**CAROL DAVILA UNIVERSITY OF MEDICINE AND PHARMACY,
BUCHAREST
DOCTORAL SCHOOL
MEDICINE**

THE SUMMARY OF THE DOCTORAL THESIS

PhD supervisor:
PROF. UNIV. DR. ARAMĂ VICTORIA

PhD student:
COBASCHI MIHAELA

2024

**CAROL DAVILA UNIVERSITY OF MEDICINE AND PHARMACY,
BUCHAREST
DOCTORAL SCHOOL
MEDICINE**

EVALUATION OF THE CHARACTERISTICS OF OCULAR MANIFESTATIONS IN HIV/AIDS PATIENTS IN ROMANIA

THE SUMMARY OF THE DOCTORAL THESIS

PhD supervisor:
PROF. UNIV. DR. ARAMĂ VICTORIA

PhD student:
COBASCHI MIHAELA

2024

TABLE OF CONTENTS

LISTA DE LUCRĂRI PUBLICATE ÎN REVISTE DE SPECIALITATE

Articole avizate confrom contractului încheiat cu școala doctorală:

1. **Cobaschi M,** Loghin II, Dorobăț VD, et al. Ophthalmological Manifestations in People with HIV from Northeastern Romania. *Medicina (Kaunas)*. 2023;59(9):1605. Published 2023 Sep 6. doi:10.3390/medicina59091605
   IF 2.4
   https://www.mdpi.com/1648-9144/59/9/1605
   (4.3.2 Evaluation of ocular characteristics of HIV infected patients by region)

2. **Cobaschi M**, Dorobăț CM, Dorobăț VD, et al. Ocular involvement in highly treatment-experienced patients with HIV. *Rom J Ophthalmol*. 2024;68(2):152-157. doi:10.22336/rjo.2024.28
   PubMed
   https://rjo.ro/ocular-involvement-in-highly-treatment-experienced-patients-with-hiv/
   (4.3.1Evaluation of ocular impairment in the general patient cohort, in the cohort versus other transmission routes)

3. **Cobaschi M**, Negru AR, Dorobăț VD, et al. Miscellaneous ophthalmic conditions related to low CD4 cell count in HIV-positive patients. *Rom J Ophthalmol*. 2024;68(2):202-209. doi:10.22336/rjo.2024.38
   PubMed
   https://rjo.ro/miscellaneous-ophthalmic-conditions-related-to-lowcd4-cell-count-in-hiv-positive-patients/
   (4.3.3 Theoretical applicability and practical Considerations, notable cases from current practice)

4. Loghin II, Rusu ȘA, Vâţă A, **Cobaschi M**, Cecan I, Manciuc C, Dorobăţ CM. Antiretroviral Therapy Switch in HIV-Infected Adults from a Regional HIV/AIDS Center in NE Romania. *Medicina*. 2024; 60(6):854. https://doi.org/10.3390/medicina60060854
   IF 2.4
   https://www.mdpi.com/1648-9144/60/6/854

5. Loghin II, Vâță A, Miftode EG, **Cobaschi M**, Rusu ȘA, Silvaș G, Frăsinariu OE, Dorobăț CM. Characteristics of Tuberculous Meningitis in HIV-Positive Patients from Northeast Romania. *Clinics and Practice*. 2023; 13(6):1488-1500. https://doi.org/10.3390/clinpract13060131
   IF 2.4
   https://www.mdpi.com/2039-7283/13/6/131

6. **Cobaschi M,** Loghin II, Silvaș G, et al. Interdisciplinary in managing HIV/AIDS infected patients – the importance of teamwork. Revista Medico-Chirurgicala. 2022;126. 495-503. 10.22551/MSJ.2022.04.05.
   ESCI*, 2021 JCI**: 0.07.
   https://www.revmedchir.ro/index.php/revmedchir/article/view/2661

GENERAL PART

Chapter 1. Etiopathogenesis and epidemiology of HIV/AIDS infection

The Human Immunodeficiency Virus (HIV) is the infectious agent that led to the onset of the HIV pandemic in 1981. HIV is responsible for infecting 88.4 million people and the deaths of 42.3 million both through the direct action of the virus and the complications it causes (World Health Organization, 2024; United Nations Programme on HIV/AIDS, 2024). The multisystemic effects caused by HIV infection are known as Acquired Immune Deficiency Syndrome (AIDS) (Sudharsan et al., 2020; Patel et al., 2021).

The evolution of HIV infection in Romania has certain characteristics, differentiating it from the global epidemiological profile. The first diagnosed case of HIV infection was in 1985, but the real onset of the epidemic in Romania occurred in 1989 when about 10,000 children were diagnosed with HIV infection, forming the "pediatric cohort." The route of HIV transmission was parenteral, through inadequately sterilized medical equipment or through blood transfusions. Another peculiarity for Romania was the isolation of the F1 subtype of HIV-1, rare in Europe but more commonly found in South America and Africa.

Globally, the average age of patients living with HIV infection has increased due to the optimization of therapeutic management (Loghin et al., 2019). From the group considered the pediatric cohort composed of young adults, currently, 40% of the patients are alive under antiretroviral treatment (ART).

In developed countries, the management of people living with HIV infection has evolved through the creation of a complex system of viro-immunological and multidisciplinary clinical monitoring, which has increased the survival rate from years to decades (Hidalgo et al., 2000).

The introduction of antiretroviral therapy regimes in 1996 marked the first step in controlling the HIV pandemic, significantly reducing the death rate compared to previous therapeutic approaches, and also the prevalence of multisystemic comorbidities (Easterbrook and Meadway, 2001).

Chapter 2. Ocular manifestations in patients with HIV/AIDS infection

Ophthalmological conditions arising in the context of HIV infection, some with the potential to lead to vision loss, have a major impact on the quality of life of patients, thus early diagnosis and appropriate treatment are essential.

Ocular pathology found among patients with HIV infection can be classified into two categories: the first is associated with HIV infection and ART treatment, which is specific, and the second is present among the general population affecting those with HIV infection, independent of the virus's presence. Given the evolution of ART in the last two decades and increased life expectancy, ophthalmologic pathology increasingly encountered is also due to aging (Pathai et al., 2013).

