic
OR	Odds ratio
OST	Opiate Substitution Treatment
RNA	Ribonucleic acid
USA	United States of America

Table of contents

Index of Tables and Figures

Introduction

According to the report on *Infeção VIH e SIDA em Portugal 2020*[1], Portugal stands out for the high rates of new cases of HIV infection and AIDS among Western European countries, with 7.6 new cases of HIV per 100,000 inhabitants in 2019. From the cases where the mode of transmission could be identified, only 2.1% are attributed to Intravenous Drug Users (IVDU). Nevertheless, IVDU transmission constitutes 7.6% of new AIDS cases and 31.5% of deaths in the same year. In fact, according to the Centers for Disease Control and Prevention (CDC)[2], in 2018, in 41 states of the United States of America (USA) and in the District of Columbia, the percentage of patients with HIV viral load suppressed by six months after diagnosis was lower among IVDU (53%) than in patients with other HIV transmission modes (68%). Late testing, poor adherence to antiretroviral therapy (ART) and treatment interruptions or failures related to intravenous drug use are extensively documented in this population[3].

We know that IVDU can achieve excellent virological outcomes, particularly with early ART adherence and when psychosocial support is available[3]. The attendance of HIV/HCV co-infected IVDU in Opiate Substitution Treatment (OST) programs is associated with a reduction in heroin use, increased adherence to antiretroviral therapy, as well as with the achievement of viral load suppression and increased T-CD4+ lymphocyte counts[3,4].

In patients with substance use disorders and infectious diseases such as HIV infection and Hepatitis C, which are often associated with other psychiatric disorders, healthcare is often provided separately by different doctors in spatially distant locations. This separation of medical treatment by specialities constitutes a systemic barrier to treatment for these patients, who have a lower likelihood of receiving ART compared to other HIV-infected patients[3]. The integration of the healthcare for these multiple comorbidities in a single service enables a holistic view of the patient and optimization of their treatment[3,5,6].

Acknowledging the daily visit of OST patients for Direct Observation Therapy (DOT) as an opportunity to increase retention in HIV therapy, several authors advocate the integration of OST with ART, as well as the treatment of other comorbidities, and the need for further studies in this area[3–5,7–10].

This model, called one-stop shopping, is considered ideal since it allows overcoming the political, bureaucratic and financial barriers that separate people with substance dependence problems from the necessary medical care, and it solves the problem of geographical separation, patient disorganization and low motivation that inhibit this population from accessing medical

appointments[5,6]. On the other hand, this model allows for the logistical simplification of services, better management of the adverse effects of medication, the avoidance of unfavourable drug interactions, greater synergy between the team providing care, an increase in the coherence and continuum of care and, of course, a better relationship between the patient and the care providers[5]. In short, one-stop shopping drives an increase in access to healthcare, increased adherence to medical appointments and therapeutic regimes and an improvement in the health outcomes of this population[6].

The studies already available in this area show that the integration of ART with OST leads to greater adherence[9], retention in treatment[8,9] and greater viral suppression[3,8,9]. The conjugation of ART and OST in DOT should also be seen as a long-term intervention since the benefits of adherence and viral load suppression cease once the therapy is discontinued[9]. Moreover, the integration of anti-tuberculosis treatment in this context is considered fundamental[3,11].

The Center for Integrated Therapeutics (Centro de Terapêutica Combinada - CTC) at Hospital Joaquim Urbano was initially set up in 1998, within the scope of a study[12] that aimed to solve the problem of low adherence to anti-tuberculosis treatment among people living with HIV who underwent OST. In this project, in which anti-tuberculosis treatment and opioids were administered under direct observation, the experimental group, despite initially showing greater immunological depletion than the control group, had significantly higher rates of adherence to treatment (81.3% vs. 35.7%), a significantly lower 12-month mortality rate (22% vs. 40%) and eliminated tuberculosis as the main cause of death. It also demonstrated an improvement in the immunological and virological status of the patients, which are important factors for delaying the progression of HIV infection. Nowadays, Opiate Substitution Treatment, antiretroviral therapy and anti-tuberculosis treatment are offered daily under direct observation and managed by a multidisciplinary team including infectious disease specialists, psychiatrists, psychologists, social workers, nurses and social workers who follow combined protocols, enabling integrated and co-located healthcare. Treatment for Hepatitis C and chronic medication for other comorbidities, namely psychiatric, are also carried out on site. There is a field team that provides home treatment to patients physically unable to attend medical appointments. In order to facilitate therapeutic adherence, several social services are offered, such as the monthly bus ticket, daily breakfast, internet and computer access, sports activities and laundry and hairdressing services. Participation in this project requires a daily visit to the CTC[13].

Despite the existing evidence on the benefits of integrating HIV, tuberculosis and Hepatitis C treatment into Opiate Substitution Treatment, the existing studies only assess goals such as viral

suppression, T-CD4+ lymphocyte count and adherence to therapy. Thus, we found no studies relating this model to medium-term clinical outcomes, such as the development of AIDS, the number of opportunistic infections and mortality. Furthermore, although the research behind the CTC shows the clear advantage of this approach, 24 years after its creation it is still the only centre of its kind in Portugal. It is now important to carry out a new assessment of the results obtained, particularly in the battle against HIV and HCV infection, at a time when the paradigm of these pathologies has undergone a major revolution thanks to new antiretroviral drugs and the principle of early treatment in all patients[14].

This study is a cohort study in HIV-infected patients undergoing Opiate Substitution Treatment, aiming to assess whether patients receiving integrated therapy for various comorbidities have better health outcomes than patients receiving therapy separated by medical specialities and services.

Methodology

The study was designed and conducted within the framework of the Dissertation Course Unit of the Integrated Master's Degree in Medicine of the School of Medicine and Biomedical Sciences of the University of Porto. The study was authorized by the Board of Directors and by the Ethics Committee of the Centro Hospitalar e Universitário do Porto (CHUPorto), with the authorization number 2021.297(243-DEFI/251-OC).

Background

The study was carried out at the CHUPorto and the data collected refer to the period between 2015 and 2020. The study was designed between May and December 2021 and the permission to conduct it was obtained on 3 May 2022. All the data was collected in May 2022.

Participants

The group exposed to the integrated therapy for the various comorbidities was composed of all the patients receiving antiretroviral and OST at the Centro de Terapêutica Combinado of the CHUPorto in 2015, henceforth referred to as the CTC group. The non-exposed group, which received the therapy separated by medical specialities, was composed of all the patients receiving treatment for HIV infection in the Outpatient Clinic of the CHUPorto's Infectious Diseases Service and receiving OST in the Centros de Resposta Integrada (CRI's) Ocidental, Central and Oriental of the city of Porto in 2015, henceforth referred to as the Outpatient Clinic (OC) group. The participants were then followed up for five years.

