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CLINICAL AND PROGNOSTIC SIGNIFICANCE OF IMMUNO - HEMATOLOGICAL DETERMINATIONS AND INFLAMMATORY MARKERS IN HIV POSITIVE PATIENTS

PHD THESIS SUMMARY

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CONSTANȚA

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Chapter I. HIV - Epidemiological data

Data on the epidemiology of HIV / AIDS worldwide

From the first 5 cases published on July 5, 1981 by the CDC in the "Morbidity and Mortality Weekly Report MMWR" (1, 2) to the pandemic caused by the human immunodeficiency virus, the epidemiology of HIV infection shows a unified pattern. The exponential growth of infections and the generation of secondary cases in almost all countries is based on human behavior, with man becoming the source of the spread of infection.

The HIV epidemic in Romania has, as the global pandemic, an interesting evolution: it started with the first case reported in Romania in 1985, the next 22 cases found and reported to the WHO before December 1989 and virtually exploded in the next year. The Ministry of Health counts 1168 AIDS cases, of which 93.7% (1096 cases) in the pediatric population (mostly - children under 4 years). There is a discrepancy in these three statements: although the first cases mentioned above in Romania were adults, the epidemiological explosion affected the pediatric population (27).

There were not many published data about the local situation of the circulating subtypes in Constanța. The first study that tried to specify this type is the one in 1998, published by C. Apetrei (46), in which the situation of Constanta cases also appears, however the number was relatively low: 34 adults and 8 children.

Chapter II. Pathophysiology of anemia in HIV patients

Despite numerous publications on the proper use of blood and blood products, few are particularly considering the role of transfusion in HIV management. This project aims to analyze the situations encountered in the management of HIV-infected patients and the cases in which blood transfusions or blood products may be indicated in these patients. The current state of research, which is still in progress, shows that the principles of transfusion hematology are the same for HIV-negative and HIV-positive patients (17). The overall incidence of anemia in HIV-positive patients ranges from 10% in asymptomatic patients to 92% in individuals with AIDS. Although it is rarely a fatal complication of HIV disease, anemia can significantly increase morbidity in this population. Several attempts have recently been made to assess the negative impact of anemia on HIV-positive people. Thus, it has been reported that HIV patients suffering from anemia have a significantly higher risk and a poorer prognosis (54).

II.1 Characteristics of anemia in HIV infection

It is noteworthy that antianemic therapies can accelerate the evolution of HIV disease. Primary infection or reactivation of known or unknown pathogens is still a major concern associated with blood transfusions and can lead to a poor prognosis (54). In addition, the pervasive risk of immunological reactions from allogeneic transfusions has been shown to have an immunomodulatory effect by regulating humoral immunity and regulating cellular immunity (55). Inter alia, there is evidence that blood transfusions lead to a decrease in type 1 T helper lymphocytes and an increase in type 2 T helper lymphocytes by increasing cytokine production, as well as increasing the number of CD8 cells and decreasing the number of CD4 cells (56, 57).

Treatment of anemia in HIV patients may require antiretroviral reduction and anti-opportunistic strategies. As anti-HIV therapy has become much more effective and life expectancy in recent years has increased dramatically, as far as possible, the use of this therapy should not be limited due to anemia (60-62).

II.2 Causes of anemia in HIV patients

Anemia is not a diagnosis, and its management should focus on investigating and treating major causes, regardless of HIV status. Anemia refers to a reduction in erythrocyte mass that is reflected in a low level of hematocrit and / or hemoglobin. It is a clear clinical sign that reflects a process of underlying disease that requires investigation and proper management, which is specific to the basic process. Anemia is common to a wide range of pathologies and various therapeutic options. Therefore, generic treatment (e.g., blood transfusion or iron administration) without knowing the specific cause is considered an erroneous practice (69).

Anemia can occur at any stage of HIV infection, and 63-95% of infected people will develop anemia during the disease; In addition, the incidence of anemia increases progressively. The presence of anemia is a predictable and reversible indicator of HIV mortality (54).

Chapter III. Human Immunodeficiency Virus Infection

Pathogenesis and immunopathology

In the early and middle stages of HIV infection, the virus multiplies in the lymph nodes, their viral load vastly exceeds the circulating load. As a result of the T CD8+ *cell immune response*, after 2-4 months, the level of plasma viral load decreases by 10 to 100 times, reaches low values, relatively stable for months / years, ("set point", balance point, stable), and this level is predictive of the rate of progression of the infection (111, 112). The marked turnover of CD4+T lymphocytes is maintained even years, it reaches of certain balance between destruction and production rates, however – *and this is specific* – in time, the level drops from normal values to 200-300 cells / mm3 (immunosuppression increases) (113).

III.1 CD4 / CD8 ratio

Recent studies demonstrate the early impairment in HIV infection of T cells with memory in the gut-associated lymphoid tissue (GALT). Thus, within a short period of time from infection onset, 20% of CD4+T cells in GALT are infected and, of these, 80% are destroyed by virus-mediated direct cytotoxicity or Fas-mediated apoptosis. When the maximum viremia level is reached ~ 60% of CD4+T memory cells are infected. It should be noted that most CD4+T cells reside in the GALT, and the number of these circulating cells does not reflect the magnitude of the destruction of T lymphocytes in the digestive tract. In the light of these new data, it could be assumed that the initial increase in viral load is caused by the exaggerated proliferation and infection of CD4+T cells in GALT, and the subsequent decrease of viral load up to a certain plateau value indicates the depletion of the most CD4+T cells in the body(21).

Chapter IV. ABO and Rh systems: regular and immune antigens and antibodies

About 400 different erythrocyte antigens are known to date. In the erythrocyte membrane, the number of antigenic determinants and their ability to induce the immune response varies from antigen to antigen (17).

At 5-6 days after an incompatible transfusion, in the serum of an unimmunized patient may appear alloantibodies to the antigen absent on its erythrocytes, but present on the donor's erythrocytes. In case of an incompatible transfusion, these alloantibodies may cause post-transfusion accidents. For this reason, compatibility tests should be performed on fresh serum, sampled before each transfusion (17).

IV.1 Rules of ABO and Rh Compatibility and Identity in Blood Transfusion

The immunological rules of transfusion and the immunological effects of transfusion apply according to international guidelines also to HIV patients (17).

