"OVIDIUS" UNIVERSITY OF CONSTANȚA
DOCTORAL SCHOOL OF MEDICINE
FIELD OF MEDICINE
ACADEMIC YEAR 2024

DOCTORAL THESIS - SUMMARY

INSIGHTS ON HUMAN IMMUNODEFICIENCY VIRUS-INDUCED LIVER FIBROGENESIS

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CONTENTS

Key words: HIV monoinfection, HIV co-infection, liver fibrosis, APRI score, FIB4 score, FORNS score.

GENERAL PART

1. INTRODUCTION

Human immunodeficiency virus infection remains a global public health problem. Following the introduction of active antiretroviral therapy in the mid-1990s, HIV infection has become a chronic infection, the correct management of which has led to an increase in the life expectancy of these patients. HIV infection causes multisystem damage, depending on the degree of immunosuppression. Liver damage may occur as a direct consequence of HIV infection or through indirect mechanisms triggered by the human immunodeficiency virus. Chronic inflammation of the liver as a result of HIV per se or in association with exposure to hepatotoxic factors leads to hepatic fibrosis, resulting in progression of liver disease.

2. CURRENT KNOWLEDGE

2.1 Effects of HIV infection on liver cells

HIV infects liver cells and causes intrahepatic apoptosis, activation and fibrosis. It also alters permeability in the gastrointestinal tract, increasing circulating lipopolysaccharides with consequences for liver function. Viral antigens 'per se', in the absence of viral infection of liver cells, can stimulate various liver cells and thus elicit cellular responses. Viral antigens can be components of infectious virions, defective virions that cannot infect any type of liver cell, or viral proteins that have been released from virions and are freely circulating (1): glycoprotein coat 120 (gp120) and the transactivator protein Tat. There is evidence of HIV infection of liver cell populations (16), with HIV-HIV RNA detected in primary liver cells both in vivo (2-4) and in vitro (5,6). HIV has been shown in vivo to infect resident liver macrophages and Kupffer cells more than hepatocytes. (2-4) Although the nature of the receptors that allow viral attachment and entry into hepatocytes is not fully understood, it may be the CXCR4 and CCR5 receptors on Huh 7.5 cells (7), which induce hepatocyte apoptosis and activation of stellate cells, both of which contribute to fibrosis. Suppression of PPAR-Ɣ (peroxisome proliferator-activated receptor-activated receptor) activity by HIV through its two accessory proteins, Vpr and Nef (8,9), is another mechanism by which HIV contributes to the progression of liver damage. Infection of hepatocytes and other cell lines is independent of CD4+ cells as most of these, like primary hepatocytes, do not express CD4+ (10-12). Infection of hepatocytes can

occur via endocytosis mediated by alternative receptors or coreceptors. Hepatocytes can act as transient reservoirs for HIV and can promote CD4+ T cell infection through intercellular contact. (13) Viral entry into hepatocytes may also be facilitated by plasma membrane glycosphingolipids, such as the glycolipid galactosyl ceramide. (14)

2.2 How HIV affects the liver

Intestinal microbial translocation increases liver levels of bacterial lipopolysaccharide (LPB). These induce liver inflammation by recruiting and activating inflammatory cells - Kupffer cells and hepatic stellate cells, indirectly inducing the systemic immune response and promoting cell apoptosis (15), and increasing the production of proinflammatory cykines and acute phase reactants (TGFB-1 transforming growth factor beta-1; IL-6; IL-10).(16) In acute infection, intestinal lymphoid tissue is initially affected, with depletion of CD22, CD4+ and TH17 lymphocytes. (17) Under the influence of HIV viral proteins, the production of inflammatory cytokines by the intestinal epithelium increases, leading to epithelial cell apoptosis and disruption of tight junctions (17-19).

Systemic inflammation can cause liver fibrosis by inducing oxidative stress, mitochondrial dysfunction or accelerated senescence. (20) Decreased CD4/CD8 ratio as a consequence of HIV action will lead to underexpression of the anti-fibrotic cytokine IFN gamma. This favours a profibrotic state in the liver by reducing stellate cell apoptosis. (21-24)

Regenerative nodular hyperplasia. The pathogenic mechanism is triggered by intestinal bacterial translocation leading to vascular endothelial injury, stenosis and portal hypertension. Endothelial injury occurs either by direct action of HIV or is immune-mediated. (25)

As a result of **oxidative stress**, oxygen free radicals cause activation of Kupffer cells. This is followed by activation of stellate cells via nuclear factor kappa-beta (NF-kB) and activator protein 1, which increases the production of proinflammatory and profibrotic cytokines, leading to liver injury, fibrosis and then cirrhosis. (26) In HIV infection, hepatic stellate cells are activated via the gp120 receptor, activating metabolic pathways leading to the release of oxygen free radicals.

Mitochondrial damage is caused by increased stress on the endoplasmic reticulum (ER), initiated by activation of the IRE 1/ TRAF 2 (inositol REquiring 1/TNF receptor-associated factor 2) pathway. This results in increased production of inflammatory cytokines, activation of macrophages and beta-oxidation of fatty acids accumulated in the liver. (27, 28)

Immune-mediated liver damage. Through the gp120 receptor, HIV binds to and activates hepatic stellate cells. (29) This increases the production of collagen and monocyte

chemoattractant protein (MCP-1). (30) HIV also acts by decreasing the number of Kupffer cells, which reduces the ability of the liver to remove microbial translocation products from the portal blood. (3, 4, 31) The reduced CD4/CD8 ratio alters the cytokine profile. Thus, the decrease in interferon (IFN) gamma in Th1 cells and the increase in profibrotic cytokines (IL-4, IL-5, IL-10 and IL-13) due to a relative increase in TH2 signalling will cause a decrease in antifibrotic cytokines (32).

Cytotoxicity. HIV has a direct cytopathic effect on hepatocytes, inducing apoptosis via the gp 120 receptor signalling pathway.(33)

Lipotoxicity. Liver damage may be secondary to an increase in free fatty acids in the liver. These are peroxidised, increasing oxidative and endoplasmic reticulum stress. The ultimate consequence will be the development of fibrosis. (21)

Accumulation of toxic metabolites may be the result of drugs used both as components of antiretroviral therapy, especially older representatives, and for the treatment of opportunistic infections. (20)

Senescence is a progressive process in which telomere shortening during DNA transcription leads to the expression of a senescent cellular phenotype, resulting in the disproportionate secretion of the pro-inflammatory cytokines IL-6 and IL-8 (21).