Ocular impairment observed in people living with HIV infection can occur through several mechanisms: direct action, either by the appearance of opportunistic infections facilitated by HIV-induced immunosuppression, as well as through chronic inflammation that can lead to neoplasms or toxicity induced by ART. Ocular manifestations that occur in the context of HIV infection can affect any of the structures of the ocular globe or its annexes.

Involvement of the ocular globe's annexes may include:

- Trichomegaly
- Blepharitis
- Ophthalmic herpes zoster
- Molluscum contagiosum
- Kaposi's sarcoma (eyelid, conjunctiva)
- Involvement of the lacrimal glands and ducts (dacryoadenitis, lymphocytic infiltration)
- Extraocular muscle (functional anomalies, cranial nerve palsies) (Dian, Ganiem, and Ekawardhani, 2023; Almagro et al., 2003; Feroze and Wang, 2022; Nguyen, 2020; Venkateswaran, 2021; Pineles, 2012; Achdiat et al., 2023).

Involvement of the anterior segment of the ocular globe includes:

- Conjunctival microvasculopathy
- Surface ocular neoplasms
- Keratoconjunctivitis sicca
- Infectious keratitis, frequently involving Herpes Simplex Virus (HSV)

- Anterior uveitis (triggered by opportunistic infections or following ART initiation) (Feroze and Wang, 2023; Yang et al., 2023; Biswas and Sudharshan, 2008).

Involvement of the posterior segment of the ocular globe includes:

- HIV retinopathy
- Retinitis caused by cytomegalovirus (CMV), HSV, varicella-zoster virus (VZV), *Toxoplasma gondii, Treponema pallidum, Pneumocystis jirovecii, Mycobacterium tuberculosis, Cryptococcus neoformans* (Feroze & Wang, 2022; Koundanya and Tripathy, 2024; Freeman et al., 2008; Kalogeropoulos, 2022).

Neuro-ophthalmic involvement includes:

- Papilledema
- Papillitis
- Optic neuritis
- Cranial nerve palsies (Feroze & Wang, 2022).

Orbital involvement includes:

- Orbital cellulitis
- Orbital lymphoma
- Orbital pseudotumors

The therapeutic approach to ocular pathology depends on the type of ocular involvement present (Govender et al., 2011).

Immune reconstitution inflammatory syndrome (IRIS) can occur within the first few months of initiating ART and is more likely to manifest in patients with low CD4 values at the time of ART initiation. Rapid restoration of immunity can lead to exacerbated inflammation in cases of pre-existing ocular impairment or aggravation of subclinical ocular conditions (Sereti, 2020).

The management of ocular pathology in patients infected with HIV is interdisciplinary, with treatment predictability achieved by optimizing collaboration and communication among the members of the interprofessional team who must ensure that HIV-associated ocular manifestations are diagnosed early and all efforts are made to ameliorate or halt their progression.

PERSONAL PART

Chapter 3. Evaluation of clinical, biological, and viro-immunological characteristics of HIV-infected patients with ophthalmological manifestations: in the general group of infected patients, cohort group versus patients infected through other transmission routes

3.1 Study objectives

The study objectives were as follows:

- To evaluate the clinical and paraclinical characteristics of all patients included in the study at the general group ;
- To compare characteristics of HIV-infected patients from the cohort versus those infected through other transmission routes (sexual transmission/intravenous drug use (IVDU)).

3.2 Materials and Methods

This retrospective study included 62 patients (out of a total of 1,190 patients examined) aged over 18 years, diagnosed with HIV/AIDS who presented with various ocular pathologies. Patients were selected from two centers, Bucharest (24 out of 915 patients evaluated) and Iași (38 out of 275 patients evaluated), and the study period was from January 1, 2019, to December 1, 2023.

HIV infection was diagnosed using two immunoenzymatic tests (ELISA), confirmed by the Western blot (WB) test.

The "cohort" is defined as the group of adult patients with HIV infection acquired in childhood, during the years 1985-2000, through parenteral transmission.

The group of HIV patients infected through "other transmission routes" includes patients infected in adulthood through sexual transmission and intravenous drug use.

Demographic information (age and sex), personal pathological history, clinical and viro-immunological characteristics, potential opportunistic infections, disease stage, history of antiretroviral treatment, and the evolution of HIV/AIDS patients were included in the database.

HIV infection staging was performed according to the Centers for Disease Control and Prevention (CDC): stage 1 when CD4+ T lymphocyte levels are above 500 cells/mm3; stage 2 when they are between 200-499 cells/mm3; and stage 3 when they are below 200

cells/mm3. HIV infection is represented by stages 1 and 2, while AIDS is represented by stage 3. HIV viremia was measured using RT-PCR for HIV-1, with a viral load above 40 copies/mL considered detectable.

Clinical, biological, and viro-immunological evaluation: "initial" was defined as the time of HIV diagnosis, "in evolution" was defined as the evaluation at the time of study enrollment, and "current" was the last documented evaluation in 2023.

The following data regarding the characteristics of HIV-infected patients – from both the general group and the cohort group – were analyzed: age group, sex, duration of HIV infection, CD4 levels, HIV viremia (HIV VL), and co-infections.

Statistical analysis was performed using XLSTAT software version 2019. The Pearson test and Kendall's Tau correlation coefficients were used.

3.3 Results

The gender distribution does not show significant differences (p=0.07); there is a higher proportion of men in the general group of HIV-infected patients (59.7% versus 40.3%), a trend that is also maintained in the sexual transmission sub-group (71% versus 29%), compared to the cohort group where the female sex predominates (51.6%/48.4%). (p=0.07)

In the general group, there are significant differences in the proportion of patients in the 30-39 age group compared to the other age groups (p<0.001*), with a predominance of individuals aged 30-39 years in the cohort (87.1%) and a more balanced distribution in the sexual transmission group.

Patients from the cohort have a longer duration of HIV infection compared to the group of HIV-infected patients through sexual transmission/IVDU.

Regarding the age distribution by possible HIV transmission route, there are significant differences between the groups. In the cohort, the median age is 34.06 years, while in the group of HIV-infected patients through sexual transmission, the median age is higher, at 40.21 years.