The main immunological rules of transfusion are:

- Avoid the meeting of erythrocyte antigens of the donor and the corresponding blood donors to the recipient of the blood.
- Avoid immunization of the recipient by administering a blood product that contains additional antigens to the recipient's blood. Isogroup transfusion is the priority indication. Non-isogroup compatible transfusion is performed according to rules 1 and 2

In any case, compatible CER / ST O RH (D) may be administered without hemolysins; ST / CER selection in case of positive compatibility test (AGGLUTINATION); The decision is made by the attending physician, possibly advised by the transfusion unit coordinator.

If the transfusion is vital and there are no compatible units, the unit is chosen for which the weakest positive reaction to the compatibility test was obtained ("the least incompatible").

Chapter V. Inflammatory Markers

V.1 Fibrinogen

Fibrinogen (coagulation factor I) is a dimeric glycoprotein with a molecular weight of approximately 340kD, present in plasma and platelet α-granules. It is synthesized in the liver at a rate of 1.7-5 g / day (125). Each of the two subunits contains three polypeptide chains: Aα, Bβ and γ. The fibrinogen has a trinodular structure, the central globular domain being called the E domain, which comprises the N-terminal ends of the six polypeptide chains, and the D domains contain the C-terminal ends of the three chains. The half-life of fibrinogen is 3-5 days (122).

V.2 C-reactive protein - general information

CRP is a non-glycosylated protein in the pentameric structure that migrates electrophoretically near the gamma zone. It is an fast-acting non-specific acute phase reactant, that responds to tissue damage and inflammation, being a more sensitive and prompt indicator than ESR [74].

CRP is a proinflammatory "trigger" in itself, because it stimulates monocyte production of IL-1, IL-6 and TNF-α. Although the main source of CRP is the liver, recent data indicate that arterial tissue can produce both CRP and proteins belonging to the complement system (133). The CRP level can increase very much (100 times or even more) after severe trauma, bacterial infections, inflammation, surgery or neoplastic proliferations (134, 135).

Chapter VI. Material and method

Study design

In this study we tried to achieve the objectives through a comprehensive analysis of the available data using established statistical methods. In the descriptive analysis we tried to compare the two groups (transfused and non-transfused) depending on the performance of the known biochemical scores in each group of patients.

This paper is a retrospective study and we gathered information from 678 patients hospitalized at the Department of HIV for adults of the Infectious Diseases Clinical Hospital of Constanța, between 2015 and 2017. We analyzed inflammatory markers (C-reactive protein and fibrinogen value), CD4, CD8 count (CD4 / CD8 ratio), viral load (HIV1 RNA quantitative test) for the assessment of the clinical condition of HIV patients before and after administration of blood products, together with demographic data (sex, age, urban / rural environment), comorbidities and compatibility tests performed before the blood transfusion (enzyme test, saline test and Coombs test).

VI.1 Presentation of study methods

The medical equipment used to determine inflammatory, immune and hematological markers belong to the Medical Test Laboratory of the Infectious Diseases Hospital of Constanța. All blood samples were collected taking standard precautions at the Infectious Diseases Clinical Hospital of Constanța.

VI.1.1 For the evaluation of CRP (C-reactive protein) levels

The venous blood was sampled in a vacutainer without anticoagulant with/without a separating gel, and the test was performed on the THERMO Conelab Prime 30i chemical analyzer that uses as measuring method colorimetric and turbidimetric assay with kinetic modes and end-point, and spectral range (wave length) of 340 - 800 nm. Reference values - <0.5 mg / dL

VI.1.2 Fibrinogen samples

Samples were collected in a vacutainer with Na citrate 0.105M (sodium citrate-blood ratio = 1/9) and processed on the ThrombalyzerCompass X automatic coagulation analyzer using the photometric method. The samples were immediately brought to the immunology laboratory for processing. Adult reference values - 200-400 mg / dL.

VI.1.3 Complete blood count

For the complete blood count, we used venous blood collected on the anticoagulant: EDTA tripotassium / dipotasium / disodium (vacutainer with purple / lavender stopper - K3 EDTA), before and after the administration of blood products, the value of hemoglobin being the most important criterion. For the complete blood count with leukocyte formula was used the BC-5300 Auto Hematology Analyzer, a semi-automatic analyzer, with the principle of flow cytometry and fluorescence, using a semiconductor laser with hydrodynamic focusing, the erythrocytes being counted by the analyzer during their passing through an hole through which they are directed in a single row by a hydrodynamic focusing method.

VI.1.4 Hemoglobin value:

Method of determination - Hb is determined automatically by the photometric method after conversion to SLS-Hb with a sodium lauryl sulphate surfactant (117).

Critical values - Hb <5g / dL leads to congestive heart failure and death may occur - a concentration of Hb> 20 g / dL may lead to blockage of the capillaries due to hemoconcentration (6)

VI.1.5 T helper CD4, CD8 lymphocyte count

It was performed with the Aquios CL semi-automatic analyzer (Beckman Coulter) which uses a flow cytometry identification technique. We tried to collect the blood samples at the same time of day was due to the fact that the absolute number of lymphocyte populations is influenced by a number of biologic factors, including hormones, temperature and environment. Studies of circadian rhythm variations have shown a progressive increase in the number of CD4+ cells during the day, while CD8+ cells have only grown in the first part of the day, while their number remained unchanged during afternoon.

VI.1.6 HIV RNA viral load quantitative test

Viremia monitoring was necessary to evaluate the response to treatment and the durability of viral suppression. The goal of antiretroviral therapy, both in "naive" patients and in patients with previous exposure to originator, is to suppress HIV-RNA to undetectable levels. Viremia was determined before initiating therapy, after 2-8 weeks and then at intervals of 4-8 weeks until HIV-RNA was undetectable or decreased considerably (usually this protocol is used at first presentation or recording). At 4 weeks after the start of treatment, a decrease in viremia of 1 log10 should be obtained, and after 16-24 weeks it is necessary to reach undetectable values.

At clinics where viremia monitoring is not available, the use of only clinical criteria and possibly CD4-positive cell counts may result in a lack of identification of antiviral resistance and the continuation of only partially effective treatment. Pre-preparation of the patient was not necessary, venous blood (at least 2 mL blood) was sampled in a vacutainer containing EDTA as anticoagulant, and the plasma was separated by centrifugation. Plasma samples are stable for 1 month at -20°C. To determine the viral load, we used the Real-time PCR method (chain polymerization reaction and real-time detection of the accumulated PCR product, by measuring the emitted fluorescence (quantitative test) the samples being placed in the Conelab Prime 30i analyzer.

Reference values: HIV - 1 RNA: undetectable

Detection limit - 40 copies / mL4.