2.3 Estimation of liver damage

The best way to assess the extent of liver damage is to perform a puncture biopsy of the liver (PBH). In current practice, the degree of liver injury can be assessed using non-invasive imaging techniques (abdominal ultrasound, transient hepatic elastography, MRI elastography), biochemical tests (TGP, TGO, GGT, bilirubin, cholesterol, INR, prothrombin concentration, albumin) and indirect markers (calculated using scores based on routine tests - APRI, FIB4, FORNS, AAR index, AP index, Fibroindex, Bonacini index, Fibro Q, HUI score, Fibromax examination). These allow an immediate, accurate diagnosis of liver damage and the possibility of monitoring the progression of liver disease.

APRI score - AST to Platelet Ratio Index

((AST/upper normal value)/platelets(10^9/l)) x 100)

((APRI < 0.5: minimal or absent fibrosis, APRI 0.5 – 1.5: moderate or significant fibrosis, APRI > 1.5: severe liver fibrosis or cirrhosis)**.**

FIB-4 score – Fibrosis index based on the 4 factor

(age (years) xAST (U/l)) / (platelets (10^9/ l) xALT (U/l)$^{1/2}$)

(FIB4 < 1.45: Ishak 0-1, FIB4 between 1.45 – 3.25: Ishak 2-3, FIB4 > 3.25: Ishak 4-6)

FORNS score

(7.811-3.131 xln(platelets) + 0.781 x ln(GGT) + 3.467 x ln(age) – 0.014 xcholesterol)

(FORNS < 4.25: F0-F1, FORNS > 6.9: F2-F3-F4, FORNS between 4.25 şi 6.9 does not distinguish between different stages of liver fibrosis).

2.4 Liver involvement in HIV infection. Evidence from clinical studies.

Several clinical and epidemiological studies have reported that HIV induces hepatic fibrogenesis (34) in the absence of co-infection with other hepatitis viruses. Therefore, detectable viremia is essential to determine liver damage. (35) A close correlation between AST and HIV-HIV RNA levels has been demonstrated. (36) In addition, mathematical modelling of elevated liver enzymes in HIV monoinfection has shown that a significant increase in ALT correlates with increased viral load. (37) Data from the CFAR (Centre for AIDS Research) have shown that untreated HIV monoinfection is an independent risk factor for liver fibrosis. (38) A large clinical study conducted in North America showed that in patients with HIV monoinfection, elevated plasma HIV-HIV RNA levels were associated with significant fibrosis (FIB-4).(39) Three other studies using transhepatic elastography to assess the degree of liver fibrosis have shown a direct correlation between liver damage (40-44) and elevated HIV RNA levels. (45) Elevated plasma HIV viremia results in chronically elevated ALT levels (45, 46) and hepatic steatosis (47). The association between detectable HIV-HIV RNA levels and APRI scores above 1.5 directly correlates with the risk of developing liver disease and significant liver fibrosis.(48) The mechanisms by which HIV is involved in causing liver damage depend to some extent on the functionality of the immune system. (49) For example, in a retrospective study, elevated ALT levels correlated with CD4+ levels < 200 cells/mm3. (46) In another cross-sectional study, a CD4+ count < 200/mm3 was considered a predictor of abnormal liver stiffness. (42)

PERSONAL CONTRIBUTION

I. Working hypothesis/ Objectives

The research hypothesis is that liver damage may be a direct or indirect consequence of HIV infection, with liver fibrosis being present in newly diagnosed patients or in those with virological and immunological failure after antiretroviral therapy, in the absence of other risk factors.

The main objectives of the thesis were: to determine the degree of liver damage induced by HIV, to determine how the patient's virological status influences the stage of liver damage, to establish the usefulness of APRI, FIB4, FORNS scores in assessing the degree of liver damage in HIV-infected patients.

The secondary objectives were: to determine how liver damage varies according to the type of infection (HIV monoinfection or HIV/hepatitis co-infection), to determine the best predictor of CD4+, HIV-HIV RNA and type of infection in determining liver fibrosis.

II. General methodology

We conducted a retrospective and prospective longitudinal study, including all patients, at least 18 years of age, newly diagnosed and confirmed with HIV infection at the Clinical Hospital of Infectious Diseases Constanța between 1 January 2015 and 30 June 2024. One year after starting antiretroviral therapy, the remaining patients were re-evaluated clinically and biologically to assess the degree of liver damage after starting antiretroviral therapy. In 2024, FibroScan was used to assess the stage of liver fibrosis in a proportion of these patients on antiretroviral therapy.

A. Evaluation of newly diagnosed HIV-infected patients for liver impairment at the start of antiretroviral therapy and after one year of therapy

1. Material and Methods

Between 1 January 2015 and 30 June 2024, 351 patients were diagnosed with HIV infection, 313 with HIV monoinfection and 37 with HIV/hepatitis co-infection (one patient had no AgHbs or AcVHC results for objective reasons). Biological and viro-immunological evaluation was performed one year after initiation of antiretroviral therapy in 278 patients.

Patients were biohumorally assessed for immunological status, co-infections, liver and kidney function, lipid profile and staged according to CDC criteria. The degree of liver involvement was assessed using the APRI, FIB4 and FORNS liver fibrosis scores. For their calculation and interpretation, we used the formulas available on the Internet:
https://www.mdcalc.com/calc/3094/ast-platelet-ratio-index-apri
https://www.mdcalc.com/calc/2200/fibrosis-4-fib-4-index-liver-fibrosis
https://www.rccc.eu/calculadoras/Forns.html.

We entered into the database the year of confirmation, sex and age of the patients, background - urban/rural, CDC HIV infection status and the following variables at both points of assessment: HBsAg, HCVsAg, HDVsAg (positive/negative), CD4+ (cells/mm3), HIV-RNA

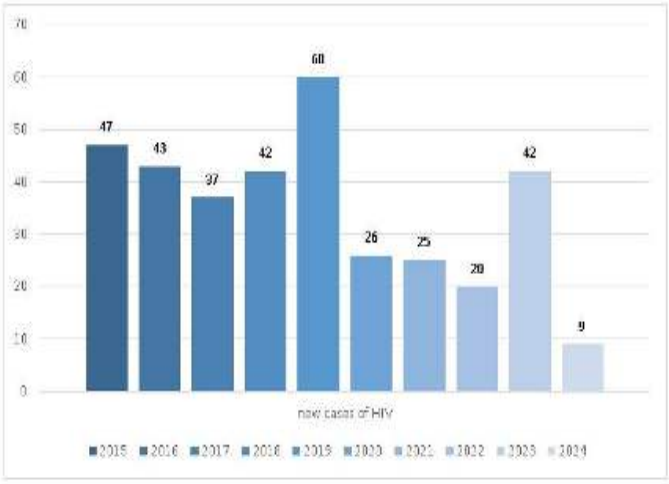
(copies/ml), platelets/mm3, TGP, TGO, GGT (U/l), total cholesterol (mg/dl), APRI, FIB4 and FORNS score values, antiretroviral therapy initiated after diagnosis.