Comparing the mean duration of HIV infection between the investigated groups, in relation to the transmission route, in the cohort, the mean duration of HIV infection is 23.71 years, while for the group of HIV-infected patients through sexual transmission, the mean duration of HIV infection is significantly lower, at 9.10 years. For the intravenous drug use transmission route, only one case was presented, with an infection duration of 24 years.

Overall, the mean duration of infection for all groups is 16.58 years. In terms of HIV infection duration, the cohort group has a longer duration compared to the group of patients infected through sexual transmission/IVDU.

In the general group of patients, at the initial evaluation, 46.8% of patients had CD4 lymphocyte levels below 200 cells/mm3; in the cohort group, 45.2% of patients had initial CD4 levels below 200 cells/mm3; in the group of HIV-infected patients through sexual transmission, 48.4% of patients, and in the group of patients with unknown and IVDU transmission, all had initial CD4 levels below 200 cells/mm3.

The distribution of CD4 levels in evolution after ART initiation in the general group shows that 51.6% had levels above 500 cells/mm3; in the cohort group, 64.5% had levels above 500 cells/mm3; and in the group of HIV-infected patients through sexual transmission, 38.7% had levels above 500 cells/mm3.

The current CD4 levels in the general group show that 43.5% of patients have values below 200 cells/mm3, 37.1% have values between 200-500 cells/mm3, in the group of HIV-infected patients through sexual transmission/IVDU, 54.9% of patients have CD4 levels below 200 cells/mm3, and in the cohort group, 32.2% have CD4 values <200 cells/mm3.

The initial viral load distribution shows that 72.6% of patients had values below 1,000,000 copies/mL, and 27.4% had values between 1,000,000-10,000,000 copies/mL (Chi2 = 0.809, p = 0.847); there were no statistically significant differences between patients with viremia < 1,000,000 copies/mL and those with viremia > 10,000,000 copies/mL.
Regarding the initial HIV viremia levels, in the cohort group, 77.4% of patients had initial viral load values below 1,000,000 copies/mL; in the group of HIV-infected patients through sexual transmission/IVDU, 67.7% of patients had values below 1,000,000 copies/mL.

The distribution of viral load values in evolution after ART initiation shows that 87.1% of patients from the general group had values below 1,000,000 copies/L (Chi2 = 0.897, p = 0.639). No statistically significant differences were identified between patients with viremia below 1,000,000 copies/mL and those with viremia above 1,000,000 copies/mL.
In the cohort group, 83.9% of patients had viral load values below 1,000,000 copies/mL, while in the group of HIV-infected patients through sexual transmission, 90.3% of patients had values below 1,000,000 copies/mL.

The distribution of current viremia values shows that 80.6% of patients had values below 1,000,000 copies/mL (Chi2 = 0.194, p = 0.907); in the cohort group, 87.1% of patients had viral load values below 1,000,000 copies/mL, while in the group of HIV-infected

patients through sexual transmission/IVDU, 74.2% of patients had values below 1,000,000 copies/mL.

The mean initial CD4 values are higher in patients from the cohort (350.84) compared to the sexual transmission/IVDU group (259.65), but the difference is not statistically significant ($p = 0.134$). The initial CD4 values show considerable variation in both groups, with large standard deviations. The mean CD4 values in evolution under ART increased in both groups, being higher in patients from the cohort (444.61) compared to the sexual transmission/IVDU group (382.66), with the difference not being statistically significant ($p = 0.290$).

The median CD4 values in evolution are also higher in patients from the cohort. The median current CD4 values are significantly higher in the cohort group (368.29) compared to the sexual transmission/IVDU group (209.97), with the difference being statistically significant ($p = 0.038$). This indicates a more consistent improvement in CD4 values in the cohort group compared to the group of HIV-infected patients through sexual transmission/IVDU. Both in the evaluation in evolution and in the current evaluation, patients from the cohort tend to have higher CD4 values than the sexual transmission/IVDU group.

The average initial viral load is lower in patients from the cohort (1,008,334.71 copies/mL) compared to the sexually transmitted group (1,950,979.58 copies/mL). The standard deviations are large in both groups, reflecting considerable variability. The medians indicate a higher value in patients from the cohort (522,000 copies/mL) than in the sexually transmitted group (288,000 copies/mL).

The mean viral load values under ART decreased significantly in both groups, being higher in patients from the cohort (347,983.77 copies/mL) compared to the sexually transmitted group (222,735.67 copies/mL). The median is the same in both groups (40.00 copies/mL), indicating that antiretroviral treatment reduced the viral load to very low levels. The standard deviations decreased, reflecting a reduction in variability between patients.

The mean viral load value in the current evaluation is higher in patients from the cohort (499,466.47 copies/mL) compared to the sexually transmitted group (380,862.94 copies/mL). The median is lower in patients from the cohort (29,100 copies/mL) compared to the sexually transmitted group (62,500 copies/mL), indicating a different distribution of values in the two groups. The standard deviations remain large, indicating the variability in response to treatment.

The study also evaluated possible correlations between co-infections and the risk of ocular involvement in patients with HIV infection. In the general group, the rate of HBV co-infection is 16.1%, indicating an average between the higher values in patients from the cohort and the lower values in the sexually transmitted/intravenous drug use group. In patients from the cohort, the rate of HBV co-infection is significantly higher (29.0%) compared to the sexually transmitted/IVDU group (3.2%).

The rate of HCV co-infection is higher in the sexually transmitted/intravenous drug use group (6.5%) compared to those from the cohort (3.2%). In the general group, the co-infection rate is 4.8%, indicating the influence of higher values in the sexually transmitted/intravenous drug use group.

The rate of Toxoplasma gondii co-infection is similar in both groups (3.2%). The rate of CMV co-infection is considerably higher in the sexually transmitted/intravenous drug use group (51.6%) compared to those from the cohort (41.9%). In the general group, the CMV co-infection rate is 46.8%, indicating an average between the higher values in the sexually transmitted/intravenous drug use group and those in the pediatric cohort.

3.4 Discussion

In the general group studied, we were able to identify percentages similar to those reported at the national level, with 40.3% females and 59.7% males, showing a predominance of males, which is also maintained in the group of patients evaluated (Cobaschi et al., 2024a). Regarding the cohort group compared to the group with other modes of transmission, a balance in gender distribution can be observed for those in the cohort and a predominance of males among those who acquired HIV through other modes of transmission, aligning with reports from Romania. The group of patients from the cohort represents a group for whom HIV infection was contracted in childhood, in the 1990s via parenteral transmission, and which did not align with global transmission trends at that time. Currently, in terms of transmission mode, a harmonization of reports in Romania with those at the European and global levels can be observed.