VI.1.7 Blood products used

Depending on the type of deficiency, we used the following for these patients: whole blood, red cell concentrate, erythrocyte mass, thrombocyte apheresis, freshly frozen plasma, leukocyte depleted blood. For the administration of blood products, the following compatibility tests were performed: group identification in ABO and Rh system (using the Diaclon ABO / Rh kit) by two BethVincent and Simonin methods, Saline test and Enzyme test.

To detect the presence or absence of A / B antigens (ABO1 / ABO2) on red blood cells, anti-A and anti-B antibodies of human or monoclonal origin directed against the corresponding antigens are used. The ABO group is incomplete without the serum sample, in which the patient's serum is tested with red blood cells-test A1, A2, B and O. The expression "Rh D positive" or "Rh D negative" refers to the presence or absence of Rh D antigen in red blood cell. An anti-D serum test is used to detect Rh D antigen, which may be of human or monoclonal origin.

Compatibility between recipient and donor exists only when the reactions are negative in all the following tests: Enzymatic Test, Coombs Test, Saline Test.

VI.1.8 For the identification of blood groups in the AB0 system

We used the Diaclon kit, the Saline Test and the Enzymatic Test whose results we attached in Annex 1:

The main principle to achieve the compatibility between the bag of administered product and the recipient, applied by the Blood Transfusion Unit of the Infectious Diseases Clinical Hospital of Constanța with blood products received from the Constanța County Transfusion Center, is the following:

Compatibility between recipient and donor exists when the reactions are negative in all tests: Enzymatic Test, Coombs Indirect Test, Saline Test.

VI.2 Statistical Analysis

The data were entered in electronic format using a Microsoft Excel spreadsheet, which included all the variables monitored in the study.

The data were then analyzed using data analysis applications, namely Microsoft Excel (used mainly to transform data - for example, based on hemoglobin values - by using formulas a new variable was generated, called Anemic Status). For the application of statistical tests and the creation of tables, we used the IBM SPSS Statistics application (136).

For the descriptive statistical analysis, we calculated the mean, standard deviation, median, minimum value and maximum value.

In order to compare, we used, depending on the type of data, the recommended statistical tests. Thus, for continuous variables, we used, in case the data had a normal distribution, the t test (when we compared two categories) or the ANOVA test (when there were more than two categories), accompanied by post-hoc analyzes, if the result was statistically significant.

If the distribution of values was different from a normal distribution, we used non-parametric tests (Mann Whitney U or Kruskal Wallis). To determine whether a distribution can be considered normal or not, we applied the Shapiro-Wilk's W test together with the visual analysis of the histogram.

For testing the association, in the case of qualitative variables, we used contingency tables and the Chi-square test. If the conditions for applying the Chi-square test were not met, we used the Fisher test (where the contingency table was of the 2x2 type, or the likelihood ratio (Likelihood ratio)).

To describe the correlation between the different variables and a dependent, dichotomous variable, we used logistic regression methods.

In all cases, to consider a statistically significant result we took into account a level $p \leq 0.05$.

Chapter VII. Results

VII.1 Descriptive analysis of patient groups

VII.1.1 Distribution of the analyzed cases by years

The study, conducted over a period of three years, between 2015 and 2017, includes a number of 678 cases, distributed relatively evenly over the three years of study.

VII.1.2 Distribution according to the study group

Regarding the distribution according to the study group, most of the cases analyzed are represented by the situation in which the treatment did not involve the use of blood products, these being over 90% of the situations encountered.

VII.1.3 Distribution according to the study group by years

As far as the distribution of cases in the two groups is concerned, by years (Figure 23), there are no statistically significant differences, $p = 0.961$ (Table 9). This result indicates that the proportion of cases and their number is relatively constant over time, thus indicating that historical data can be used in the design of specific health services expenses, to make projections for the next period.

VII.1.3.1 Distribution of patients according to the duration of the infection and the study group

Comparing the duration of the infection according to the study group, it is found that, in the case of transfused patients, it is significantly shorter (8.54 years and 12.98 years, respectively).

Graphically, it can be seen that in the case of transfused patients two thirds were diagnosed between 0-9 years (compared to 31.38% in the case of non-transfused patients). And in the case of non-transfused patients, about half were diagnosed more than 15 years ago.

Following the analysis of the graph and the tests for testing the normality of the data distribution, we decided to use for comparison the parametric tests, the distributions not being significantly different statistically from a normal distribution.

The result of the T test is statistically significant, $p = 0.002$. Thus, the difference observed between the two groups in terms of the duration of the infection is statistically significant, in the case of the group of transfused patients this period being statistically significantly shorter.

VII.1.4 Distribution of patients according to length of hospitalization

Regarding the length of hospitalization, we found that in the case of transfused patients group the average length of hospitalization was over 14 days, while in the case of non-transfused patients group, the average length of hospitalization was 9.24 days. The result of the descriptive statistical analysis is available in Table 25.

The graphical analysis of the distribution according to the number of hospitalization days highlights the fact that, in the case of transfused patients group, there is a higher percentage of cases with 10-19 days of hospitalization. It is also observed the existence of extreme cases in the case of non-transfused patients, with hospitalizations lasting up to 59 days (which led to a significant increase in the average length of hospitalization for this group, thus reducing the difference).

The distribution of data is statistically significantly different from a normal distribution, therefore non-parametric tests were used to determine the statistical significance.

The mean rank is higher in the case of transfused patients, being 426.52 compared to 327.58 in the case of non-transfused patients.

The result is statistically significant, $z = -2.896$, $p = 0.004$.

VII.2 Study of inflammatory factors

VII.2.1 ESR

VII.2.1.1 ESR upon admission

In the case of erythrocyte sedimentation rate (ESR), at the time of hospitalization, we found that there are significant differences between the two groups. Thus, in the case of transfused patients, the mean value was 68.84 mm / h (SD 34.87), while in the case of patients who did not receive blood products, the mean value was 38.76 mm / h (SD 31.97).

As can be seen, the way the values are distributed is different between the two groups differs / In the case of transfused patients group, the distribution seems to be Gaussian, with high percentages of cases around 60-70 mm / h, while in the group of non-transfused patients, most patients have low ESR values, thus indicating a less obvious inflammatory syndrome. In order to determine the statistical significance of the difference, following the analysis of the normality of the data distribution, we used the Mann-Whitney U test. The result is statistically significant ($p < 0.001$).

VII.2.1.2 Evolution of ESR values

From the point of view of the evolution of ESR values during hospitalization, we found that they changed, on average in a negative direction (decrease in values), with an average decrease of 10.69 mm / h (SD = 36.79) in the case of transfused patients group, respectively 3.98 mm / h (SD 17.59) in the case of the group with non-transfused patients.