For statistical processing of the survey data we used IBM SPSS Statistics for Windows, version 29.0. (30-day trial version) Armonk, NY: IBM Corp. Nominal data were presented as absolute frequencies and percentages, and continuous variables were expressed as means, medians, minimums and maximums. Analysis of associations between categorical variables was performed using cross-tabulation and the $\chi 2$ (chi-squared) test. Fisher's exact test was used when the results of the chi-square test were sufficiently skewed to be disregarded. The ANOVA test was used to compare means of parameters between groups. Stepwise multiple regression was used to select predictors of a given dependent variable based on statistical criteria. A coefficient of statistical significance of $p < 0.05$ was considered significant.

2. Results

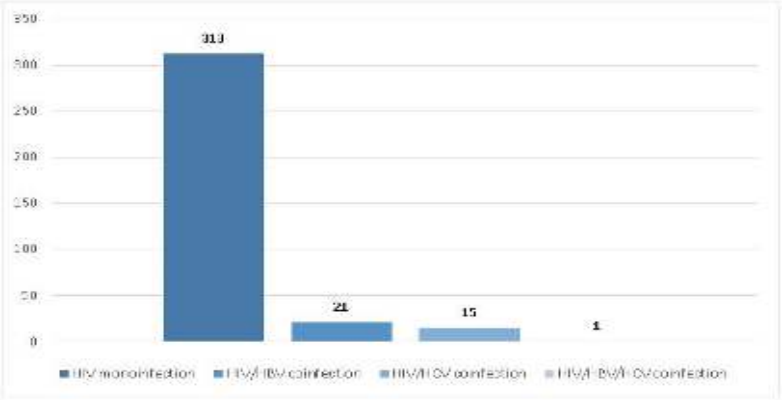
2.1 Annual distribution of cases diagnosed with HIV infection

Figure 1. Annual distribution of new HIV infections (N)



Between 01/01/2015 and 31/12/2023, 342 patients were diagnosed and another 9 in the first 6 months of 2024, 313 with HIV monoinfection and 37 with HIV/hepatitis co-infection (21 patients co-infected with HIV/HBV, 15 with HIV/HCV and one with HIV/HBV/HCV). Overall, from 2015 to 2024, with the exception of 2019, we observe a decreasing trend in the number of new cases with confirmed HIV infection.

Figure 2. Distribution of monoinfection vs. coinfection



2.2 Gender and age distribution of patients

The majority of people in the study were male - 237 patients (67.5%), from urban areas - 242 patients (68.9%), with a mean age at diagnosis of 36.09 years and a median age of 34 years.

Figure 3. Gender distribution of patients

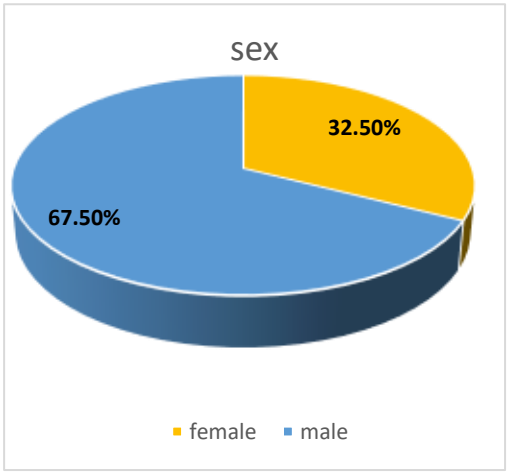
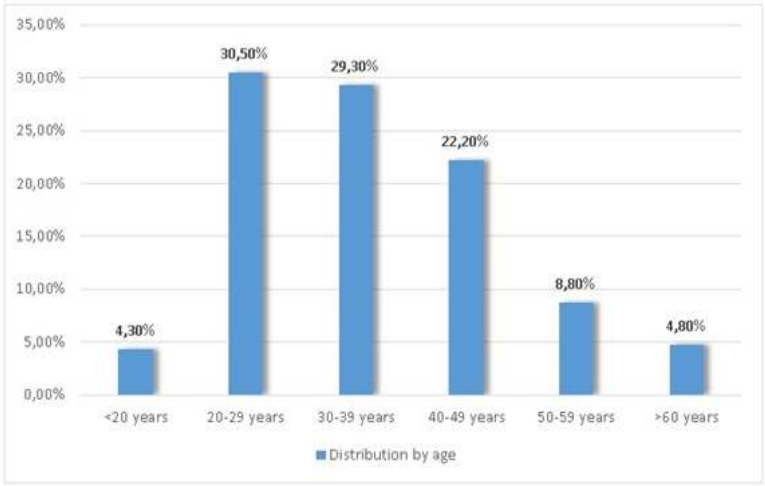
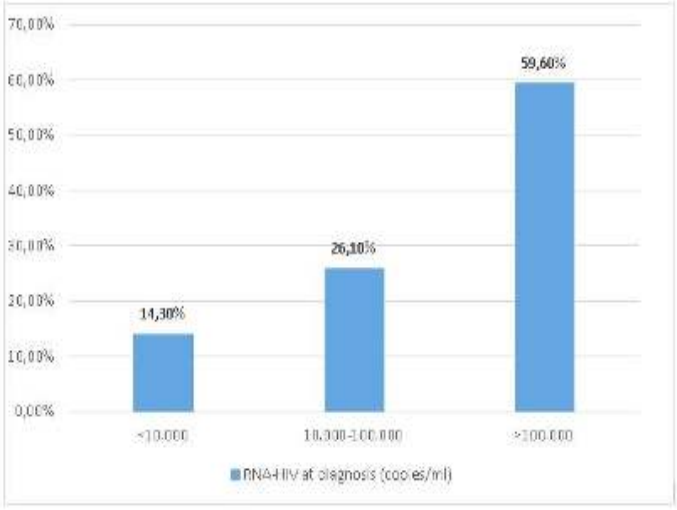


Figure 4. Distribution of cases by patient age (%)



2.3 Plasma HIV-RNA levels – particularities

Figure 5. Distribution of HIV-RNA values (copies/ml) in the general group



Plasma HIV viremia was measured in 349 patients, 59.6% (208 patients) with HIV-HIV RNA $>10^5$ copies/mL and 26.1% (91 patients) with values between 10^4 and 10^5 copies/mL.