Regarding the duration of HIV infection, it is expected that the cohort group has a longer duration of HIV infection since they are considered long-term survivors, having had access to ART medication since the 1990s.

The classification in terms of the severity stage of HIV infection, as well as subsequent monitoring, was done based on CD4 count and viral load. In the general group, the largest

proportion was of those with CD4 counts less than 200 cells/mm3. Over time, the patients from the cohort had, at the time of enrollment in the study, a considerably higher percentage of those with CD4 counts >500 cells/mm3. Considering the current status, an increase in the percentage of patients with CD4 counts <200 cells/mm3 in the general group can be observed, which could be an indicator of therapeutic fatigue.

Among the co-infections detected at the time of diagnosis or in evolution during periodic reevaluations, the most common are infections with HBV, HCV, Toxoplasma gondii, and CMV.

The prevalence of CMV infection and associated ocular involvement is reported to be 30% in other studies for patients with HIV infection and CD4 counts <50 cells/mm3 globally (Tang, 2020). Regarding HBV infection, globally, 8-10% of people living with HIV are co-infected, presenting the same modes of transmission as HIV (Corcorran and Kim, 2023). The prevalence of toxoplasmosis in Europe is high (50%) but shows significant geographic and population variability.

3.5 Conclusions

- The demographic parameters observed in the general group, as well as in the subgroups studied according to the mode of transmission, do not show significant differences compared to data reported at the national, European, and global levels.
- The increase in life expectancy of HIV-positive patients from the cohort group can be observed, with them now reaching the third or fourth decade of life.
- The occurrence of therapeutic fatigue in some patients and the variability in treatment adherence suggested by fluctuations in CD4 and HIV viral load values during the study monitoring demonstrate the need for a complex multidisciplinary strategy for monitoring the dynamics of HIV infection in Romania, which should include the ophthalmologist's perspective.

Chapter 4. Characteristics of ophthalmic manifestations in HIV-infected patients in Romania

4.1. Objectives of the study

- To assess the frequency of ocular involvement in HIV-infected patients in Romania;
- To identify specific ophthalmic characteristics in patients from the cohort compared to the group with other transmission routes;
- To identify correlations between ocular involvement and treatment regimens;
- To perform a comparative analysis of two geographical regions in Romania, the northeast and the south, to identify potential regional differences;
- To highlight relevant cases from current practice.

4.2. Materials and Methods

The retrospective study included 62 patients from two centers, Bucharest and Iași. Patients included were over 18 years old, diagnosed with both ophthalmic pathology and HIV infection, confirmed by two immunoenzymatic tests (ELISA) and Western blot (WB). The study covered the period from January 1, 2019, to December 31, 2023.

The study group for the northeastern region included 38 patients (out of 275 examined), and the southern region group included 24 patients (out of 915 ophthalmologically evaluated), from a total of 1,190 examined patients.

The "cohort" refers to the group of adult patients who acquired HIV during childhood through parenteral transmission. The "other transmission routes" group includes patients who contracted HIV through sexual contact or intravenous drug use.

The northeastern Romania group includes patients diagnosed and monitored at RCSPI Iași, while the southern Romania group includes patients under the care of INBIMB Bucharest.

The molecular biology laboratory measured CD4 counts and HIV viral load (VL) by RT-PCR. When the viral load was above 40 copies/mL, it was considered detectable. The stage of HIV infection was determined using CD4 lymphocyte counts according to the Centers for Disease Control and Prevention (CDC) criteria.

Data analyzed included general patient characteristics and cohort-specific features: age groups, gender, HIV duration, CD4 values, HIV viremia (VL), TGP, TGO, cholesterol, HDL, LDL, triglycerides, creatinine, initial and evolving treatment regimens, number of treatment schemes, opportunistic infections, and associated ophthalmic diagnoses. Periodically, the clinical and biological status of each enrolled HIV patient was evaluated for metabolic dysfunction or liver damage, considered present or absent in the statistical analysis.

The ophthalmological evaluation included the determination of corrected visual acuity, biomicroscopic examination of the anterior segment (Zeiss), fundus examination with a Volk 78 diopter lens, intraocular pressure measurement adjusted for corneal thickness (Topcon), and Schirmer test (strips). Only confirmed ophthalmic diagnoses were included in the study.

The following statistical tools were used: Pearson's test in XLSTAT version 2019, Kendall's Tau correlation coefficients, and Mann-Whitney tests for analyzing nonparametric data. Statistical analysis identified correlations between ocular manifestations, age, transmission route, HIV duration, number of treatment schemes, metabolic changes, and the degree of ocular impairment.

4.3. Results

4.3.1. Evaluation of ocular involvement in the general group, cohort vs. other transmission routes

Correlation of ocular involvement with transmission route

The most common pathologies were CMV retinitis in 46.8% of cases and HIV retinopathy in 29.0% of cases. Distribution by transmission route shows that CMV retinitis is the most prevalent in all categories, while HIV retinopathy is similarly present in both cohort patients and those with sexual transmission. Less common ophthalmic pathologies included keratoconjunctivitis sicca in 19.4%, ocular toxoplasmosis in 3.2%, and tuberculous uveitis in 1.6%.

The proportions of CMV retinitis and HIV retinopathy are similar between the cohort group and the other transmission routes group. Additionally, keratoconjunctivitis sicca is more frequent in cohort patients (22.6%) compared to those with other transmission routes

(16.1%). Ocular toxoplasmosis and tuberculous uveitis were diagnosed in similarly low percentages in both groups (3.2% and 1.6%, respectively). Chi-square analysis for both tables shows no statistically significant difference between transmission route and type of ophthalmic pathology.

Correlation of ocular involvement with age group

CMV retinitis is the most frequent ophthalmic pathology, predominantly in the 30-39 age group (48.6%). HIV retinopathy has a higher prevalence in patients aged 50 and over (55.6%). Keratoconjunctivitis sicca is more common in the 40-49 and 50+ age groups (33.3% each). Ocular toxoplasmosis and tuberculous uveitis are rarer, with 3.2% and 1.6% of cases, respectively, found only in the 30-39 age group.