From the point of view of the distribution of values, we noticed that the transfused group has a greater variability, with situations of higher decreases (starting at the same time from higher ESR values upon admission), but also situations in which ESR has grown significantly. In the case of non-transfused patients group, the changes less significant, especially in a positive direction, having at the same time decreases comparable to those observed in the transfused patients group.

For comparison, following the analysis of how the values are distributed, and based on the result of the Shapiro-Wilk's W test (Table 41), we decided to use parametric tests to make the comparison.

The Levene test is statistically significant ($p < 0.001$), therefore, we used for comparison the T test for inhomogeneous variances. The result is statistically insignificant ($p = 0.231$).

The study shows how ESR values have evolved according to their initial values. We noticed that, in the case of transfused patients, the regression slope is more accentuated (patients with higher ESR values had higher changes in the negative direction), these changes being smaller in the case of non-transfused patients.

VII.2.2 C-reactive protein

VII.2.2.1 C-reactive protein upon admission

Analyzing the results for C-reactive protein (CRP), obtained at hospitalization, we found that, for the transfused patients group, the mean value of CRP was significantly higher (61.03 vs. 41.62 mg / L), being much increased in both situations. Another aspect noticed is the median value, this being 54 mg / dL in the case of transfused patients group, and 18 mg / L in the case of non-transfused patients group.

The graph shows clearly how the values are distributed. In the case of the non-transfused group, the majority of values (56% are less than 25 mg / L, and almost 90% of the values less than 100 mg / L), while in the case of measurements performed in patients in the transfused group, a more uniform distribution of values is seen, only 28.6% of them being for values lower than 25 mg / L.

In both groups, the distribution of values is different from a normal distribution (the Shapiro-Wilk's W test is statistically significant and the histogram results are clarifying).

Thus, we found that the mean rank for the transfused patients group is significantly higher (416.04) compared to that for the non-transfused patients group (239.46), the difference being statistically significant (z = -2.59, p = 0, 01).

VII.2.2.2 C-reactive protein evolution

The evolution of CRP indicates that the values decreased in both situations, for the group with non-transfused patients the mean change was -19.29 mg / L (SD 35.94 mg / L), while for the transfused patients group, the mean change of was -25.20 mg / L (SD 31.65 mg / L). Thus, a greater decrease in CRP values can be seen in the case of transfused patients.

As far as the distribution of CRP change values is concerned, a higher percentage of patients with changes in the range -50 - -25 mg / L can be observed in the case of transfused patients.

After analyzing the data provided by the Shapiro-Wilk's W test, together with the analysis of the graph of value distribution, we concluded that they differ significantly from a normal distribution, therefore we used a non-parametric test for comparison.

In the case of non-transfused patients group, the mean rank for CRP evolution values is high, the result of the Mann Whitney U test being statistically significant (p = 0.022). Thus, it can be concluded that, in the case of transfused patients group, the decrease in CRP values was significantly higher compared to the non-transfused patients group.

The correlation between the initial values of CRP and their evolution is highlighted. A more accentuated decrease slope is observed in the case of the non-transfused group. The fact that there were smaller changes (in the sense of decreasing CRP values) is justified by the fact that there were increases, especially in the cases with relatively low CRP values.

VII.2.3 Fibrinogen

For fibrinogen, similar mean values were identified upon admission, these being 323.92 mg / dl (SD 137.52 mg / dl) for the transfused patients group, respectively 331.69 mg / dl (SD 140.72 mg / dl). dl) for the transfused patients group.

The analysis of data regarding the normality of their distribution indicates that it differs significantly statistically from a normal distribution (Shapiro-Wilk's W test, p is statistically significant for both groups), and together with the analysis of histograms (where it is observed that the distributions are asymmetric), the subsequent analysis decision was to use the Mann Whitney U non-parametric test.

Regarding the mean rank, it is observed that in the case of transfused patients group it is higher (348.37 and 333.2, respectively).

The result of the Mann Whitney U test is statistically insignificant (z = -0.453, p = 0.65), thus indicating that there is no statistically significant difference in fibrinogen values at the time of admission between the two study groups.

VII.3 Blood count

VII.3.1 Hemoglobin

At the time of admission, the group of transfused patients had significantly lower mean hemoglobin values, with an average of 10.63 g / dl (SD 2.11 g / dl), compared to the group of non-transfused patients, for whom the mean hemoglobin value of was 12.9 g / dl (SD 2.21 g / dl).

From the point of view of the way the cases are distributed, it is observed that in the case of transfused patients, the values are generally lower, but there are about a quarter of the cases that have normal hemoglobin values at the time of admission.

In the case of non-transfused patients, approximately 30% have values below the normal limit, but less than 5% have hemoglobin values lower than 8 g / dl.

Visual inspection of histograms indicates distributions similar to a Gaussian distribution, and the Shapiro-Wilk's W test confirms that the distribution in the group of transfused patients does not differ significantly statistically from a normal distribution, therefore, we decided to use the t test for comparison.

The T test is statistically significant ($p < 0.001$), indicating that the observed mean difference of 2.28 g / dl between the two groups is statistically significant.

VII.3.1.1 Evolution of hemoglobin values

Following the period of hospitalization and medical interventions, it is found that, in the case of transfused patients group, the mean value of hemoglobin change is -0.1 g / dl, while in the case of non-transfused patients there was an average change of -1.5 g / dl.

Analyzing the distribution graphs, it can be seen that, in the case of transfused patients, 50% of them showed changes in hemoglobin in the range of (-1, +1 g / dl). In the case of non-transfused patients, there was a more significant negative change, with a higher percentage of patients for whom the negative change in hemoglobin value was relatively high.

The Shapiro-Wilk's W test was statistically significant for both groups, which led, together with the visual analysis of histograms, to the decision to use non-parametric tests to determine the statistical significance of the changes.

The mean rank is higher in the group of transfused patients, thus demonstrating that the change in hemoglobin in the sense of increasing it was more significant for patients in the group of transfused patients.

The result obtained is statistically significant, ($z = -3.844$, $p < 0.001$), indicating that the observed changes and the difference between the two groups are statistically significant.

In the case of patients in the non-transfused group, there are significant increases in those with low baseline values, and significant decreases in those with high baseline values (the regression line has a steeper slope), compared to the changes observed in transfused patients,

where the regression line has a direction closer to the horizontal, indicating smaller changes in hemoglobin in their case, with significant increases among those with moderate anemia and slight decreases in those with higher Hb values.