Variables

Demographic data (gender, date of birth, race/ethnicity, highest level of education, employment status, marital status, housing status) and clinical data (smoking habit, number of years since diagnosis of HIV infection, number of years on OST, number of years since first intravenous drug injection, ART performed) were collected to characterize this population. Each patient’s period of participation on the study was defined as the time gap between the dates of collection of T-CD4+ lymphocyte counts in 2015 and 2020. For the fatalities, the collection of the T-CD4+ lymphocyte count in 2020 was replaced by the date of death and for losses to follow-up, this was replaced by the date of the last medical appointment taken.

Endpoints

The primary endpoints were the incidence of serious illness events and the mortality rate from any cause during the study period.

The events of serious illness considered are part of the criteria defined by the CDC for AIDS case surveillance[15] [Table 1], except for the criterion of T-CD4+ lymphocyte count less than 200/mm^3. Regarding recurrent pneumonia, in addition to the criterion established by the CDC, we also considered all pneumonia that resulted in hospital admission. In the case of recurrent pneumonia, in patients who met the established criteria, it was recorded as a single event, regardless of the number of pneumonia events or hospital stays during the study.

In terms of secondary endpoints, we aimed to determine the variation in T-CD4+ lymphocyte count and HIV RNA load in this period, by collecting T-CD4+ lymphocyte counts and HIV RNA load in 2015 and 2020. Considering the high prevalence of Hepatitis C virus infection in this population, the percentage of infected patients who had treatment during the study period and its outcome were determined. We also collected the number of days of hospitalization in the ward and in the Intensive Care Unit (ICU) for each event of serious illness during the study period.

Information sources

Apart from the date and cause of death, all data were was collected by consulting the Electronic Health Record in SClínico. The date and cause of death were obtained by consulting the death certificate on the SICO platform. The CD4+ lymphocyte count was obtained using flow cytometry and the HIV viral load was obtained through the RT-PCR technique.

The number of patients followed at the CTC in 2015 and the number of patients followed for HIV infection at the CHUPorto's Infectious Diseases Outpatient Clinic and undergoing OST in the CRIs of the city of Porto in 2015 determined the sample size.

Quantitative variables

For the purpose of statistical analysis, when the HIV RNA load was undetectable, we considered the value as 0.

CD4+ lymphocyte counts were grouped according to the three categories recommended by the CDC[15]:

- Category 1: ≥500 cells/mm^3;

- Category 2: 200-499 cells/mm^3;
- Category 3: ≤200 cells/mm^3.

In order to facilitate the analysis of each patient's progression through the study, it was considered a positive endpoint if, from 2015 to 2020, the patient remained with a count ≥500 cells/mm^3 or moved up to a category with higher T-CD4+ lymphocyte counts. On the contrary, if the patient remained in category two or three or moved down to a category with lower counts, it was considered a negative outcome.

HIV viral loads were grouped according to the following categories:

- Category 1: <20 copies/mL[Undetectable]
- Category 2: ≥20 copies/mL [Detectable]
    - Subgroup: ≥20-100 copies/mL [blips]

Blips were defined as having a detectable HIV viral load <100 copies/mL in a single measurement.

Statistical analysis

Statistical analysis was performed following the intention-to-treat principle.

Continuous variables were tested for normal distribution within each group using the Shapiro Wilk test (if n>100) and the Kolmogorov-Smirnov test (if 30<n<100). Continuous variables that followed a normal distribution were presented using the mean and standard deviation, while the remaining variables were presented using the median and interquartile range. Categorical variables were presented using percentages. Mortality was presented with a Kaplan-Meier curve and the association between the two groups was tested with the Mantel-Cox test. The frequency of AIDS-defining events and days of ward and ICU stay were presented as a rate per 1000 person-years and the results between groups were compared by performing generalized linear models with the Poisson distribution. The association between groups for the remaining variables was determined using the chi-square test and, when possible, logistic regressions.

Statistical analysis was performed using SPSS® Statistics 28.

Results

Participants

The information regarding sample recruitment can be found in figure 1, in the appendix. However, it is important to explain that all patients from which no record of T-CD4+ lymphocyte count and HIV RNA load could be found in their clinical records in 2020 were considered lost to follow-up.

The characteristics of the patients recruited for this study can be seen in Table II in the appendix. Of the 25 patients in the CTC group with detectable HIV viral load, 56% were blips. In the OC group, of the 36 patients with detectable HIV viral load, 33% were blips. The median nadir of T-CD4+ lymphocytes in the CTC group was 115 (IQR: 33-223) cells/mm^3 and in the OC group was 157 (IQR: 85-286) cells/mm^3, with a statistically significant difference ($p<0.001$). Also in the 2015 HIV RNA load, it was found that 82.6% of the CTC patients had an undetectable viral load, against 66.4% of the OC patients ($p=0.011$). In the remaining variables, no statistically significant differences were found between the groups. The variable smoking habit was excluded from this analysis due to the high number of patients of whom the result is unknown (39.8%).

The variables race/ethnicity (n=8), level of education (n=9), employment status (n=40), housing status (n=105), marital status (n=12), pack-year unit [smoking] (n=70), number of years on OST (n=6) and number of years since first intravenous drug use (n=35) were excluded from the statistical analysis due to the high number of missing data. The variable of treating the HCV infection only considered patients initially infected with HCV, therefore having a sample size of 187 cases. In the case of the drugs used to treat HCV infection, it was not possible to find data for three of the patients who underwent treatment. Among the deaths observed during follow-up, two occurred during the year 2020 and it was, therefore, possible to obtain clinical data for that year.

The most prescribed drugs during the study period were abacavir/lamivudine (16.5%), tenofovir disoproxil/emtricitabine (16%) and efavirenz (12.9%). A complete list of all prescribed drugs and their frequency can be found in Table III.

Follow-up

The median duration of study participation for each patient is 60 months (IQR: 58-63), with 61 months (IQR: 59-63) for the CTC group and 59 months (IQR: 54-62) for the OC group.

The follow-up of these patients occurred over 1151 person-years, 683 of which were in the CTC group and 468 person-years in the OC group.

Primary endpoints

During the study period 27 deaths occurred, 13 in the CTC group (9.1%) and 14 in the OC group (15.2%). The survival curve can be seen in figure 2 (p=0.223). The main causes of death were infectious, followed by neoplastic (table IV).

There were also 54 serious illness events, with 21 of these occurring in the CTC group (31 events per 1000 person-years) and 33 in the OC group (71 events per 1000 person-years). Using the attack rate ratio, we found that patients in the OC group had an average number of illnesses 2.29 times bigger than the patients in the CTC group (95% CI 1.33-3.96, p=0.003). Recurrent or hospitalized pneumonias were the main event accounted for, followed by *M. tuberculosis* infection in any location. A detailed list of all diagnosed events with corresponding frequencies and lengths of hospital stay can be found in Table V.