Looking at the viral load according to the type of infection, 59.2% of patients with monoinfection (184 cases), 66.7% (14 cases) of those diagnosed with HIV/HBV coinfection and 60% (9 patients) of those with HIV/HCV coinfection had HIV-HIV RNA $>10^5$ copies/mL.

Table I - Distribution of HIV-RNA values (copies/ml) according to the type of infection

			ARN-HIV at diagnosis (copies/ml)			
			$<10^4$	10^4-10^5	$>10^5$	Total
Infection type	HIV monoinfection	Frequency	44	83	184	311
		%	14,1	26,7	59,2	100,0
	HIV/VHB coinfection	Frequency	4	3	14	21
		%	19,0	14,3	66,7	100,0
	HIV/VHC coinfection	Frequency	2	4	9	15
		%	13,3	26,7	60,0	100,0
	HIV/VHB/VHC coinfection	Frequency	0	0	1	1
		%	0,0	0,0	100,0	100,0
	Total	Frequency	50	90	208	348
		%	14,4	25,9	59,8	100,0

Table II. Minimum, maximum and mean values of HIV-RNA (copies/ ml)according to type of infection

	N	Mean	Minimum	Maximum
HIV monoinfection	311	1166315,81	40	29000000
HIV/VHB coinfection	21	681375,90	1998	2421951
HIV/VHC coinfection	15	424760,73	2219	1370000
HIV/VHB/VHC coinfection	1	10000000	10000000	10000000
Total	348	1130472,76	40	29000000

Patients with HIV monoinfection had a higher mean viral load than those with co-infection. Using ANOVA, we accept the hypothesis that there are statistically significant differences between groups in HIV viral load at diagnosis (df = 3, F = 3.276 and p = 0.021), patients with HIV/hepatitis co-infection, especially those with HIV/HBV, were slightly more likely to have a very high viral load at diagnosis than those with HIV monoinfection.

Table III. HIV-RNA values (copies/ml) according to CDC stage of HIV infection and type of infection

RNA-HIV at diagnosis: copies/ml

Infection type		N	Media	Minim	Maxim
HIV monoinfection	A1	51	989544,76	40	10000000
	A2	85	270982,73	515	9740000
	A3	15	518247,73	11900	3380000
	B1	11	981219,73	136	10000000
	B2	37	651120,95	157	10000000
	B3	31	2902494,19	4220	29000000
	C1	3	441343,33	2430	1250000
	C2	13	1608328,54	7390	13279634
	C3	64	2081338,63	6090	17985698
HIV/VHB coinfection	A1	2	31607,50	2515	60700
	A2	3	156268,67	34806	236000
	B2	1	1998,00	1998	1998
	B3	4	957250,00	580000	1660000
	C1	1	89900,00	89900	89900
	C3	10	985597,50	9474	2421951
HIV/VHC coinfection	A2	2	129685,00	100807	158563
	A3	1	1160000,00	1160000	1160000
	B2	6	318111,50	2219	1240000
	B3	3	546549,00	51663	1370000
	C3	3	467908,33	17100	1290000

Regarding the distribution of HIV-RNA values according to the stage of HIV infection and the type of infection, we observed that the highest mean and maximum values were in the group of patients with HIV monoinfection, stage B3. In the group with HIV/HBV coinfection, the highest mean and maximum HIV-HIV RNA values were in stage C3, and in the group with HIV/HCV coinfection, the highest mean value was in stage A3 and the maximum value was in stage C3.

In HIV monoinfection, F was 3.053 and p was 0.003, with significant differences between HIV

infection stages and HIV-HIV RNA levels. In HIV/HBV and HIV/HCV coinfection, F = 1.827 and p = 0.168, F = 0.642 and p = 0.645, respectively, there were no significant differences.

In multiple comparisons (Bonferroni), for monoinfection, the comparison of mean HIV-RNA between different CDC stages of HIV infection showed differences between stage B3 and other stages (A2 vs. B3, mean difference -2,631,511.464 and p = 0.003, A2 vs. C3, mean difference -1,810,355.896 and p = 0.019, B3 vs. A2, mean difference 2,631,511.464 and p = 0.003). There were no significant differences for HIV/HBV and HIV/HCV co-infection.

2.4 Clinical and immunological staging of HIV infection

According to symptoms at diagnosis and immune status, most patients were classified as stage A2 (26.1 %) and C3 (22.1 %), and the fewest as stage C1 (1.1 %).

Figure 6. Distribution of cases according to CDC classification

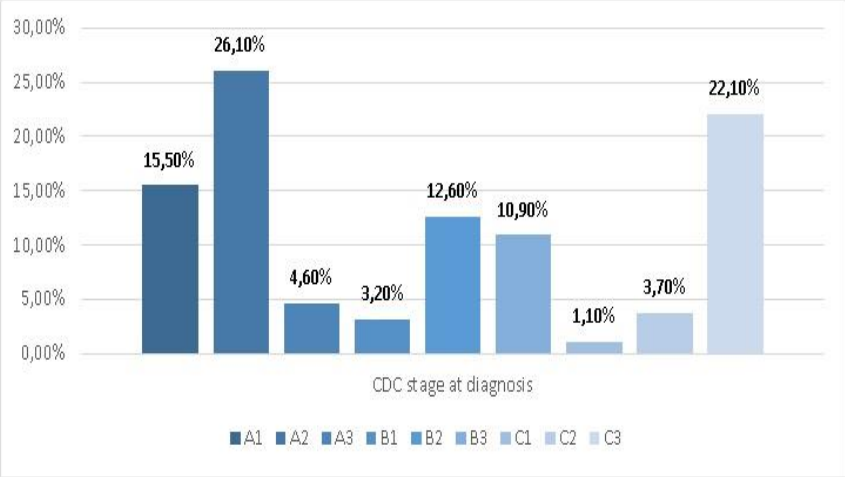
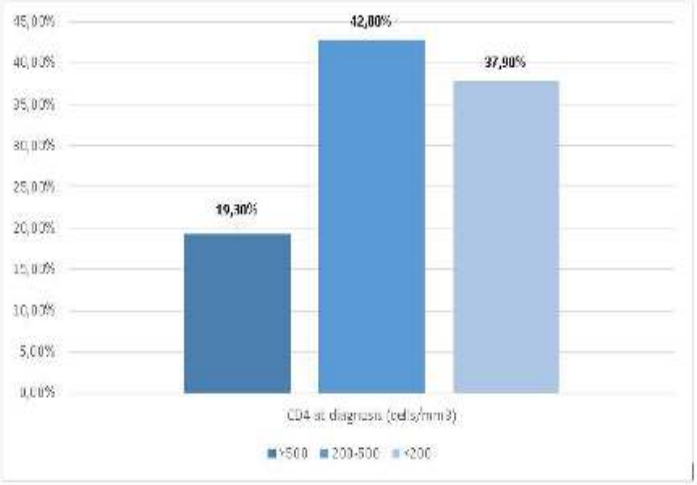