VII.3.1.2 Distribution of patients according to the presence of anemia

At the time of hospitalization, out of the total number of cases analyzed, 27.1% had some form of anemia.

VII.3.1.3 Distribution of patients according to the presence of anemia and transfusion

Of the total number of patients that upon admission had hemoglobin values indicating the presence of anemia, approximately 17% of them received transfusions with blood products, while among the patients who had normal hemoglobin values upon admission, 2.8% received blood transfusions.

VII.4 Analysis of infection indicators

VII.4.1 Viral RNA

Viral RNA showed significantly higher mean values in patients in the non-transfused group, with mean values almost twice as high (2977928.05 and 1640170.24, respectively).

In most cases, the values were less than 5,000,000 copies / ml, the maximum value for patients in the non-transfused group being almost 7 million copies / ml, while in the case of patients in the transfused group two extreme values were recorded, one of almost 20 million copies / ml, and one of almost 30 million copies / ml (represented as extreme values in the framework).

It is also observed, graphically, that, once the extreme values (marked by black stars in the graph) are removed, the distribution of values in the case of the transfused patients group indicates significantly lower values for this group.

From the point of view of the normality of data distribution, taking into account the result of the Shapiro-Wilk's W test, which indicates that the distributions of viral RNA values for the two groups differ significantly statistically ($p < 0.001$ in both cases) from a normal distribution, and taking into account of the significant number of cases with outlier values observed in one of the groups, we decided to use non-parametric tests for comparison.

By grouping the data in ascending order, the rank for each case is obtained. The mean rank for the cases of patients in the non-transfused group indicates an mean rank of 349.43, significantly higher compared to the mean rank for patients in the transfused patients group, the latter being 134.80.

VII.4.1.1 Evolution of Viral RNA values

The evolution of viral RNA values shows an average change in addition by 412086.53 copies / ml in the case of patients in the transfused patients group, respectively a decrease by 282242.32 copies / ml in the case of patients in the transfused patients group. Analyzing the median values of the changes, it is found that the difference is smaller, the median for the non-transfused group is 123459 copies / ml, while for the transfused group, the median is very close, 167920 copies / ml.

In the graph, it can also be seen the extremely close way in which the values of the viral RNA evolution are presented. Thus, if the extreme values are ignored (especially the 2 values that indicate changes in the sense of decreasing the high viral RNA values), it will be found that the differences in evolution are very small.

To determine whether these differences are statistically significant, we applied, following the decision to use non-parametric tests, the Mann Whitney U test:

Regarding the mean rank, it is found that the mean values are close, of 331.8 for the group with non-transfused patients, respectively 356.57 for the transfused patients group.

These differences are statistically insignificant, $p = 0.432$.

VII.4.2 CD4

The CD4 value upon admission indicates relatively low mean values, these being 304.62 for the group with non-transfused patients, respectively 235.5 for the transfused patients group. It is thus observed that, on average, patients in the transfused group have lower mean values of CD4 cell count.

From the point of view of the way they are distributed, it can be seen that in the case of the non-transfused patients group, there is a tendency for a higher percentage of patients to have higher CD4 values, thus the percentage of those with CD4 values higher than 200 cells / mm3 is 39.5% in the case of transfused patients, and 56.6% in the case of non-transfused patients.

We found that the distribution of cases differs significantly from a normal distribution (most cases have low values of CD4, with long queues to higher values, the distributions being asymmetric, with positive asymmetry. As a result of this aspect of the histogram and results highlighted in Table 95, we used the Mann-Whitney U test to determine the statistical significance of the differences.

The mean rank is higher for patients in the non-transfused group, which is 339.3 in their case, compared to 272.32 in the case of transfused.

The observed differences are statistically significant, $p = 0.038$. This result indicates that, in the case of patients in the transfused group, CD4 values tend to be lower compared to the measured values in non-transfused patients.

VII.4.2.1 Evolution of CD4 + values

From the point of view of the evolution of CD4 values, we found that the mean values increased significantly more in the case of non-transfused patients 338.72 and 206 cells / mm3, respectively.

The graph shows a homogeneous distribution of the way in which the values evolved for the patients from the non-transfused group, the degree of dispersion being higher in their case - SD 807.32 and 678.8 cells / mm3, respectively. In the case of transfused patients, about a quarter of them showed increases in the range 0 - 200 cells/mm3, representing the largest group. Also in the case of transfused patients, decreases were observed at 39.4%, while in the case of non-transfused patients, the percentage of patients with decreased values is only 11.2%.

The distributions in the two cases differ significantly from a normal distributions, both in terms of the Shapiro-Wilk's W test result, and the histograms for the two groups, in the case of transfused patients it is asymmetric, and in the case of non-transfused patients, platykurtic.

The mean rank was significantly higher in non-transfused patients 347.18, compared to patients in the transfused group, where the mean rank was 141.32. These values indicate a significantly lower increase in CD4 + values in the group of transfused patients. The result is statistically significant ($z = -6.368$, $p < 0.001$).

VII.4.3 CD8

Descriptive analysis of CD8 cell counts indicates approximately equal mean values for the two groups (Table 102).

Histogram analysis representing the distribution of CD8 values for the two groups provides an image of two similar distributions, with the highest percentages of cases in the range of 400-600 cells / mm3.

The distributions thus differ statistically significantly from the normal distribution curve (statistically significant Shapiro-Wilk's W test, distributions with positive asymmetry), therefore, we used the Mann-Whitney U test for comparison.

From the point of view of the mean rank, the differences are very small between the two groups, the mean rank being 334.69 for the group with non-transfused patients, respectively 348.91 for the transfused patients group.

The result of the Mann-Whitney U test (Table 105) is statistically insignificant ($z = -0.44$, $p = 0.66$), indicating that there was no statistically significant difference in the number of CD8 cells.

VII.4.3.1 Evolution of CD8 values

Regarding the evolution of the number of CD8 cells, we found that in the case of non-transfused patients, the values increased more than four times (on average by 1037.84 cells / mm3), compared to transfused patients, where the average increase in the number of CD8 cells was 239.65 cells / mm3.

The graph shows significant differences in the evolution of CD8 cells number. In the case of transfused patients, it is observed that a significant percentage of patients (over one third) showed decreases in the number of CD8 cells, and if there were increases, they were mostly in the range 0 - 750 cells/mm3.

For the statistical analysis, we considered that the distribution of values for the evolution of the number of CD8 cells does not follow the pattern of a normal distribution (Shapiro-Wilk's W test statistically significant, asymmetric distributions).