Secondary endpoints

The median T-CD4+ lymphocyte count at the end of the study period was 571 cells/mm^3 (IQR: 384-82) and the median HIV RNA load was <20 copies/mL (IQR: <20-<20) (Table VI). Regarding the category variation of T-CD4+ lymphocyte count, 71.8% of patients in the CTC group had a positive evolution, compared to 62.0% of patients in the OC group (p=0.546). At the end of the study, 80.9% of CTC and 72.2% of OC patients had an undetectable viral load (p=0.312). Of those with detectable viral load, 80% and 50% were blips in the CTC and OC groups, respectively.

The number of days of hospitalization (Table V) in the CTC group was 466 days per 1000 person-years (95% CI: 417-520) in the ward and nine days per 1000 person-years (95% CI: 4-20) in the ICU. In the OC group, there were 1216 days per 1000 person-years (95% CI: 1120-1320) in the ward and 47 days per 1000 person-years (95% CI:31-71) in the ICU. We can therefore conclude with 95% confidence that the differences found in the days of hospital stay in the two groups are statistically significant.

Thus, for a group of 150 patients, which is the average number of patients followed up annually at the CTC, patients with integrated therapy had 113 fewer days of ward stay and 6 fewer days of ICU stay than patients with therapy separated by medical speciality and services, per year.

As regards treatment for HCV infection, in the CTC group 94.2% of patients were treated, while in the OC group only 63.9% of patients were treated (Table VII). Thus, the odds ratio of taking treatment in the CTC group compared with the OC group is 7.6 (95% confidence interval [CI]: 2.9-19.9; p<0.001). The HCV infection status in 2020 can be seen in the same table. The main drug used

to treat HCV infection was the combination of Sofosbuvir and Ledipasvir, used in 68.2% of cases (Table VIII).

Further analysis

Table IX and Figure 3 were constructed to assess the distribution of patients with detectable and undetectable viral load by the different categories of T-CD4+ lymphocyte counts in both groups in 2015 and 2020. It is possible to see that in the CTC group the distribution of patients by T-CD4+ lymphocyte count categories was relatively similar regardless of whether or not they had detectable viral load ($p=0.925$ in 2015 and $p=0.882$ in 2020). However, in the OC group the distribution across T-CD4+ categories differed between the different viral loads, with patients with undetectable viral load appearing to have higher lymphocyte counts ($p=0.020$ in 2015 and $p<0.001$ in 2020).

Discussion

We set out to evaluate the effect of integrated therapy, as opposed to separate therapy by medical specialities, on health outcomes in HIV-infected patients undergoing OST.

Regarding mortality, variation in T-CD4+ lymphocyte counts and HIV RNA load at the end of the study period, no statistically significant differences were found between the two groups. However, regarding the incidence of serious illness events, treatment for HCV co-infection and the number of days spent in the ward and ICU, a statistically significant difference was found between the two groups, with better results in the group of patients who received integrated therapy.

In two studies conducted in 2004[16,17], the integration of ART with OST allowed the achievement of HIV RNA load <50 copies/mL in 60% and 41% of patients. The percentage of patients with undetectable viral load in this study was much higher, but it is difficult to directly compare the results given the small sample sizes (n=38 and n=54, respectively) and since the drugs used in ART are very different.

In California in 2012[9], 24 patients received ART integrated with OST, with 76% of these achieving an undetectable viral load after 24 weeks of treatment. At the same site in 2015[8], viral suppression after one year was compared between a group of patients on ART in an OST clinic and a group of patients on ART in an HIV specialty clinic. In the former group, 93% of 15 patients achieved a viral load <40 copies/mL, while in the latter, only 79% of 42 patients achieved this value. Although these results in the integrated therapy group are better than the results obtained in the CTC group, we must note that there are some limitations to making a direct comparison between them. We must consider that the sample size was very small and that the study population had different characteristics, namely a higher proportion of female patients and on average an older age (81% of participants were older than 45 years old).

Given the difference in the incidence of serious disease events between the two groups, one would expect a larger difference in the results of variation in T-CD4+ lymphocyte counts and viral load. This unexpected result could be related to the patients' previous therapeutic pathway. In fact, at the beginning of the study we did not know their previous course of treatment. They may have already been on ART in the same treatment model (integrated or separated by specialities) for several years, or may have just been transferred from the opposite treatment group or have just started ART. Thus, in addition to this being a possible confounding factor, if a patient has already been on ART for several years, the possibility of variation in lymphocyte load and viral load is smaller. Several studies[18,19] have found that the most significant benefits of ART in terms of increased lymphocyte counts occur early in the course of therapy, particularly in the first four years.

Moreover, since the T-CD4+ lymphocyte counts and HIV viral load results are based on two measurements taken five years apart, we should be careful in evaluating them.

The difference found in the distribution of patients with detectable and undetectable viral load among the different categories of T-CD4+ lymphocyte counts is curious. While in the OC group the two variables seem to be related - the lymphocyte count will be higher if the viral load is undetectable - no relation is found in the CTC group. This might be related to a higher prevalence of Immunological non-responders (InRs) in the CTC group. Several studies[18–22] identify that between 15-25% of patients starting ART with lymphocyte counts <200 cells/mm^3 may fail to achieve immune reconstitution despite viral load suppression. In particular, a study[23] developed in 2016 in Porto found that 7% of 401 patients were unable to obtain lymphocyte counts higher than 200 cells/mm^3 over 40 months of viral suppression. The use of intravenous drugs and lower T-CD4+ nadir values are identified as risk factors for this lack of immune response[22], which is compatible with the T-CD4+ nadir values found on the CTC group. However, we also know that InRs have higher mortality and risk of AIDS-defining disease events[24], which doesn't happen on the CTC group.

In regard to AIDS-defining diseases, there is a set of these diseases, namely oesophageal candidiasis, *Pneumocystis jirovecii* pneumonia, immunoblastic lymphoma, CMV disease and toxoplasmosis that are clearly related to more immunodepleted states[25]. Interestingly, in addition to the differences in the overall incidence of severe disease events, the OC group has a particularly high incidence of these diseases associated with a higher state of immune depletion. This fact may be related to a lower adherence to prophylaxes that are recommended in stages of greater immune depletion, such as prophylaxis against *Pneumocystis jirovecii*.

The differences found in the proportion of HCV-infected patients that received treatment for HCV infection during the study and in the final HCV infection status are very significant (19% infected in the OC group vs 6.9% in the CTC group). However, it is important to underline that both groups were treated by the same service of infectious disease physicians, and in many cases the same physicians treated both groups. Although the causes for the non-completion of treatment for Hepatitis C virus infection have not been studied, we can hypothesize that the main obstacles in the OC group were the difficulty of transport and access to hospital consultations and their exclusion due to the risk of therapeutic non-compliance. Furthermore, the significant differences found in the achievement of treatment for HCV infection may translate in the future into the incidence of and mortality from liver cirrhosis, which, given the time limit of this study, it has not yet been possible to measure.