Among cohort patients, CMV retinitis is present in the 30-39 age group (44.4%). HIV retinopathy and keratoconjunctivitis sicca have similar distributions across different age groups. In the group with other transmission routes, CMV retinitis is the most frequent pathology, with high prevalence in the under 30, 30-39, and 40-49 age groups. HIV retinopathy is more common in patients aged 50 and over (55.6%). Keratoconjunctivitis sicca is more prevalent in the 50+ age group (33.3%). Ocular toxoplasmosis is rare, occurring in only 3.2% of cases.

Chi-square analysis shows no statistically significant correlation between ophthalmic diagnosis and age group in the general group ($Chi2 = 5.404$, $p = 0.943$), the cohort group ($Chi2 = 3.622$, $p = 0.889$), or the group of patients infected with HIV through other transmission routes ($Chi2 = 13.406$, $p = 0.145$).

Correlation of ocular involvement with the duration of HIV infection

CMV retinitis is most common in patients with an HIV duration of 1-4 years and 5-9 years (66.7% each), but its frequency significantly decreases in those with a duration of ≥25 years (15.4%). HIV retinopathy is more frequent in patients with an HIV duration of ≥25 years (38.5%) and 20-24 years (36.8%). Keratoconjunctivitis sicca has higher prevalence in patients with an HIV duration of 10-19 years (44.4%) and ≥25 years (30.8%). Ocular toxoplasmosis is present in 3.2% of cases, while tuberculous uveitis is extremely rare, found in only 1.6% of cases.

When analyzing patients from the cohort, CMV retinitis is more common in those with an HIV infection duration of 20-24 years (60.0%). HIV retinopathy and keratoconjunctivitis sicca show a similar distribution across different HIV infection duration groups.

Considering the prevalence of ophthalmic pathology according to HIV infection duration in patients with sexual transmission or intravenous drug use, CMV retinitis is most common in patients with an HIV duration of 1-4 years and 5-9 years (66.7% each). HIV retinopathy is particularly frequent in patients with an HIV duration of 20-24 years (75.0%). The Chi-square analysis shows no statistically significant correlation between ophthalmic diagnosis and HIV infection duration in the overall group ($Chi2 = 23.165$, $p = 0.109$) and the cohort group ($Chi2 = 11.185$, $p = 0.191$). However, in the group with sexual/IVDU transmission, the correlation is significant ($Chi2 = 41.241$, $p < 0.001$), suggesting a possible influence of HIV duration on the prevalence of certain ophthalmic pathologies.

Correlation of ocular involvement with the number of treatment regimens

Patients with fewer than two treatment regimens and those with 2-5 regimens had a similar prevalence of CMV retinitis (55.6% and 55.2%, respectively). However, the prevalence significantly decreases in patients with more than five regimens (20.0%). HIV retinopathy was present in 29.0% of cases, with a slight increase in prevalence in patients with more than five treatment regimens (33.3%). Keratoconjunctivitis sicca had a higher prevalence in patients with more than five treatment regimens (26.7%). Ocular toxoplasmosis and tuberculous uveitis were rarer, found in 3.2% and 1.6% of cases.

For cohort patients, CMV retinitis is more prevalent in those with 2-5 treatment regimens (60.0%), compared to those with fewer than two regimens (33.3%) and more than five regimens (23.1%). HIV retinopathy and keratoconjunctivitis sicca both show a relatively constant distribution across the number of treatment regimens. Ocular toxoplasmosis and tuberculous uveitis have a uniform distribution across all treatment regimen categories.

For patients with HIV infection acquired through sexual transmission or intravenous drug use, the prevalence of CMV retinitis decreases from 60.0% in those with fewer than two treatment regimens to 50.0% in those with 2-5 regimens, and it is not present in patients with more than five regimens. HIV retinopathy shows a constant prevalence across the number of treatment regimens. The Chi-square analysis indicates no statistically significant correlation between the type of ophthalmic pathology and the number of treatment regimens in the overall group ($Chi2 = 13.108$, $p = 0.108$), for cohort patients ($Chi2 = 6.261$, $p = 0.618$),

whereas in the group with sexual/IVDU transmission, the correlation is significant (Chi2 = 16.716, p = 0.010), indicating that the number of treatment regimens may influence the prevalence of certain ophthalmic pathologies in this group.

Correlation of ocular involvement with metabolic changes

Regarding the correlation between the ophthalmic diagnosis of HIV-infected patients and the presence of metabolic changes, 55.6% of patients with CMV retinitis showed metabolic changes, and 33.3% of patients with HIV retinopathy had metabolic changes.

Considering the types of metabolic changes in the overall group, CMV retinitis is mainly associated with hepatic dysfunction (75.0%) and lipid metabolism changes (33.3%). HIV retinopathy is primarily linked to lipid metabolism changes (44.4%). Keratoconjunctivitis sicca is rarer and is mainly associated with lipid metabolism changes (22.2%).

For cohort patients, CMV retinitis presents a higher prevalence of metabolic changes (62.5%), and the majority of patients with HIV retinopathy do not have metabolic changes (34.8%).

A more detailed analysis of metabolic changes in the cohort group suggests that CMV retinitis is predominantly associated with hepatic dysfunction (100.0%). Keratoconjunctivitis sicca and HIV retinopathy are linked to lipid metabolism changes (50.0% and 25.0%).

For patients with sexual/IVDU transmission, the correlation between ophthalmic pathologies and metabolic changes revealed that metabolic changes are present in half of the patients with CMV retinitis. Patients with HIV retinopathy showed a higher frequency of metabolic changes, present in 50.0% of cases. Keratoconjunctivitis sicca is exclusively present in patients without metabolic changes. The Chi-square analysis indicates no statistically significant correlation between the presence or absence of metabolic changes and the type of ophthalmic pathology in the overall group (Chi2 = 2.698, p = 0.610), for cohort patients (Chi2 = 2.826, p = 0.587), and in the sexual/IVDU transmission group, the correlation is also insignificant (Chi2 = 5.100, p = 0.165). However, a detailed analysis of the types of metabolic changes suggests that certain ophthalmic pathologies, such as CMV retinitis and HIV retinopathy, may be more frequently associated with hepatic dysfunction and lipid metabolism changes. These results highlight the importance of a detailed metabolic

assessment in HIV patients to identify and manage potential associated ophthalmic complications.