Regarding the mean rank, it reflects the differences in the mean values of CD8 evolution and thus has values more than twice as high in non-transfused patients.

Thus, the result of the Mann Whitney U Test is statistically significant high, $z = -6.284$, $p < 0.001$, indicating an evolution that differs significantly statistically between the two groups, with a higher increase in non-transfused patients.

VII.4.4 CD4 / CD8 ratio

The mean CD4 / CD8 ratio is subunitary, being 0.504 for patients in the non-transfused group and lower, 0.396 for patients in the transfused group.

Superunitary values are found in a very small percentage of patients, 5.3% for the transfused group and 8.3% for patients in the non-transfused group.

The distribution of cases also provides an image of severely affected immune status in transfused patients, approximately 45% with a ratio of less than 0.25, compared with approximately 27% in non-transfused patients.

The data do not follow a normal distribution, which requires the use of non-parametric tests to determine the statistical significance of the differences observed between the two groups.

The mean rank is significantly higher in the group of non-transfused patients, which is 339.68 compared to 265.93, which indicates the existence of a higher number of cases with a higher value of CD4 / CD8 ratio.

The difference is statistically significant, $z = -2.282$, $p = 0.023$. Thus, it can be concluded that patients who received blood products during hospitalization had a statistically significantly lower CD4 / CD8 ratio compared to patients who did not receive blood products.

VII.4.4.1 Evolution of the CD4 / CD8 ratio

The evolution of the CD4 / CD8 ratio indicates a slight increase in the ratio in the case of non-transfused patients (increase of 0.052) and a slight decrease in the ratio for transfused patients (-0.0166).

Analyzing the distribution graph of how the value of the CD4 / CD8 ratio varied, it is observed that the two histograms are very similar, in both situations most values being within the range 0 - 0.25, and with some extreme values of decreasing ratio (values extremes around -1.5.

No superunitary increases were observed in any of the groups.

Due to the way the values are distributed, and considering the result of the Shapiro-Wilk's W test, we considered that their distribution differs from a normal distribution.

The mean rank for the evolution of CD4 / CD8 shows close values, of 336.93 and 302.11, respectively, indicating small differences between the two groups. This is confirmed by the fact that the result of the Mann-Whitney U test is statistically insignificant ($z = -1.065$, $p = 0.287$).

Thus, regarding the evolution of the values of the CD4 / CD8 ratio, we found that between the two study groups, respectively the non-transfused patients group and the transfused patients group, there are no statistically significant differences.

VII.5 Viral RNA Correlation - Inflammation Factors

VII.5.1 Viral RNA - Fibrinogen Correlation

At the time of hospitalization, we found that there is no statistically significant correlation between viral RNA and fibrinogen values ($p = 0.801$), the correlation level being -0.01, which indicates practically an almost total independence of the two indicators analyzed.

VII.5.2 Viral RNA – C-reactive protein correlation

The analysis of the correlation between viral RNA and C-reactive protein indicates that between the two analyzed variables, for the analyzed study group, there is no direct linear correlation. The correlation level is -0.003 (an extremely low value, which indicates a high independence of values), and $p = 0.937$, indicating the lack of statistical significance.

The graph shows that, as it emerged from the correlation analysis, no correlation is identified between the two variables, they evolve independently of each other.

VII.5.3 Viral RNA - ESR correlation

Between the values of Viral RNA and ESR we found that there is a negative linear correlation, weak (r = -0.128), statistically significant (p = 0.001).

Thus, it can be seen in the graph (Figure 65) that ESR values tend to have a negative evolution (decrease), whereas viremia values increase.

VII.6 Transfusions

VII.6.1 Number of transfusions

The group of transfused patients was represented by cases. Table 121 shows the data on the number of transfusions performed. It is observed that the vast majority (over 85%) received a single transfusion of blood or blood products, but there were also patients that received 2, 3 or 4 transfusions during hospitalization.

VII.6.2 Distribution according to transfused products

In most cases, over 40% received resuspended red cell concentrate without other products. Table 122 lists all transfusion situations and types of preparation used (alone or in combination).

The most commonly used is resuspended red cell concentrate, this type of preparation being used alone or together with other types of preparations in almost half of the cases (46.6%). As a frequency of use, we used standard platelet concentrate (SPC) (20.7%) and freshly frozen plasma (FFP) (17.8%).

VII.6.3 Survival analysis

In the analysis of survival, we took into account the deaths recorded during the study period. We found that, in the case of transfused patients, the average duration of monitoring was significantly shorter, the main reason for this being the occurrence of the death event among them.

There is a significantly lower probability of survival if the HIV-infected patient received blood products.

VII.6.4 Hemoglobin influence - death analysis

Comparison of hemoglobin values upon admission between patients who survived the study and those who died indicates that lower mean hemoglobin values (10.61 g / dl) are associated with a higher risk of death.

The graph shows how they are distributed, having higher values in the case of those who did not die, but also a wider distribution of values, which is also objectified by the standard deviation (2.21 for the group of survivors, respectively 1.82 for those who died).

Also, for the group of survivors, there is a significant number of outliers, which indicates that the distribution of values is significantly different from a normal distribution. The Shapiro-Wilk's W test confirms this aspect, the result being statistically significant for both groups of patients.

The non-parametric Mann-Whitney U test is statistically significant ($p < 0.001$) (Table 130), the mean rank for hemoglobin values being significantly lower in deceased patients (which represents a higher number of lower hemoglobin values).

VII.6.5 Hemoglobin values according to death and transfusion

Next, we introduced in the analysis the transfused status of patients. The role is to gain a better understanding of how hemoglobin values are associated with the risk of death. Thus, four categories of patients were created, by grouping according to Death (No / Yes) and Transfusion (No / Yes).

We observed that the highest mean hemoglobin values that are in the normal range, were observed in non-transfused patients who did not die. For the other three categories, the mean hemoglobin values were relatively equal, ranging from 10,469 to 10,658 g / dl.

Graph shows how hemoglobin values are distributed to the four groups of patients. Approximately identical distributions are observed in the case of the deceased. In the case of the patients who did not die, but with transfusions we observed a wide distribution of hemoglobin values, and in the case of the non-transfused, we observed a majority distribution of hemoglobin values in the normal range, there are also some "abnormal" values (patients with abnormally low values hemoglobin levels).

To determine the statistical significance of the observed values, we ran the ANOVA test, followed by a post hoc analysis. The result of the ANOVA test is statistically significant, with $p < 0.001$. This result indicates that there are statistically significant differences in hemoglobin between the four groups.