Figure 7. Immunologic staging of HIV infection



The CD4+ cell count, in the overall lot, was determined in 348 patients: 19.3 % (67 patients) had more than 500 cells/mm3, 42.8 % (149) between 200-500 cells/mm3, 37.9 % (132) < 200 cells/mm3. The mean was 309.8 cells/mm3 and the median 269.5 cells/mm3.

Table IV. Distribution of CDC stages of HIV infection by infection type

		HIV monoinfection		HIV/VHB coinfection		HIV/VHC coinfection		HIV/VHB/VHC coinfection	
		Frequency	%	Frequency	%	Frequency	%	Frequency	%
CDC stage at diagnosis	A1	52	16,7	2	9,5	0	0,0	0	0,0
	A2	85	27,3	3	14,3	2	13,3	1	100,0
	A3	15	4,8	0	0,0	1	6,7	0	0,0
	B1	11	3,5	0	0,0	0	0,0	0	0,0
	B2	37	11,9	1	4,8	6	40,0	0	0,0
	B3	31	10,0	4	19,0	3	20,0	0	0,0
	C1	3	1,0	1	4,8	0	0,0	0	0,0
	C2	13	4,2	0	0,0	0	0,0	0	0,0
	C3	64	20,6	10	47,6	3	20,0	0	0,0
Total		311	100,0	21	100,0	15	100,0	1	100,0

According to the clinico-immunological staging and to the type of infection, we observed that the majority of patients with HIV monoinfection were in stage A (48.9 %) and had $CD4^+$ between 200 and 500/mm3 (135 patients), 110 patients were severely immunocompromised ($CD4^+$ below 200/mm3). Those with HIV/HBV co-infection were mostly in stage C (52.4%) and had $CD4^+ < 200$/mm3 (14 patients, respectively 66.7 %), and those with HIV/HCV co-infection were mostly in stage B (60%), all patients with $CD4^+$ cell counts below 500/mm3, 7 patients (46.7 %) with $CD4^+$ cell counts even below 200/mm^3.

Table V. $CD4^+$ cell values (cells/mm3) according to infection type

Infection type	Mean	Median	Minimum	Maximum
HIV monoinfection	320,48	281,00	1	1423
HIV/VHB coinfection	204,10	148,00	8	668
HIV/VHC coinfection	237,47	254,00	6	472
HIV/VHB/VHC coinfection	295,00	295,00	295	295

HIV monoinfected patients had the highest mean $CD4^+$ cell count (320.48 cells/mm^3), while co-infected patients had lower mean $CD4^+$ cell counts. Using the ANOVA test, we obtained F values of 1.826, df =3 and p of 0.142, indicating that there were no statistically significant differences between the mean CD4+ cell counts at diagnosis for the patient groups included in the study.

2.5 Transaminase levels by type of infection

Figure 8. TGP values according to infection type

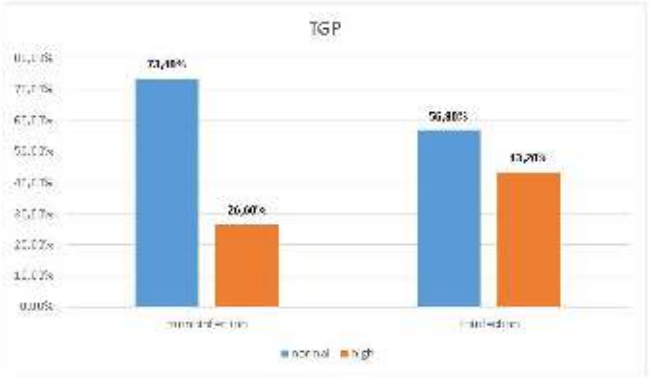
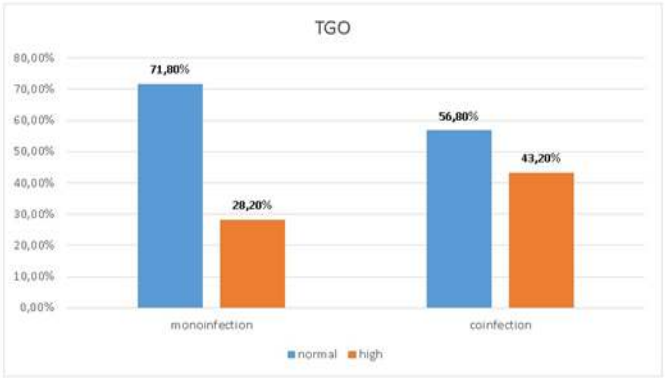


Figure 9. TGO values according to infection type



Transaminases were measured in 338 of the patients: TGP was elevated in 26.6 % (80) of HIV monoinfected patients and 43.2 % (16) of co-infected patients. TGO was elevated in

28.2 % (85) of HIV monoinfected patients and 43.2 % (16) of co-infected patients. Both mean and median TGP and TGO levels were higher in the co-infected group.