Results regarding the association between treatment protocols and ophthalmic diagnosis in HIV-infected patients

For patients diagnosed with CMV retinitis, the most common ART combinations administered as initial treatment were NNRTI + NRTI (31.0%), followed by INSTI + NRTI and PI + NRTI (24.1%). "One Single Tablet" (OST) was used for 10.3% of patients. For patients diagnosed with HIV retinopathy, the most common initial treatment was NNRTI + NRTI and PI + NRTI (27.8%), followed by INSTI + NRTI (16.7%). For patients diagnosed with keratoconjunctivitis sicca, the combination NNRTI + NRTI was most frequently administered initially (50.0%), followed by NNRTI + 2NRTIs and PI + NRTI (16.7%). The Pearson Chi-square test (Chi2 = 20.774, p = 0.652) indicates that there is no statistically significant association between ART combinations administered as initial treatment and ophthalmic diagnoses.

For patients diagnosed with CMV retinitis, the most common ART combinations were OST (44.8%), followed by PI + NRTI (27.6%) and INSTI + NRTI (20.7%). For patients diagnosed with HIV retinopathy, the most common ART combinations were INSTI + NRTI (33.3%), followed by PI + NRTI and OST (27.8%). For patients diagnosed with keratoconjunctivitis sicca, the most common ART combinations were NNRTI + NRTI (41.7%), followed by INSTI + NRTI, PI + NRTI, and OST (16.7%). The Pearson Chi-square test (Chi2 = 35.440, p = 0.309) suggests that there is no statistically significant association between ART regimen families and ophthalmic diagnoses.

4.3.2. Evaluation of ocular characteristics of patients in the northeast and south regions of Romania

In the Northeast region of Romania, men experienced these manifestations most frequently (65.7%) compared to the South region, where the gender distribution of cases was balanced (Cobaschi et al., 2023). In the Northeast region, the majority of cases involved young adults aged between 30 and 39 years (65.8%), followed by the age groups 20-29 and 40-49 years with 4 patients each (10.53%), 50-59 years - 2 patients (5.26%), over 60 years - 2 patients (5.26%), and 0-19 years - 1 patient (2.63%).

In the South region, Bucharest, the majority of cases also involved young adults aged between 30 and 39 years, with 12 patients (50%), followed by the age group of 20-29 years with 5 patients (20.83%), 50-59 years - 3 patients (12.5%), and the age groups 40-49 and over 60 years with 2 patients each (8.33%).

In the Northeast region of Romania, the most common route of HIV transmission was parenteral, with a total of 24 cases (63.2%) originating from the cohort, while in the South region of Romania, the most frequent route of HIV transmission was sexual, accounting for 16 patients (67%).

Regarding the comparison of the prevalence of ophthalmologic diagnosis between the Northeast and South regions, the prevalence of CMV retinitis is similar in both centers, with 47.4% in the Northeast and 45.8% in the South. This indicates a uniform distribution of this diagnosis between the two groups. In the case of HIV retinopathy, the South region has a higher prevalence of HIV retinopathy (33.3%) compared to the Northeast region (26.3%), suggesting a difference (which is not statistically significant) in the prevalence of this diagnosis between the two locations. Regarding keratoconjunctivitis sicca, its prevalence is higher in the South (20.8%) compared to the Northeast (18.4%) (Cobaschi et al., 2023).

In the Northeast, HBV co-infections were the most frequent (21.05% of patients with co-infections), while in the South, CMV co-infections were the most common (61.5% of patients with co-infections).

4.3.3 Notable clinical cases from current practice

The first case presented is that of a 34-year-old patient from the cohort group, who, due to lack of adherence to ART, developed systemic infectious and neurological complications that led to exogenous endophthalmitis with multidrug-resistant *Pseudomonas aeruginosa.*

The second case is of a newly diagnosed HIV patient who presented at the time of diagnosis with CMV neuroretinal damage, which led to total compromise of visual acuity.

The last case presented is of a patient who concurrently manifested HIV infection, syphilis, and two episodes of herpes zoster, one of which involved the ophthalmic branch of the trigeminal nerve, causing corneal damage in one eye and ocular inflammation in the fellow eye (Cobaschi et al., 2024b).

4.4. Discussions

Ophthalmologic impairment represents one of the complications associated with HIV infection and can be underestimated. Following the analysis of the studied cohorts, including the general group that included all enrolled patients, subsequently the cohort group, the group with patients infected through other transmission routes, and the groups at HIV centers in Romania, the frequency of types of ophthalmologic impairments in patients with HIV infection was identified.

Compared to other studies regarding the frequency of ophthalmologic impairments, similarities can be noted concerning the most prevalent ophthalmologic pathology in the analyzed cohorts: keratoconjunctivitis sicca, HIV retinopathy, and CMV retinitis with the highest percentages.

Regarding ophthalmologic impairments by age, it represents a complex issue; age plays an important role in how these manifest and evolve. Analyzing the distribution of ophthalmologic pathologies by age group in the general lot, CMV retinitis is the most frequent ophthalmologic pathology, predominantly in the age group of 30-39 years. HIV retinopathy has increased prevalence in patients aged 50 years and over (55.6%). Keratoconjunctivitis sicca is more common in the age groups of 40-49 and ≥50 years.

The CD4 count is considered an independent risk factor for ocular pathology in HIV-infected patients (Kim et al., 2015). A decrease in CD4 count (CD4 number <200 cells/mm3) is a risk factor and predictor for ocular pathology (Bekele et al, 2013). Regarding the initial viral load values, they are similar across all lots. The initial viral load values showed a similar distribution, with most patients having values under 1,000,000. Over time and at the current moment, the proportion of patients with a viral load under 1,000,000 has significantly increased in all lots, reflecting the efficacy of treatment.

Regarding the co-infections present in the lots investigated in the first part, patients from the cohort have higher rates of HBV co-infection but similar rates for HCV.