Following the post hoc analysis, we found that statistically significant differences are seen between the group of patients who did not die and were not transfused and all other groups, with different values of statistical significance.

No statistically significant differences in hemoglobin values were observed between deceased and non-transfused patients, respectively deceased patients who were transfused.

In patients with anemic status, we looked at whether there was an association between the use of blood products and survival. The result indicated that there is a statistically significant association, with patients with anemia receiving transfusions of blood products having a statistically significantly higher risk of death (OR 139.82 (95% CI 38.28 - 510.66).

A possible explanation for this result is that, in the case of those deceased, hemoglobin values tended to be lower. It can be seen that patients with anemia, who survived without the need for blood transfusions, had the highest levels of hemoglobin. It can also be seen that in the case of those who did not survive and received blood transfusions, the hemoglobin values were higher compared to those who were transfused but survived, or those who died without receiving blood transfusions.

The observed differences are statistically significant ($p = 0.001$)

Between groups, statistically significant differences are observed between anemic patients who did not die and were not transfused and those who did not die give were transfused, and also between patients who did not die and were transfused respectively those who died and were transfused.

These results indicate that, in addition to the value of hemoglobin, there are other factors that influence the risk of death of patients.

VII.6.6 Comparative analysis of viral markers and death

VII.6.6.1 Viral RNA Analysis

Viral RNA values indicate that patients in the group who survived had higher viremia values. In the case of the deceased, most cases had viremias below 1,000,000 copies / ml (three quarters of cases), but also had extreme values, of over 20,000,000 copies / ml (two cases).

In the case of patients who did not die during the study, their viremias were relatively evenly distributed, with two peaks for the range 0 - 1,000,000 (a quarter of cases) and the range 3 million - 4 million with an approximately equal percentage. The maximum value in the case of those who survived was almost 7 million copies / ml.

Data are not normally distributed, the Shapiro-Wilk's W test is statistically significant ($p < 0.001$) for both groups, and histogram analysis also indicates that the way the data is distributed differs from a normal distribution.

The mean score for viral RNA is significantly higher in patients who did not die, arguing that patients who died generally had lower viremia values.

VII.6.6.2 CD4 analysis

The mean CD4 values were 304.29 (SD 234.61) for the surviving patients and 242.84 (SD 214.71) for the deceased patients, respectively. There is a small difference between the two groups, in that the deceased patients had lower CD4 values.

The distribution of values provides a clear picture of how cases are distributed. It is observed that, in the case of deceased patients, 60% had CD4 values lower than 200, while for the patients who survived, less than 45% had similar values.

The two distributions show a deviation to the right, a fact confirmed by the Shapiro-Wilk's W test, being significantly different from a normal distribution.

The mean rank for CD4 in patients who died is lower, being 282.79, compared to the mean rank for patients who survived, namely 340.88.

The result is statistically insignificant, according to the criterion chosen in this research, the p value indicating a limit value of 0.053. This value does not reach the threshold defined for statistical significance of $p < 0.05$.

VII.6.6.3 CD8 analysis

The mean CD8 lymphocyte count was 657.17 (SD 380.59) for the group of patients who survived the study period, and 591.09 (SD 312.00) for the group of deceased patients.

It is noted that these differences are relatively small. Comparing the median values of 503 and 491, respectively, it can be seen that they are even closer.

The graph shows that the distributions are similar, with a peak frequency between 400 and 600. Also, for both groups it is observed that the values have a right asymmetry, with extreme values reaching over 1500 cells / mm3 for the group of deceased patients, and 2000 for the group of surviving patients, respectively.

The distribution thus differs significantly from a normal distribution, a fact confirmed by the result of the Shapiro-Wilk's W test (statistically significant).

The analysis of the mean rank indicates that the differences are small, 338.43 and 317.07, respectively. Thus, we found that there is no statistically significant difference ($z = -0.712$, $p = 0.476$) of CD8 values between the two groups studied.

Chapter VIII. Discussions

Evaluation of the number of CD4 and CD8 lymphocytes, correlated with hemoglobin values in the two study groups, showed that, although at the time of hospitalization the differences observed were relatively small (in the case of CD4) or statistically insignificant (in the case of CD8), following the administration of blood products, both the evolution of CD4 values and the evolution of CD8 values showed statistically significantly lower values in the case of transfused patients. In the case of CD4, following transfusions of blood products, approximately 40% of patients experienced decreases (compared to the time of hospitalization), while for CD8, approximately 35% showed decreases. In the case of patients who did not require blood transfusions, the percentage of those who had higher values of CD4 and CD8, respectively, is statistically significantly higher. This is confirmed by the fact that blood transfusions lead to a decrease in type 1 T helper lymphocytes and an increase in type 2 T helper cells, by increasing cytokine production, as well as increasing the number of CD8 cells and decreasing the number of CD4 cells (56).

In our study the correlation between viral load and administration of blood products was not demonstrated, although in some international reports it has been shown that HIV replication can be stimulated by heterologous blood components. Thus, no statistically significant difference was identified in terms of the evolution of viremia values, depending on whether or not the administration of blood products.

More than a quarter (27.1%) of HIV-infected adult patients who required acute medical hospitalization at the Infectious Diseases Hospital were anemic, with a significant number suffering from severe anemia or life-threatening anemia. Nearly one-fifth of patients with anemia received at least one blood transfusion.

Although one study found a relatively small increase in viral load in HIV-infected transfused patients, the clinical significance of the small increase in viral load was not determined [20]. In previous studies, it was believed that leukocytes in the blood products can (1) directly activate HIV cell reservoirs, (2) which leads to increased proviral transcription, (3) can cause immunosuppression, and (4) may be associated with higher mortality. Between the groups with transfused and non-transfused patients, although at the time of hospitalization there was a statistically significant difference, the patients in the transfused group showing statistically significantly higher values of viremia, following the treatments performed no evolution was noticed that differs statistically significantly between the two groups. Although, the literature describes situations in which there was a small increase in viral load in HIV-infected transfused patients, the clinical significance of growth and viral load has not been yet determined (58). In some previous studies, it is believed that blood products can activate HIV cell reservoirs, which could lead to increased proviral transcription, cause immunosuppression, and may be associated with higher mortality. We and other studies suspect the correlation between receiving a blood transfusion and the increased risk of mortality reported in previous studies more or less reflects an inherent higher risk of mortality among patients with advanced

stage of HIV who are likely to be transfused compared to those who are not. In this way, receiving a blood transfusion serves as a marker of the general risk of disease severity and mortality. Therefore, low survival rate may simply be a confusion by indication.