Some limitations of this study should be noted. Firstly, the baseline characteristics of the two groups were not equal. While the OC group had better T-CD4+ lymphocyte nadir values than the CTC group, the latter had a higher percentage of patients with undetectable HIV RNA load. It is therefore not surprising that the CTC group has a lower incidence of serious illness events and fewer days of hospitalisation. However, the difference between the percentage of patients with initial undetectable HIV RNA load in the CTC group (82.6%) and the OC group (66.4%) may not fully explain the latter having an average number of illnesses 2.29 times bigger, and the same applies to the very discrepant differences in ICU and ward length of stay.

Secondly, it is possible that the integrated therapy teams, due to their specialization and frequent contact with these patients, may have gained some desensitization to the severity of their problems (as shown by the absence of records on smoking habits in the clinical files of this group), and for this reason they may have undervalued some events, particularly pneumonia, not recording them in the clinical files and therefore generating an underreporting of events in this group.

Another limitation of this study is the lack of data supporting the socio-economic characterisation of the two groups, which may be a confounding variable in the results obtained.

It should also be kept in mind that the two groups of patients are not randomly drawn from a pool of HIV and OST patients in Porto. Patients in the OC group, when they become treatment defaulters and are admitted to hospital for opportunistic infections, are proposed for integration in the CTC group, to increase therapeutic adherence. In a way, we may state that the CTC group consists of patients from the OC group who, at the outset, would have a worse prognosis. Therefore, it would be more appropriate to compare the population of this study with a group from another city, where integrated therapy does not exist.

Considering the results and the limitations already addressed, we suggest that further research should be carried out, enhancing the effects of an adequate socio-economic characterisation of the population and of serial analytical studies. It would also be interesting to extend the investigation, addressing the possible consequences in the treatment of other cardiovascular comorbidities.

We know that this type of approach, due to the frequency of contacts with the health system, will not be the most appropriate for all patients. However, we would like to highlight the value of the integrated approach in boosting health outcomes. Thus, what we propose is not the replacement of the traditional model of separate treatment by medical speciality with this model of integrated treatment, but rather its complementarity.

As it is difficult to generalise these results to other countries, given the epidemiological specificities of the Portuguese HIV and on OST patients and the unique legal framework of decriminalisation of drug use in Portugal, we can perhaps generalise them to the rest of the Portuguese population and thus substantiate the suggestion to implement more services with this model of operation in the Portuguese territory.

Conclusion

We set out to evaluate the effect of integrated, as opposed to separate specialty-based, therapy on health outcomes in HIV-infected patients on OST.

Statistically significant differences were found supporting the benefit of integrated therapy, also called one-stop shopping, in reducing the incidence of serious illness, treating for HCV infection, and reducing days of hospital stay. However, no differences were found to demonstrate significant advantages in terms of mortality, viral suppression and T-CD4+ lymphocyte levels.

The results obtained allow us to substantiate the suggestion of implementing more services with this model of operation in Portugal, complementing the traditional model of treatment separated by medical specialities.

Apendix

Table I – Criteria for serious illness event, adapted from the 1993 CDC AIDS case surveillance definition[15].

Table I – Criteria for serious illness event
Candidiasis of bronchi, trachea, or lungs
Candidiasis of esophagus
Cervical cancer, invasive
Coccidioidomycosis, disseminated or extrapulmonary
Cryptococcosis, extrapulmonary
Cryptosporidiosis, chronic intestinal (>1 month's duration)
Cytomegalovirus disease (other than liver, spleen, or nodes), onset at age >1 month
Cytomegalovirus retinitis (with loss of vision)
Encephalopathy attributed to HIV
Herpes simplex: chronic ulcers (>1 month's duration) or bronchitis, pneumonitis, or esophagitis (onset at age >1 month)
Histoplasmosis, disseminated or extrapulmonary
Isosporiasis, chronic intestinal (>1 month's duration)
Kaposi sarcoma
Lymphoma, Burkitt (or equivalent term)
Lymphoma, immunoblastic (or equivalent term)
Lymphoma, primary, of brain
Mycobacterium avium complex or *Mycobacterium kansasii*, disseminated or extrapulmonary
Mycobacterium tuberculosis of any site, pulmonary†, disseminated, or extrapulmonary
Mycobacterium, other species or unidentified species, disseminated or extrapulmonary
Pneumocystis jirovecii (previously known as "*Pneumocystis carinii*") pneumonia
Pneumonia, recurrent or with hospital admission criteria
Progressive multifocal leukoencephalopathy
Salmonella septicemia, recurrent
Toxoplasmosis of brain, onset at age >1 month
Wasting syndrome attributed to HIV