Regarding the association between ophthalmologic diagnosis and metabolic changes, there was no statistically significant correlation found between the presence or absence of metabolic changes and the type of ophthalmologic pathology in the general lot, in patients from the cohort, or in those with other HIV transmission routes.

4.5. Conclusions

- Identification of ocular impairment in a percentage between 40-50%, regardless of the clinical form and route of transmission of the infection
- Advanced age, despite its inherent changes, represents a potential risk factor for ocular pathology
- The viro-immunological status is an important predictor of progression and is closely linked to treatment adherence.
- The use of first-generation ART correlates with an increased prevalence of ocular concerns.

Chapter 5. Conclusions and personal contributions

- The analysis of the subgroups: the cohort and patients infected through other means (sexual, intravenous drug use) or subgroups from distinct centers, outlined the differences in the medical approach as well as specifics of their ophthalmological pathology.
- From our data, the demographic parameters of the studied lot show no significant differences compared to data reported at the European and global levels.
- Regarding the duration of HIV infection, it is evidently longer in patients from the cohort (average 23.71 years), with values ranging from 18 to 31 years, and shorter in patients infected through other means, 5 years for those with transmission through intravenous drug use, and 10 years for sexual transmission.
- The viro-immunological status has been an important element to monitor from the time of diagnosis to the present. Initially, CD4 values were below 200 cells/mm³ for nearly half of the patients (46.8%) and 27.4% had CD4 values between 200-500 cells/mm³; subsequently, the trend is favorable, so that under treatment, 19.4% have below 200 cells/mm³ and 29% between 200-500 cells/mm³, highlighting the initial character of treatment adherence, which later changes over time and influences the occurrence of ocular impairment.
- The presence of ocular impairment in a percentage between 40-50% of the general lot, regardless of the transmission route, once again proves the high frequency of ophthalmological impairment among patients with HIV infection.
- Due to the phenomenon of 'aging', and also the decrease in CD4 values, the prevalence of ocular impairment through angiopathic changes, HIV retinopathy, is predominantly present in the age group > 50 years.
- The presence of opportunistic infections (CMV retinitis or Toxoplasma gondii) is correlated, as expected, with the moment of viro-immunological failure.
- The analysis highlights the importance of the viro-immunological status, as a marker of immune status, which is crucial in the risk of occurrence of opportunistic infections including at the level of the ocular globe.
- The statistical study illustrated in tables and figures is intended to demonstrate the important role of ocular impairment in HIV patients and hence the necessity of involving the ophthalmologist in managing such a patient.
- Whether it is changes in the ocular globe through the direct action of the virus or through associated infections - as can be seen through the three cases presented illustratively in this

respect, their occurrence has a major and immediate impact on the patient's quality of life (Cobaschi et al., 2024b).

- I believe that the results obtained have managed to outline the ophthalmological profile of the HIV-infected patient in Romania, facilitating the understanding of the incidence of associated ocular pathologies and the importance of the ophthalmological consultation including in the evaluation of immunological status and adherence to treatment. Also, the predictability of the appearance of ocular manifestations based on viro-immunological parameters leads to better management of HIV patients.
- The recommendation to include an ophthalmological screening consultation in the investigation plan of the HIV-infected patient would undoubtedly lead to early detection of ocular involvement.
- The statistical study conducted was able to highlight the importance of correlations between the cohort versus other transmission routes. Looking at the transmission routes, we can consider it a premiere; patients from the cohort represent a peculiarity for Romania (subsection 4.3.1). This special group that makes up the cohort represents the authenticity element of the study, which marks an example of the dynamics and temporal evolution of HIV/AIDS infection.
- The recommendation to include an ophthalmological screening consultation in the investigation plan of the HIV-infected patient would undoubtedly lead to early detection of ocular impairment. In this regard, I have developed an ophthalmological management algorithm for the HIV/AIDS infected patient (subsection 4.6), with the aim of synthesizing the steps of initial evaluation and long-term monitoring. Currently, the favorable prognosis of an HIV patient depends on the promptness of diagnosis, the correctness of treatment, and the existence of a team and good collaboration between medical specialties congruent in its management.

BIBLIOGRAPHY

1. Achdiat PA, Andiani S, Hindritiani R, et al. Molluscum Contagiosum in HIV Patient Treated with 20% Topical Glycolic Acid After Resistance with Topical Tretinoin. *Clin Cosmet Investig Dermatol*. 2023;16:2749-2755. Published 2023 Sep 29. doi:10.2147/CCID.S423304
2. Almagro M, del Pozo J, García-Silva J, Martínez W, Castro A, Fonseca E. Eyelash length in HIV-infected patients. *AIDS*. 2003;17(11):1695-1696. doi:10.1097/00002030-200307250-00015
3. Bekele S, Gelaw Y, Tessema F. Ocular manifestation of HIV/AIDS and correlation with CD4+ cells count among adult HIV/AIDS patients in Jimma town, Ethiopia: a cross sectional study. *BMC Ophthalmol*. 2013;13:20. Published 2013 May 27. doi:10.1186/1471-2415-13-20
4. Biswas J, Sudharshan S. Anterior segment manifestations of human immunodeficiency virus/acquired immune deficiency syndrome. *Indian J Ophthalmol*. 2008;56(5):363-375. doi:10.4103/0301-4738.42412
5. **Cobaschi M**, Dorobăț CM, Dorobăț VD, Loghin II, Macovei ML, Marinescu A, Aramă V. Ocular involvement in highly treatment-experienced patients with HIV. Rom J Ophthalmol. 2024a Apr-Jun;68(2):152-157. doi: 10.22336/rjo.2024.28. PMID: 39006338; PMCID: PMC11238857.
6. **Cobaschi M**, Loghin II, Dorobăț VD, Silvaș G, Rusu ȘA, Hârtie V, Aramă V. Ophthalmological Manifestations in People with HIV from Northeastern Romania. Medicina (Kaunas). 2023 Sep 6;59(9):1605. doi: 10.3390/medicina59091605. PMID: 37763724; PMCID: PMC10536229.
7. **Cobaschi M**, Negru AR, Dorobăț VD, et al. Miscellaneous ophthalmic conditions related to low CD4 cell count in HIV-positive patients. *Rom J Ophthalmol*. 2024b;68(2):202-209. doi:10.22336/rjo.2024.38
8. Corcorran MA, Kim N. Chronic hepatitis B and HIV coinfection. *Top Antivir Med*. 2023;31(1):14-22.)
9. Dian S, Ganiem AR, Ekawardhani S. Cerebral toxoplasmosis in HIV-infected patients: a review. *Pathog Glob Health*. 2023;117(1):14-23. doi:10.1080/20477724.2022.2083977
10. Dwivedi R, Prakash P, Kumbhar BV, Balasubramaniam M, Dash C. HIV-1 capsid and viral DNA integration. *mBio*. 2024;15(1):e0021222. doi:10.1128/mbio.00212-22