2.6 Correlations between transaminases, CD4+ cell counts and infection type

Table VI. Distribution of TGP values according to $CD4^+$ and infection type

Infection type	TGP		CD4+ (cells/mm³) >500	200-500	<200
HIV monoinfection	normal	Frequency	47	106	68
		%	21,3	48,0	30,8
	high	Frequency	14	28	38
		%	17,5	35,0	47,5
HIV/hepatitis viruses coinfection	normal	Frequency	3	7	11
		%	14,3	33,3	52,4
	high	Frequency	0	6	10
		%	0,0	37,5	62,5

Table VII. Distribution of TGO values according to $CD4^+$ and infection type

Infection type	TGO		CD4+ (cells/mm³) >500	200-500	<200
HIV monoinfection	normal	Frequency	49	111	56
		%	22,7	51,4	25,9
	high	Frequency	12	23	50
		%	14,1	27,1	58,8
HIV/hepatitis viruses coinfection	normal	Frequency	3	7	11
		%	14,3	33,3	52,4
	high	Frequency	0	6	10
		%	0,0	37,5	62,5

Using the chi-squared test, we observed that in HIV monoinfection there was a significant association between $CD4^+$ cell count and TGP at diagnosis (p=0.026), with levels below 200 cells/mm^3 associated with increased TGP levels. Although the same was observed in the co-infected group, the association was not statistically significant (p = 0.287). Similar results were obtained for TGO, which increased with decreasing CD4+ cell count, with a significant association in the monoinfected group (p < 0.001).

2.7 Correlations between transaminase values, HIV-HIV RNA values and infection type

Tabelul VIII. TGP values * HIV-RNA values*type of infection

Infection type	TGP		ARN-HIV (copies/ml) $<10^4$	10^4-10^5	$>10^5$
HIV monoinfection	normal	Frequency	35	60	125
		%	15,9	27,3	56,8
	high	Frequency	8	20	52
		%	10,0	25,0	65,0
HIV/hepatitis viruses coinfection	normal	Frequency	4	6	11
		%	19,0	28,6	52,4
	high	Frequency	2	1	13
		%	12,5	6,3	81,3

Tabelul IX. TGO values * HIV-RNA values* type of infection

Tipul infecției	TGO		ARN-HIV (copies/ml) $<10^4$	10^4-10^5	$>10^5$
HIV monoinfection	normal	Frequency	34	67	114
		%	15,8	31,2	53,0
	high	Frequency	9	13	63
		%	10,6	15,3	74,1
HIV/hepatitis viruses coinfection	normal	Frequency	5	6	10
		%	23,8	28,6	47,6
	high	Frequency	1	1	14
		%	6,3	6,3	87,5

Irrespective of the type of infection, we observed an increase in the percentage of patients with high transaminase values in proportion to the HIV-RNA value. The association between elevated TGP values and HIV-RNA level was not statistically significant (p=0.331 in monoinfection and p=0.150 in

coinfection) but there was a significant association between TGO level and HIV-RNA value. IncreasedTGO values were more frequent in patients with HIV-RNA > 105 copies/ ml (df=2, p=0.003, Cramer`s V of 0.196 in monoinfection and df=2, p=0.042, Cramer`s V of 0.414 in coinfection). The association was stronger in coinfection.

2.8 Correlations between transaminase values, clinical stage of HIV infection and infection type

Patients with advanced HIV infection (B, C) were more likely to have elevated TGP and TGO levels. In HIV monoinfection there was a statistically significant association between these variables (p=0.014, df=2, Cramer's V of 0.168, respectively p<0.001, df=2, Cramer's V of 0.327). In co-infected patients, the association was not statistically significant.

Table X. TGP values * clinical stage of HIV infection * type of infection

Infection type	TGP		Clinical stage of HIV infection A	B	C
HIV monoinfection	normal	Frequency	119	55	47
		%	53,8	24,9	21,3
	high	Frequency	29	23	28
		%	36,3	28,8	35,0
HIV/hepatitis viruses coinfection	normal	Frequency	6	6	9
		%	28,6	28,6	42,9
	high	Frequency	3	8	5
		%	18,8	50,0	31,3

Table XI. TGO values * clinical stage of HIV infection * type of infection

Infection type	TGO		Clinical stage of HIV infection A	B	C
HIV monoinfection	normal	Frequency	126	53	37
		%	58,3	24,5	17,1
	high	Frequency	22	25	38
		%	25,9	29,4	44,7
HIV/hepatitis viruses coinfection	normal	Frequency	6	7	8
		%	28,6	33,3	38,1
	high	Frequency	3	7	6
		%	18,8	43,8	37,5

2.9 Distribution of $CD4^+$ cell values according to APRI, FIB4 and FORNS score values in the overall group

Table XII. $CD4^+$ values * APRI * liver fibrosis stage

			APRI at diagnosis < 0.5 F0F1	0.5-1.5 F2	>1.5 F3F4
$CD4^+$ (cells/mm^3)	>500	Frequency	51	12	1
		%	79,7	18,8	1,6
	200-500	Frequency	111	30	6
		%	75,5	20,4	4,1
	<200	Frequency	56	45	26
		%	44,1	35,4	20,5
Total		Frequency	218	87	33
		%	64,5	25,7	9,8

In the study group, moderate/severe immunosuppression was associated with higher liver fibrosis scores - APRI, FIB4 and FORNS - and a higher risk of liver fibrosis progression. Patients with lower $CD4^+$ cell counts were more likely to be in more advanced stages of liver fibrosis (F3F4), while patients with higher $CD4^+$ cell counts were more likely to be in stages F0F1. As the p-value for each of these

Table XIII. $CD4^+$ values * FIB4 * liver fibrosis stage

			FIB4		
			<1.45	1.45-3.25	>3.25
			Ishak 0-1	Ishak 2-3	Ishak 4-6
$CD4^+$ (cells/mm^3)	>500	Frequency	53	9	2
		%	82,8	14,1	3,1
	200-500	Frequency	126	15	6
		%	85,7	10,2	4.1
	<200	Frequency	69	39	19
		%	54,3	30,7	15
Total		Frequency	248	63	27
		%	73,37	18,63	8

associations was less than 0.001, the correlation is statistically significant, and the association between the two variables is moderate (Cramer's V of 0.258 for APRI, 0.242 for FIB4 and 0.296 for FORNS).

Tabelul XIV. $CD4^+$ values * FORNS * liver fibrosis stage

			FORNS		
			<4.25	>6.9	4.25-6.9
			F0-F1	F2-F3-F4	P=NS
$CD4^+$ (cells/mm^3)	>500	Frequency	49	3	12
		%	76,6	4,7	18,8
	200-500	Frequency	109	4	34
		%	74,1	2,7	23,1
	<200	Frequency	43	23	59
		%	34,4	18,4	47,2
Total		Frequency	201	30	105
		%	59,8	8,9	31,3

The observation made on the FORNS score was that, for values between 4.25 and 6.9, it could not differentiate the stage of liver fibrosis in 105 of the 336 patients evaluated (31.3%), with a higher percentage of patients with $CD4^+$ < 200 cells/ mm^3.