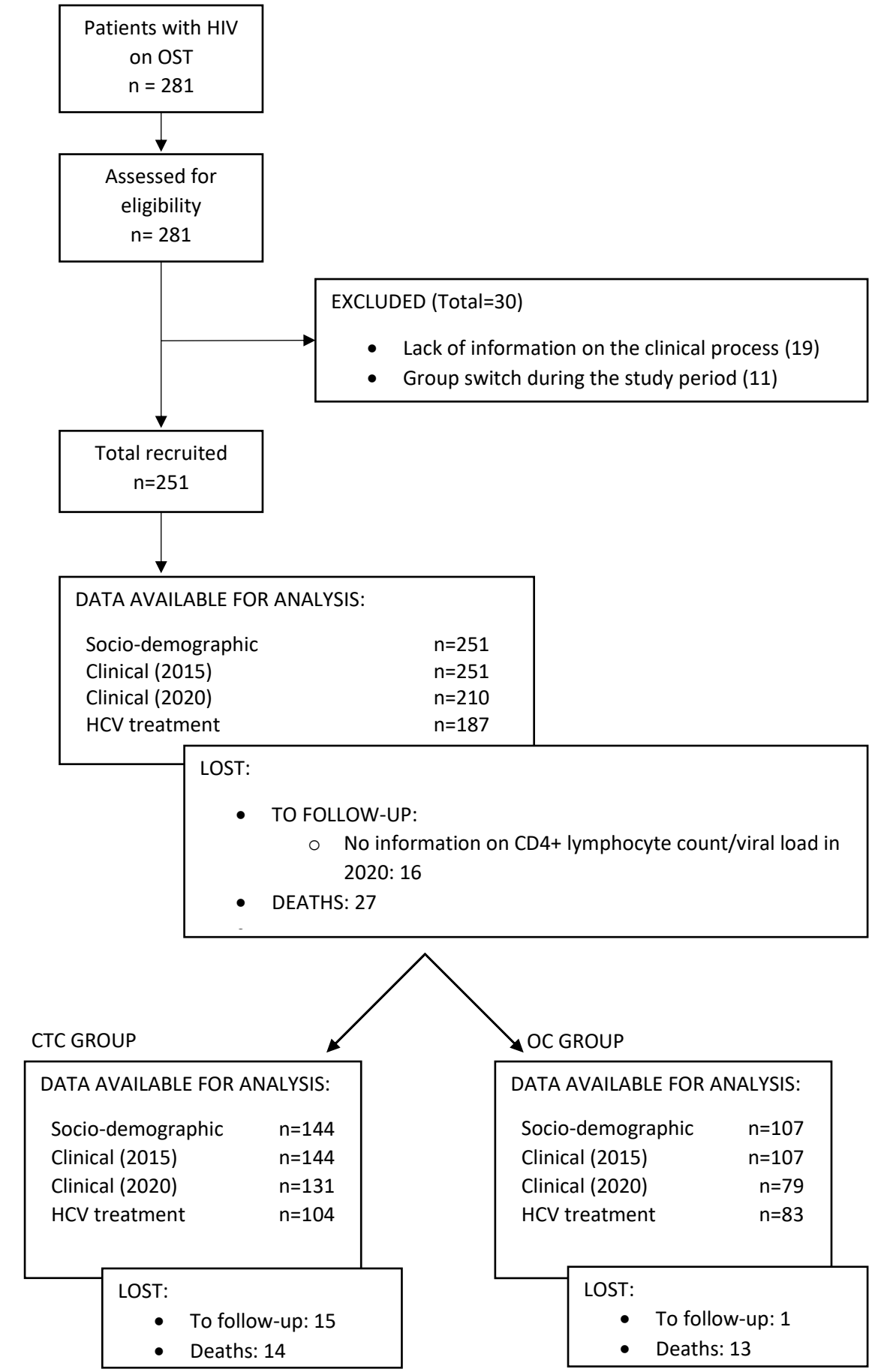

Figure 1. Diagram of patient recruitment for the study.

Table II - Baseline characteristics of the patients recruited to the study.

Characteristics	CTC Group (n=144)	OC Group (n=107)	Total (n=251)
Average age (sd) – years *	44.4 (6.6)	43.7 (7.1)	44.1 (6.8)
Female sex – n.º (%) *	18 (12.5)	14 (13.1)	32 (12.7)
Median time since diagnosis of HIV (IQR) – years *	14.0 (11.0-17.8)	15.0 (10.0-18.0)	14.0 (11.0-18.0)
Initial median CD4+ count (IQR) – cells/mm^3	530 (349-728)	547 (329-774)	531 (346-745)
Initial CD4+ count – nº (%) *			
≥500 cells/mm^3	83 (57.6)	62 (57.9)	145 (57.8)
200-499 cells/mm^3	45 (31.3)	35 (32.7)	80 (31.9)
<200 cells/mm^3	16 (11.1)	10 (9.3)	26 (10.4)
Median initial HIV RNA load (IQR) – cópias/mL	<20 (<20-<20)	<20 (<20-54)	<20 (<20-<20)
Initial HIV RNA load – nº (%) *			
Indetetável	119 (82.6)	71 (66.4)	190 (75.7)
Detetável	25 (17.4)	36 (33.6)	61 (24.3)
Median nadir T-CD4+ count (IQR) – células/mm^3 *	115 (33-223)	157 (85-286)	132 (50-254)
Smoking habits – nº (%)			
Smoker	68 (47.2)	81 (75.7)	149 (59.4)
Former smoker	1 (0.7)	1 (0.9)	2 (0.8)
Unknown	75 (52.1)	25 (23.4)	100 (39.8)
HCV infection – nº (%) *			
Infected	102 (70.8)	83 (77.6)	185 (73.7)
Cured	14 (9.7)	7 (6.5)	21 (8.4)
Spontaneous clearence	21 (14.6)	10 (9.3)	31 (12.4)
Under treatment	1 (0.7)	1 (0.9)	2 (0.8)
Liver cirrhosis – nº (%) *	28 (19.4)	20 (18.7)	48 (19.1)
Place of OST – nº (%)			
CTC	144 (100)	-	144 (57.4)
CRI Ocidental	-	38 (35.5)	38 (15.1)
CRI Central	-	33 (30.8)	33 (13.1)
CRI Oriental	-	36 (33.6)	36 (14.3)

* Variables were tested for statistically significant differences between the two groups using a logistical regression. A statistically significant difference was found in the variables Initial HIV RNA Load (p=0.011) and Nadir of T-CD4+ lymphocytes count (p<0.001).

Table III – ART drugs prescribed during the study.

Drugs – nº (%)	CTC Group (n=522)	OC Group (n=353)	Total (n=875)
Abacavir/Lamivudine (ABC/3TC)	82 (15.7)	62 (17.6)	144 (16.5)
Tenofovir disoproxil/Emtricitabine (TDF/FTC)	84 (16.1)	56 (15.9)	140 (16)
Efavirenz (EFV)	69 (13.2)	44 (12.5)	113 (12.9)
Dolutegravir (DTG)	51 (9.8)	27 (7.6)	78 (8.9)
Darunavir + Ritonavir (DRV/r)	44 (8.4)	33 (9.3)	77 (8.8)
Raltegravir (RAL)	41 (7.9)	23 (6.5)	64 (7.3)
Rilpivirina (RPV)	25 (4.8)	15 (4.2)	40 (4.6)
Darunavir + Cobicistat (DRV/c)	28 (5.4)	5 (1.4)	33 (3.8)
Atazanavir + Ritonavir (ATV/r)	19 (3.6)	10 (2.8)	29 (3.3)
Tenofovir disoproxil (TDF)	21 (4)	8 (2.3)	29 (3.3)
Nevirapine (NVP)	18 (3.4)	9 (2.5)	27 (3.1)
Iopinavir + Ritonavir (LPV/r)	9 (1.7)	11 (3.1)	20 (2.3)
Etravirine (ETV)	5 (1)	12 (3.4)	17 (1.9)
Tenofovir alafenamide/Emtricitabine (TAF/FTC)	8 (1.5)	9 (2.5)	17 (1.9)
Zidovudine (AZT)	7 (1.3)	6 (1.7)	13 (1.5)
Abacavir (ABC)	8 (1.5)	1 (0.3)	9 (1)
Zidovudine/Lamivudine (AZT/3TC)	-	7 (2)	7 (0.8)
Lamivudine (3TC)	2 (0.4)	4 (1.1)	6 (0.7)
Elvitegravir + cobicistat (EVG/c)	-	2 (0.6)	2 (0.2)
Maraviroc (MVC)	-	2 (0.6)	2 (0.2)
Saquinavir (SQV)	-	2 (0.6)	2 (0.2)
Saquinavir + Ritonavir (SQV/r)	-	2 (0.6)	2 (0.2)
Estavudine (d4T)	-	1 (0.3)	1 (0.1)
Didanosine (ddi)	-	1 (0.3)	1 (0.1)
Fosamprenavir + Ritonavir (FPV/r)	-	1 (0.3)	1 (0.1)
Emtricitabine (FTC)	1 (0.2)	-	1 (0.1)