11. Easterbrook P, Meadway J. The changing epidemiology of HIV infection: new challenges for HIV palliative care. *J R Soc Med*. 2001;94(9):442-448. doi:10.1177/014107680109400907
12. Feroze KB, Wang J. Ocular Manifestations of HIV. [Updated 2023 Jul 17]. In: StatPearls [Internet]. Treasure Island (FL): StatPearls Publishing; 2024 Jan-. Available from: https://www.ncbi.nlm.nih.gov/books/NBK441926/
13. Feroze KB, Wang J. Ocular Manifestations of HIV. 2022 Jul 18. In: StatPearls [Internet]. Treasure Island (FL): StatPearls Publishing; 2022 Jan–. PMID: 28722955.
14. Freeman WR, Van Natta ML, Jabs D, et al. Vision function in HIV-infected individuals without retinitis: report of the Studies of Ocular Complications of AIDS Research Group. *Am J Ophthalmol*. 2008;145(3):453-462. doi:10.1016/j.ajo.2007.10.013
15. Gore MR. Orbital pseudotumor as a result of chronic sinusitis in an HIV-positive patient. *Clin Case Rep*. 2017;5(11):1793-1796. Published 2017 Sep 18. doi:10.1002/ccr3.1186
16. Govender-Poonsamy, Pirindhavellie & Hansraj, Rekha & Naidoo, Kovin & Visser, Linda. (2011). Ocular manifestations of HIV/AIDS: A literature review (Part 2)*. African Vision and Eye Health. 70. 10.4102/aveh.v70i2.97.
17. Hidalgo JA, MacArthur RD, Crane LR. An overview of HIV infection and AIDS: etiology, pathogenesis, diagnosis, epidemiology, and occupational exposure. Semin Thorac Cardiovasc Surg. 2000 Apr;12(2):130-9.
18. Kalogeropoulos D, Sakkas H, Mohammed B, et al. Ocular toxoplasmosis: a review of the current diagnostic and therapeutic approaches. *Int Ophthalmol*. 2022;42(1):295-321. doi:10.1007/s10792-021-01994-9
19. Kim YS, Sun HJ, Kim TH, Kang KD, Lee SJ. Ocular Manifestations of Acquired Immunodeficiency Syndrome. Korean J Ophthalmol. 2015 Aug;29(4):241-8.
20. Koundanya VV, Tripathy K. Syphilis Ocular Manifestations. [Updated 2023 Aug 25]. In: StatPearls [Internet]. Treasure Island (FL): StatPearls Publishing; 2024 Jan-. Available from: https://www.ncbi.nlm.nih.gov/books/NBK558957/
21. Loghin I.I., Dorobăţ C-M., Mărdărescu M. Cascada îngrijirilor medicale în infecţia cu HIV (In : Abord Interdisciplinar în infecţia cu HIV ;editori: Cercel A.S., Dorobăţ C-M.). Edit.TehnoPress, Iasi, 2019.
22. Nguyen BN, Chung AW, Lopez E, et al. Meibomian gland dropout is associated with immunodeficiency at HIV diagnosis: Implications for dry eye disease. *Ocul Surf*. 2020;18(2):206-213. doi:10.1016/j.jtos.2020.02.003

23. Nguyen BN, Chung AW, Lopez E, et al. Meibomian gland dropout is associated with immunodeficiency at HIV diagnosis: Implications for dry eye disease. *Ocul Surf*. 2020;18(2):206-213. doi:10.1016/j.jtos.2020.02.003
24. Pathai S, Shiels PG, Weiss HA, et al. Ocular parameters of biological ageing in HIV-infected individuals in South Africa: relationship with chronological age and systemic biomarkers of ageing. *Mech Ageing Dev*. 2013;134(9):400-406. doi:10.1016/j.mad.2013.08.002
25. Pineles SL, Demer JL, Holland GN, Ransome SS, Bonelli L, Velez FG. External ophthalmoplegia in human immunodeficiency virus-infected patients receiving antiretroviral therapy. *J AAPOS*. 2012;16(6):529-533. doi:10.1016/j.jaapos.2012.08.005
26. Sereti I. Immune reconstruction inflammatory syndrome in HIV infection: beyond what meets the eye. *Top Antivir Med*. 2020;27(4):106-111.
27. Sudharshan S, Nair N, Curi A, Banker A, Kempen JH. Human immunodeficiency virus and intraocular inflammation in the era of highly active anti retroviral therapy - An update. *Indian J Ophthalmol*. 2020;68(9):1787-1798. doi:10.4103/ijo.IJO_1248_20
28. Tang Y, Sun J, He T, et al. Clinical Features of Cytomegalovirus Retinitis in HIV Infected Patients. *Front Cell Infect Microbiol*. 2020;10:136. Published 2020 Apr7 doi:10.3389/fcimb.2020.00136).
29. Venkateswaran N, Ramos JC, Cohen AK, et al. Spotlight on ocular Kaposi's sarcoma: an update on the presentation, diagnosis, and management options. *Expert Rev Ophthalmol*. 2021;16(6):477-489. doi:10.1080/17469899.2021.1962294
30. World Health Organization. (2024a). HIV data and statistics. Available at: https://www.who.int/teams/global-hiv-hepatitis-and-stis-programmes/hiv/strategic-information/hiv-data-and-statistics (Accessed: august 2024)
31. Yang M, Kamoi K, Zong Y, Zhang J, Ohno-Matsui K. Human Immunodeficiency Virus and Uveitis. *Viruses*. 2023;15(2):444. Published 2023 Feb 5. doi:10.3390/v15020444