2.10 Distribution of RNA-HIV values according to APRI, FIB4 and FORNS score values in the overall group

Table XV. RNA-HIV values * APRI * liver fibrosis stage

			APRI at diagnosis		
			<0.5	0.5-1.5	>1.5
			F0F1	F2	F3F4
RNA-HIV (copies/ml)	$<10^4$	Frequency	45	4	0
		%	91,8	8,2	0,0
	10^4-10^5	Frequency	71	14	2
		%	81,6	16,1	2,3
	$>10^5$	Frequency	101	69	31
		%	50,2	34,3	15,4

Table XVI. RNA-HIV values * FIB4 * liver fibrosis stage

			FIB4		
			<1.45	1.45-3.25	>3.25
			Ishak 0-1	Ishak 2-3	Ishak 4-6
RNA-HIV (copies/ml)	$<10^4$	Frequency	44	4	1
		%	89,8	8,2	2,0
	10^4-10^5	Frequency	76	6	5
		%	87,4	6,9	5,7
	$>10^5$	Frequency	127	53	21
		%	63,2	26,4	10,5

We observed that as the plasma HIV load increased, so did the liver fibrosis scores. Thus, the proportion of patients with advanced liver fibrosis (F3F4) increased significantly and the proportion of patients with no fibrosis or mild fibrosis (F0F1) decreased. The chi-squared test

used to determine the statistical significance of this relationship indicated an association between these variables, with a p-value < 0.001. This result suggests that plasma HIV viremia is associated with the degree of liver fibrosis at diagnosis, although the association between the two variables is moderate (Cramer's V of 0.264 for APRI, 0.200 for FIB4 and 0.263 for FORNS).

Regarding the distribution of HIV-RNA levels according to FORNS score values, we observed that at HIV plasma viremia greater than 10^5 copies/ml the FORNS score increased, but in a significant percentage of patients the stage of liver fibrosis could not be differentiated (42 % or 84 patients).

Tabelul XVII. RNA-HIV values * FORNS * liver fibrosis stage

			FORNS		
			<4.25	>6.9	4.25-6.9
			F0-F1	F2-F3-F4	P-NS
RNA-HIV (copies/ml)	$<10^4$	Frequency	43	1	5
		%	87,8	2,0	10,2
	10^4-10^5	Frequency	67	3	16
		%	77,9	3,5	18,6
	$>10^5$	Frequency	90	26	84
		%	45,0	13,0	42,0

2.11 Distribution of APRI, FIB4 and FORNS score values according to CD4+, HIV-RNA, HIV infection type and stage

Table XVIII. APRI values * liver fibrosis stage* infection type

Infection type	APRI	Fibrosis	Frequency	Percent
HIV monoinfection	<0.5	F0F1	202	67,1
	0.5-1.5	F2	75	24,9
	>1.5	F3F4	24	8,0
HIV/hepatitis viruses coinfection	<0.5	F0F1	16	43,2
	0.5-1.5	F2	12	32,4
	>1.5	F3F4	9	24,3

The majority of patients enrolled in the study, including those with HIV/hepatitis co-infection, had mild or no fibrosis (F0F1). However, there was a significant proportion with moderate/severe fibrosis in both groups (24.9 % with F2, 8 % with F3F4 in HIV monoinfected patients and 32.4 % with F2, 24.3 % with F3F4 in the co-infected group), with co-infected patients tending to have a more severe degree of liver fibrosis compared to HIV monoinfected patients.

Table XIX. Mean, median, minimum, maximum APRI values * infection type

Infection type	Mean	Median	Minimum	Maximum
HIV monoinfection	0,726	0,310	0,100	39,400
HIV/hepatitis viruses coinfection	1,920	0,540	0,150	18,280

The mean APRI score was 1.920 in patients with coinfection, significantly higher than in the HIV monoinfected group (0.726), corresponding to liver

fibrosis stage F3F4. The median value of 0.540 in coinfection suggests that more than half of these patients have at least stage F2 liver fibrosis.

To determine whether there were statistically significant differences in the stage of liver fibrosis (assessed by APRI score values) between the two groups of patients according to HIV-RNA and $CD4^+$ levels, we used the ANOVA test.

Table XX. APRI * Liver fibrosis stage * RNA-HIV values * $CD4^+$ values * infection type

		APRI	Fibrosis	N	Mean	Minimum	Maximum
HIV monoinfection	RNA-HIV (copies/ml)	<0.5	F0F1	201	581977,93	40	29.000.000
		0.5-1.5	F2	75	1819216,20	157	17.985.698
		>1.5	F3F4	24	3152614,08	20.300	16.420.000
	CD4+ (cells/mm³)	<0.5	F0F1	202	370,44	3	1176
		0.5-1.5	F2	75	251,60	1	1423
		>1.5	F3F4	24	139,75	2	501
HIV/hepatitis viruses coinfection	RNA-HIV (copies/ml)	<0.5	F0F1	16	451998,00	1.998	2.421.951
		0.5-1.5	F2	12	1310377,50	9.474	10.000.000
		>1.5	F3F4	9	858200,78	100.807	1.370.000
	CD4+ (cells/mm³)	<0.5	F0F1	16	276,63	8	668
		0.5-1.5	F2	12	205,67	21	365
		>1.5	F3F4	9	138,78	6	393

In HIV monoinfection, the F value of 11.618 and $p < 0.001$ suggest that there are statistically significant differences between these parameters, with the distribution of HIV viremia varying significantly according to these variables. Similarly, the F value of 13,209 and $p < 0.001$ suggest significant differences between fibrosis groups according to $CD4^+$ cell count. Patients with co-infection had an F-value of 0.898 and $p = 0.417$ and an F-value of 2.046 and $p = 0.145$, respectively, indicating that the differences between HIV- RNA and $CD4^+$ and stage of liver fibrosis were not statistically significant.