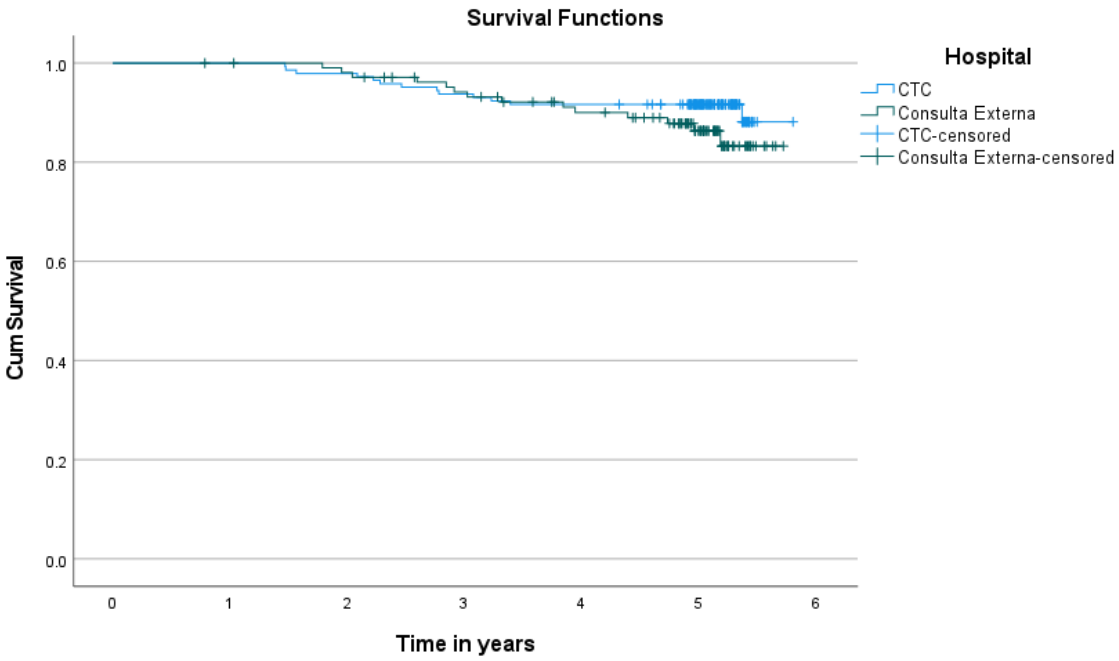

Figure 2. Kaplan-Meier curve for survival.

Table IV - Causes of death.

	CTC Group	OC Group	Total
Infectious - Nº (%)	**4 (30.8)**	**5 (35.7)**	**9 (33.3)**
Respiratory failure in the context of pneumonia	2	3	5
Septic shock due to bacterial peritonitis secondary to gastric ulcer perforation	-	1	1
Hepato-renal failure in the setting of leptospirosis	-	1	1
Septic shock with bacteraemia due to MSSA	1	-	1
Gangrene of the right trans-femoral stump	1	-	1
Neoplasic - Nº (%)	**2 (15.4)**	**3 (21.4)**	**5 (18.5)**
Epidermoid carcinoma of the tonsil	-	1	1
Lung carcinoma	-	1	1
Large B-cell lymphoma	-	1	1
Keratinising laryngeal epidermoid carcinoma	1	-	1
Multiorganic shock following porto-mesenteric bypass thrombosis in surgery for pancreatic head neoplasm	1	-	1
Hepatic - Nº (%)	**-**	**3 (21.4)**	**3 (11.1)**
Upper Digestive Haemorrhage in the context of Liver Cirrhosis	-	2	2
Cirrhosis of the liver	-	1	1
Addiction related - Nº (%)	**1 (7.7)**	**1 (7.1)**	**2 (7.4)**
Toxic intoxication	-	1	1
Withdrawal syndrome	1	-	1
Vascular - Nº (%)	**2 (15.4)**	**-**	**2 (7.4)**
Myocardial infarction	1	-	1
Cerebral haemorrhage	1	-	1
Diverse - Nº (%)	**4 (30.8)**	**2 (14.3)**	**6 (22.2)**
Asphyxia due to airway obstruction by food	1	-	1
Multiorgan failure associated with metformin intoxication	1	-	1
Unknown	2	2	4
Total – Nº (%)	**13 (100)**	**14 (100)**	**27 (100)**

Table V – Severe illness events that occurred during the study period, with respective days of stay in the ward and ICU, in absolute frequency and incidence rate per 1000 person-years.

	CTC Group (n=683)			OC Group (n=468)			Total (n=1151)		
n (/10^3 person-years)	Events	Ward days	ICU days	Events	Ward days	ICU days	Events	Ward days	ICU days
Recurrent pneumonia or with criteria for hospitalisation	14 (20)	239 (350)	6 (9)	17 (36)	249 (532)	22 (47)	31 (27)	488 (424)	28 (24)
M. tuberculosis in any location	4 (6)	39 (57)	-	3 (6)	223 (476)	-	7 (6)	262 (228)	-
Oesophageal candidiasis	2 (3)	5 (7)	-	3 (6)	13 (28)	-	5 (4)	18 (16)	-
Pneumocystis jirovecii pneumonia	-	-	-	3 (6)	62 (132)	-	3 (3)	62 (54)	-
Kaposi's sarcoma	-	-	-	2 (4)	-	-	2 (2)	-	-
HIV-related encephalopathy	-	-	-	2 (4)	15 (32)	-	2 (2)	15 (13)	-
Immunoblastic lymphoma	-	-	-	2 (4)	44 (94)	-	2 (2)	44 (38)	-
CMV disease	-	-	-	1 (2)	-	-	1 (1)	-	-
Cerebral toxoplasmosis	1 (1)	35 (51)	-	-	-	-	1 (1)	35 (30)	-
Total	21 (31)	318 (466)	6 (9)	33 (71)	606 (1295)	22 (47)	54 (47)	924 (803)	28 (24)

Table VI - Final clinical data for the year 2020.