The analysis of the data on the risk of death according to the stage of the disease and transfusion status showed that there is no statistically significant correlation. Following the analysis of data from the group of patients studied, we found that there is no statistically significant correlation between the stage of the disease (clinical or CDC) and the risk of death (regardless of the presence or absence of blood transfusion).

The analysis of secondary diagnoses of hospitalized patients revealed that among the most common secondary diagnoses are toxic status, oropharyngeal candidiasis, and with a proportion of over 11% pulmonary TB (6.6%) and sequelae pulmonary TB (5%). In addition, the patients in our study often had advanced stage of HIV disease, opportunistic infection, or major organ dysfunction. Thus, malignancies, nutritional deficiencies and drug toxicities could have further contributed to the high prevalence and severity of anemia.

The evaluation of the correlation between the level of viremia and the level of inflammatory biomarkers showed that there is no linear correlation between viremia and Fibrinogen and C-reactive protein, however, this being identified in the case of ESR. However, even in the case of the viremia - ESR correlation, the correlation is low, in the sense of decreasing ESR values as viremia values increase. A recent study from the ATHENA cohort shows that low levels (HIV RNA 50-400 copies / ml) and high levels of viremia (HIV RNA 400 copies / ml) were associated with a risk of death 1.6-fold and 1.5-fold higher (respectively) compared to undetectable viremia, but the correlations did not reach statistical significance, and the study did not adjust for inflammatory and coagulation markers (137). In the present study, the results indicate that there is no statistically significant correlation between viremia values and patients' risk of death.

Our discovery that viral load is not correlated with CRP and fibrinogen is somewhat unexpected. Data from studies of specific populations of HIV-infected patients suggest a possible correlation based on other indices of inflammation (138, 139).

After analyzing the data from this study, regarding the risk of death, we found that the main risk factor identified is the presence of anemia. Thus, the risk of death of HIV-infected patients increases with decreasing hemoglobin levels. Regarding the correlation between transfusion and death, this is most likely a confusing factor, the main cause being the low level of hemoglobin in transfused patients.

In the present study, many of the transfused patients died shortly after receiving a transfusion, but these patients were terminally ill, with advanced stage of HIV disease and multiple comorbidities. We carefully analyzed the co-variables that could confuse the correlation between blood transfusions and mortality, and we adjusted accordingly. The risk of death for transfused patients remained significantly high, even if viremia, CD4 and CD8 values, hemoglobin and patients' age criteria were introduced in the model analyzed. A possible explanation for this is the fact that patients presented to the hospital in a severely affected

state of health, which made medical interventions, including those consisting of blood transfusions, no longer provide the expected benefit.

Even after the test was performed, there was no evidence to suggest that there was an independent correlation between blood transfusion and higher mortality. Previous studies have shown that the correlation between transfusion and low survival rate has persisted, although adjustments for potential mismatches may also have had a residual confusion that is present to some extent in all observational studies. However, there are studies that have argued that the risk of early mortality has been increased in HIV-infected patients who needed transfusions (140, 141), and several hypotheses have been issue about this outcome.

Lack of guidance to provide accurate information on treatment decision through blood transfusions in HIV-infected patients. Also, the fact that, following the transfusions of blood products, there is no increase in the survival rate of these patients, the use of these methods remains, for the time being, a solution that does not have a real scientific basis.

Chapter IX. Conclusions

1. In the three years included in the database, we found that patients do not fully receive the benefits of transfusion due to the lack of a personalized transfusion procedure for HIV patients. However, these patients need a guide that covers all their needs that are different from other pathologies.
2. An impediment to the correct and timely administration of blood transfusion in these patients is the lack of a modern method of immunocompatibility, these patients receive blood products with delay due to the impossibility of a correct determination of Rh group in these immunosuppressed patients. In this study, patients with low hemoglobin values, despite receiving a transfusion, had a poor prognosis due to delayed administration.
3. Most of the patients that died had low values of CD4 (<200), but also a considerably lower viremia (due to the way HIV replicated), so the study shows that the risk of death of the patient increases with the decrease in hemoglobin, but the prognosis is not statistically significantly influenced by CD4, CD8 and viremia values.
4. The risk of death in patients with low hemoglobin levels is significantly increased, regardless of immunological status.
5. The statistical analysis shows that patients who received blood products are diagnosed more recently and had a poorer prognosis, although the duration of infection was shorter, which means that these non-compliant patients underwent the transfusion protocol with delay.
6. Patients with multiple comorbidities also have low hemoglobin values but delayed administration of blood products makes anemia the leading cause of death.
7. Of all the inflammatory markers evaluated, the highest statistical significance was the erythrocyte sedimentation rate, showing that patients who did not require blood transfusion had a more discrete inflammatory syndrome, fibrinogen and C-reactive protein did not have similar statistical significance.
8. In the present study, it is not clear if the patients had adequate support for transfusion, because some patients with anemia were not transfused and many of the patients who were transfused received only 1-2 units of resuspended red cell concentrate. This can reflect several factors, including the fact that patients who show up late for medical care die before the transfusion, and also the fact that the doctors often prescribe blood transfusions based on the patient's clinical examination and not just the hemoglobin level. There was no standardized institutional protocol for blood transfusions; thus, some of these differences are partly influenced by the variability of transfusion practices between physicians, departments, and hospitals. In addition, local physicians' perceptions of the lack of supplies of blood products and their high cost could have led to the delayed use of some blood products.
9. Increased availability of blood products can lead to lower mortality and healthcare costs. The prescribing of blood transfusions should be based on the best practice guidelines

where available and further studies are needed to assess the impact of ART on the use of blood products (especially large ART cohort studies), as well as additional strategies for reducing the blood transfusion needs in both HIV-infected and non-infected patients.

10. We also consider it necessary, although in this study it was not possible (due to high costs), to use groups with the same criteria as in our study and the administration of human factors of erythrocyte series stimulation or granulocyte-forming colonies (Epoetin alfa, Filgrastim, Pelgras), drugs that (according to Anemia in HIV Infection - Clinical impact and Evidence - based Management strategies, Paul. A Volderbing Alexandra M. Levine etc) have led to a decrease in the number of transfusions, improved quality of life and led to a better prognosis.
11. We believe that this database can be a starting point for a subchapter annexed to the National Blood Transfusion Guide.
12. These patients need a multidisciplinary team and closer collaboration between the infectious disease specialist, hematologist and laboratory physician, so that the time until receipt of the blood product is considerably reduced. This multidisciplinary team can develop, based on this study, an internal procedure for administering blood products to HIV patients.

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