	CTC Group (n=131)	OC Group (n=79)	Total (n=210)
Median final CD4+ count (IQR) - cells/mm^3	556 (394-798)	582 (330-853)	571 (384-821)
Final CD4+ count - no. (%)			
≥500 cells/mm^3	86 (65.5)	47 (59.5)	133 (63.3)
200-499 cells/mm^3	37 (28.2)	22 (27.8)	59 (28.1)
<200 cells/mm^3	8 (6.1)	10 (12.7)	18 (8.6)
CD4+ category variation - no. (%)			
Positive	94 (71.8)	49 (62.0)	143 (68.1)
Negative	37 (28.2)	30 (38.0)	67 (31.9)
Median final HIV RNA load (IQR) - copies/mL	<20 (<20-<20)	<20 (<20-21)	<20 (<20-<20)
Final HIV RNA load – no (%)			
Undetectable	106 (80.9)	57 (72.2)	163 (77.6)
Detectable	25 (19.1)	22 (27.8)	47 (22.4)

Table VII - Data on completion of treatment for HCV infection and status for HCV infection in the year 2020.

	CTC Group	OC Group	Total
Treatment for HCV – no (%) *	(n=104)	(n=83)	(n=187)
Yes	98 (94.2)	53 (63.9)	151 (80.7)
No	6 (5.8)	30 (36.1)	36 (19.3)
HCV infection status 2020 – no (%)	(n=131)	(n=79)	(n=210)
Sustained Viral Response	84 (64.1)	45 (57.0)	129 (61.4)
Infected	3 (2.3)	15 (19.0)	18 (8.6)
Under treatment	3 (2.3)	1 (1.3)	4 (1.9)
Re-infected	6 (4.6)	-	6 (2.9)
Not infected	35 (26.7)	18 (22.8)	53 25.2)

*The variable of treating the HCV infection only considered patients initially infected with HCV.

Table VIII - Drugs used for treatment of HCV infection, in absolute frequency and percentage.

Drugs n (%)	CTC Group (n=96)	OC Group (n=52)	Total (n=148)
Sofosbuvir/Ledipasvir	73 (76.0)	28 (53.8)	101 (68.2)
Sofosbuvir/Velpatasvir	9 (9.4)	5 (9.6)	14 (9.5)
Sofosbuvir/Ledipasvir + Ribavirine	3 (3.1)	7 (13.5)	10 (6.8)
Grazoprevir/Elbasvir	1 (1.0)	5 (9.6)	6 (4.1)
Glecaprevir/Pibrentasvir	1 (1.0)	3 (5.8)	4 (2.7)
Sofosbuvir/Daclatasvir + Ribavirine	2 (2.1)	2 (3.8)	4 (2.7)
Sofosbuvir + Ribavirine	3 (3.1)	-	3 (2.0)
Sofosbuvir/Daclatasvir	1 (1.0)	1 (1.9)	2 (1.4)
Ombitasvir + Paritaprevir + Ritonavir	-	1 (1.9)	1 (0.7)
Grazoprevir/Elbasvir + Ribavirine	1 (1.0)	-	1 (0.7)
Sofosbuvir + IFNpeg + Ribavirine	1 (1.0)	-	1 (0.7)
Ombitasvir + Paritaprevir + Ritonavir + Pasabrevir	1 (1.0)	-	1 (0.7)

Table IX - Distribution of patients with undetectable and detectable HIV RNA load by the different CD4+ lymphocyte count categories in the two study groups in 2015 and 2020.

	2015 n (%)				**2020 n (%)**			
	CTC		OC		CTC		OC	
	Undet	Det	Undet	Det	Undet	Det	Undet	Det
<200 cells/mm^3	13 (10.9)	3 (12.0)	3 (4.2)	7 (19.4)	7 (6.6)	1 (4.0)	2 (3.5)	8 (36.4)
200-499 cells/mm^3	38 (31.9)	7 (28.0)	22 (31.0)	13 (36.1)	30 (28.3)	7 (28.0)	17 (29.8)	5 (22.7)
≥500 cells/mm^3	68 (57.1)	15 (60.0)	46 (64.8)	16 (44.4)	69 (65.1)	17 (68.0)	38 (66.7)	9 (40.9)
Total	119	25	71	36	106	25	57	22
	p=0.926		p=0.020		p=0.882		p<0.001	

Undet – undetectable HIV RNA load
Det – detectable HIV RNA load

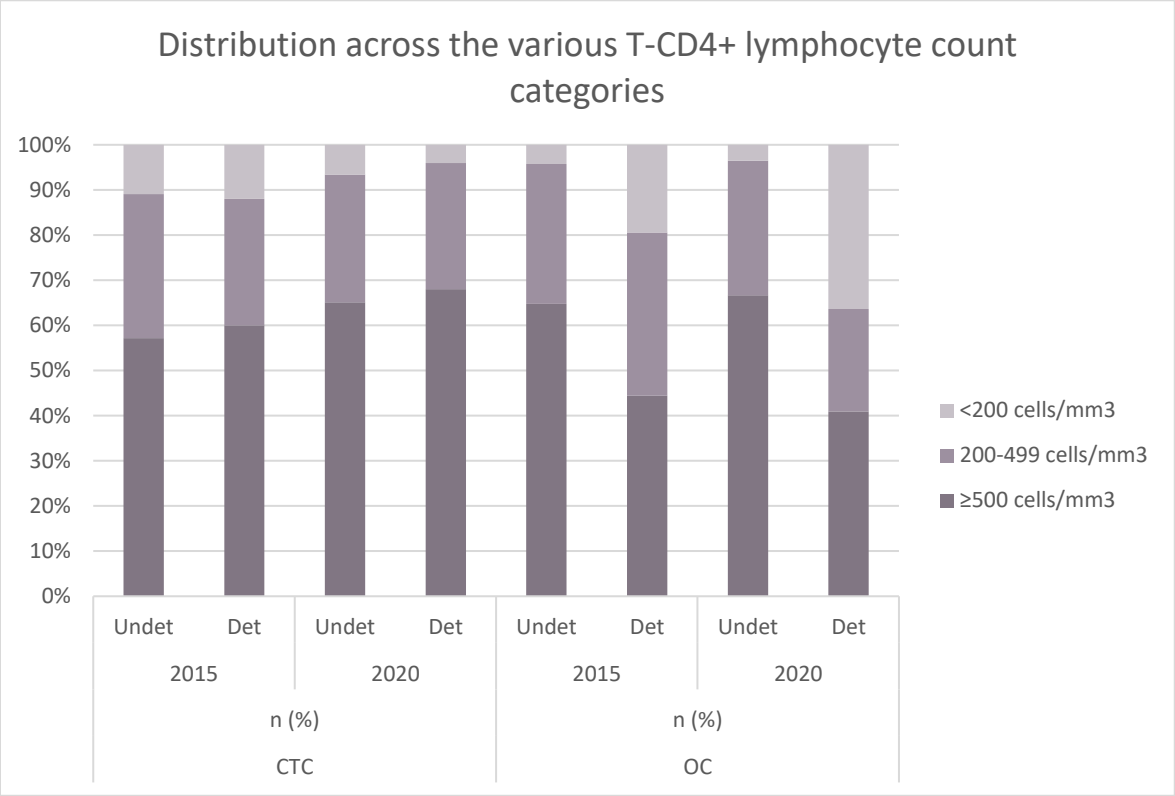

Figure 3. Bar chart with the distribution of patients with undetectable and detectable HIV RNA load by the different CD4+ lymphocyte count categories in the two study groups in 2015 and 2020.

Bibliography

1. DGS - Programa Nacional para a Infeção VIH e SIDA, INSA - Departamento de Doenças Infecciosas. *Infeção VIH e SIDA Em Portugal - 2019*. (DGS/INSA, ed.). Lisboa; 2019. https://www.sns.gov.pt/wp-content/uploads/2019/11/Relat-VIH-SIDA-2019.pdf.

2. Centers for Disease Control and Prevention. Monitoring Selected National HIV Prevention and Care Objectives by Using HIV Surveillance Data — United States and 6 Dependent Areas — 2018. *HIV Surveill Suppl Rep*. 2020;18(5).

3. Wolfe D, Carrieri MP, Shepard D. Treatment and care for injecting drug users with HIV infection: A review of barriers and ways forward. *Lancet*. 2010;376(9738):355-366. doi:10.1016/S0140-6736(10)60832-X

4. Palepu A, Tyndall MW, Joy R, et al. Antiretroviral adherence and HIV treatment outcomes among HIV/HCV co-infected injection drug users: The role of methadone maintenance therapy. *Drug Alcohol Depend*. 2006;84(2):188-194. doi:10.1016/j.drugalcdep.2006.02.003

5. Sylla L, Bruce RD, Kamarulzaman A, Altice FL. Integration and co-location of HIV/AIDS, tuberculosis and drug treatment services. *Int J Drug Policy*. 2007;18(4):306-312. doi:10.1016/j.drugpo.2007.03.001

6. Samet JH, Friedmann P, Saitz R. Benefits of linking primary medical care and substance abuse services: Patient, provider, and societal perspectives. *Arch Intern Med*. 2001;161(1):85-91. doi:10.1001/archinte.161.1.85

7. Sambamoorthi U, Warner LA, Crystal S, Walkup J. Drug abuse, methadone treatment, and health services use among injection drug users with AIDS. *Drug Alcohol Depend*. 2000;60(1):77-89. doi:10.1016/S0376-8716(00)80010-1

8. Simeone C, Shapiro B, Lum PJ. Integrated HIV care is associated with improved engagement in treatment in an urban methadone clinic. *Addict Sci Clin Pract*. 2017;12(1):1-6. doi:10.1186/s13722-017-0084-y

9. Sorensen JL, Haug NA, Larios S, et al. Directly administered antiretroviral therapy: Pilot study of a structural intervention in methadone maintenance. *J Subst Abuse Treat*. 2012;43(4):418-423. doi:10.1016/j.jsat.2012.08.014

10. Lucas GM, Gebo KA, Chaisson RE, Moore RD. Longitudinal assessment of the effects of drug and alcohol abuse on HIV-1 treatment outcomes in an urban clinic. *Aids*. 2002;16(5):767-774. doi:10.1097/00002030-200203290-00012

11. Grenfell P, Baptista Leite R, Garfein R, de Lussigny S, Platt L, Rhodes T. Tuberculosis, injecting drug use and integrated HIV-TB care: A review of the literature. *Drug Alcohol Depend*. 2013;129(3):180-209. doi:10.1016/j.drugalcdep.2012.11.013

12. Sarmento-Castro R, Barros AS, Gonzalez J, et al. Tuberculose em doentes com Sida . Tratamento completo sob observação directa. *Rev Port Pneumol*. 2001;VII(3).

13. Grenfell P, Carvalho AC, Martins A, Cosme D, Barros H, Rhodes T. Accessibility and integration of HIV, TB and harm reduction services for people who inject drugs in Portugal. *World Heal Organ Reg Off Eur*. 2012;(April). http://www.euro.who.int/__data/assets/pdf_file/0005/165119/E96531-v6-Eng.pdf.

14. Lundgren J. Initiation of Antiretroviral Therapy in Early Asymptomatic HIV Infection. *N Engl J Med*. 2015:150720091517005. http://www.nejm.org/doi/abs/10.1056/NEJMoa1506816.

15. Centers for Disease Control. Revised Surveillance Case Definition for HIV Infection. *Morb Mortal Wkly Rep*. 2014;63(3):29-31.

16. Lucas GM, Weidle PJ, Hader S, Moore RD. Directly administered antiretroviral therapy in an urban methadone maintenance clinic: A nonrandomized comparative study. *Clin Infect Dis*. 2004;38(SUPPL. 5):409-413. doi:10.1086/421405

17. Conway B, Prasad J, Reynolds R, et al. Directly observed therapy for the management of HIV-infected patients in a methadone program. *Clin Infect Dis*. 2004;38(SUPPL. 5):402-408. doi:10.1086/421404

18. Pantazis N, Papastamopoulos V, Paparizos V, et al. Long-term evolution of CD4+ cell count in patients under combined antiretroviral therapy. *Aids*. 2019;33(10):1645-1655. doi:10.1097/QAD.0000000000002248

19. Lok JJ, Bosch RJ, Benson CA, et al. Long-term increase in CD4+ T-cell counts during combination antiretroviral therapy for HIV-1 infection. *Aids*. 2010;24(12):1867-1876. doi:10.1097/QAD.0b013e32833adbcf

20. Moore RD, Keruly JC. CD4+ cell count 6 years after commencement of highly active antiretroviral therapy in persons with sustained virologic suppression. *Clin Infect Dis*. 2007;44(3):441-446. doi:10.1086/510746

21. 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(6):787-794. doi:10.1086/597093

22. 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(9):1312-1321. doi:10.1093/cid/ciu038

23. Duro R, Rocha-Pereira N, Figueiredo C, et al. Routine CD4 monitoring in HIV patients with viral suppression: Is it really necessary? A Portuguese cohort. *J Microbiol Immunol Infect*. 2018;51(5):593-597. doi:10.1016/j.jmii.2016.09.003

24. Young J, Psichogiou M, Meyer L, et al. CD4 cell count and the risk of AIDS or death in HIV-infected adults on combination antiretroviral therapy with a suppressed viral load: A longitudinal cohort study from COHERE. *PLoS Med*. 2012;9(3). doi:10.1371/journal.pmed.1001194

25. Jung AC, Paauw DS. Diagnosing HIV-related disease: Using the CD4 count as a guide. *J Gen Intern Med*. 1998;13(2):131-136. doi:10.1046/j.1525-1497.1998.00031.x

INSTITUTO DE **CIÊNCIAS BIOMÉDICAS ABEL SALAZAR**