Table XXI. APRI * liver fibrosis stage * clinical stage of HIV * infection type

				Clinical stage of HIV infection		
Infection type	APRI	Fibrosis		A	B	C
HIV monoinfection	< 0.5	F0F1	Frequency	119	48	35
			%	58,9	23,8	17,3
	0.5-1.5	F2	Frequency	24	23	28
			%	32,0	30,7	37,3
	>1.5	F3F4	Frequency	5	7	12
			%	20,8	29,2	50,0
HIV/hepatitis viruses coinfection	< 0.5	F0F1	Frequency	4	7	5
			%	25,0	43,8	31,3
	0.5-1.5	F2	Frequency	4	3	5
			%	33,3	25,0	41,7
	>1.5	F3F4	Frequency	1	4	4
			%	11,1	44,4	44,4

For APRI values > 1.5, we observed an increased percentage of patients with advanced HIV infection. The p-value < 0.001 indicates that in the HIV monoinfected group there is a statistically significant association between the stage of liver fibrosis and the severity of the clinical form, the correlation between the two variables being moderate (Cramer's V of 0.219). This suggests that clinical stage (A, B, C) has a significant effect on APRI. In the co-infected group, these differences were not statistically significant, p was 0.707, Cramer's V of 0.171, indicating a weak correlation between the two variables.

When we staged liver fibrosis according to FIB4 score, we obtained similar results: regardless of the type of infection, most patients had FIB4 scores below 1.45, corresponding to minimal or mild fibrosis stages (Ishak 0-1). Moderate and advanced fibrosis (FIB4 score

between 1.45 and 3.25 and above 3.25, respectively) were associated with a higher percentage of co-infected cases. This suggests that patients with coinfection may have a higher risk of developing advanced liver fibrosis than those with monoinfection.

Table XXII. FIB4 * liver fibrosis stage * infection type

Infection type	FIB4	Fibrosis	Frequency	Percent
HIV	<1.45	Ishak 0-1	226	75.1
monoinfection	1.45-3.25	Ishak 2-3	55	18.3
	>3.25	Ishak 4-6	20	6.6
HIV/hepatitis	<1.45	Ishak 0-1	22	59.5
viruses	1.45-3.25	Ishak 2-3	8	21.6
coinfection	>3.25	Ishak 4-6	7	18.9

Table XXIII. Mean, median, minimum and maximum FIB4 score values * infection type

Infection type	Mean	Median	Minimun	Maximum
HIV monoinfection	1,414	0,830	0,220	42,350
HIV/hepatitis viruses coinfection	2,854	1,170	0,290	29,950

Comparing the FIB4 score values for the two categories of patients, we observed that the mean FIB4 was higher in the co-infected group, indicating a higher severity of liver fibrosis in these patients. As the median is also higher (still below 1.45), this confirms that co-infected patients tend to have higher values of this score and therefore more advanced stages of liver fibrosis.

Table XXIV. Mean, median, minimum and maximum $CD4^+$ values * FIB4 * liver fibrosis stage * infection type

Infection type	FIB4	Fibrosis	Mean	Min.	Max.
HIV	<1.45	Ishak 0-1	355,73	2	1176
monoinfection	1.45-3.25	Ishak 2-3	234,33	1	1423
	>3.25	Ishak 4-6	188,45	2	649
		Total	322,44	1	1423
HIV/hepatitis	<1.45	Ishak 0-1	268,41	8	668
viruses coinfection	1.45-3.25	Ishak 2-3	165,25	32	315
	>3.25	Ishak 4-6	130,86	6	393
		Total	220,08	6	668

To determine whether the stage of liver fibrosis varied with the degree of immunosuppression of the patient, i.e. HIV plasma viremia levels, we followed the distribution of these levels in each group of patients. Although we observed a trend of decreasing mean CD4+ cell counts with increasing severity of liver fibrosis, regardless of the type of infection, this correlation was statistically significant only in those with HIV monoinfection ($p < 0.001$, $F = 8,071$) and not statistically significant in those with co-infection ($p = 0.104$, $F = 2,418$).

Table XXV. Mean, minimum and maximum RNA-HIV values * FIB4 * liver fibrosis stage * infection type

Infection type	FIB4	Fibrosis	N	Mean	Min.	Max.
HIV	<1.45	Ishak 0-1	225	856001,20	40	29.000.000
Monoinfection	1.45-3.25	Ishak 2-3	55	2178897,02	157	16.420.000
	>3.25	Ishak 4-6	20	832095,55	2430	4.680.000
		Total	300	1096938,39	40	29.000.000
HIV/hepatitis	<1.45	Ishak 0-1	22	912275,77	1998	10.000.000
viruses	1.45-3.25	Ishak 2-3	8	631303,88	34806	1.240.000
coinfection	>3.25	Ishak 4-6	7	794258,14	100807	1.370.000
		Total	37	829197,43	1998	10.000.000

HIV-RNA levels varied significantly by FIB4 score in the HIV monoinfected group (p of 0.012 and F of 4.516 indicating a statistically significant difference between groups). In coinfection, HIV-RNA levels were lower on average than in monoinfection, regardless of the stage of liver fibrosis, but the association was not statistically significant. F of 0.080 and p of 0.923 indicated that there was no significant difference between the mean HIV-RNA levels in these patients according to FIB4 level.

Table XXVI. Clinical stages of HIV infection * FIB4 * liver fibrosis stage * infection type

				Clinical stage of HIV infection		
Infection type	FIB4	Fibrosis		A	B	C
HIV	<1.45	Ishak 0-1	Frequency	127	57	42
monoinfection			%	56,2	25,2	18,6
	1.45-3.25	Ishak 2-3	Frequency	13	16	26
			%	23,6	29,1	47,3
	>3.25	Ishak 4-6	Frequency	8	5	7
			%	40,0	25,0	35,0
		Total	Frequency	148	78	75
			%	49,2	25,9	24,9
HIV/hepatitis	<1.45	Ishak 0-1	Frequency	6	8	8
viruses			%	27,3	36,4	36,4
coinfection	1.45-3.25	Ishak 2-3	Frequency	2	4	2
			%	25,0	50,0	25,0
	>3.25	Ishak 4-6	Frequency	1	2	4
			%	14,3	28,6	57,1
		Total	Frequency	9	14	14
			%	24,3	37,8	37,8

For the studied group we observed that the stage of liver fibrosis was more advanced with the severity of HIV infection. In patients with monoinfection the association between these variables was moderate (Cramer`s V of 0.206) and statistically significant, p being < 0.001. In the case of coinfection, Cramer`s V of 0.160 indicated a weak association, without statistical significance (p = 0.753).

Using the FORNS score, the results were similar to the previous ones: cases with mild or no liver fibrosis were more common in both groups. However, significant fibrosis was more common in patients with coinfection than in those with monoinfection. Also, in a higher percentage of patients with co-infection, the FORNS score could not differentiate the stages of liver fibrosis. The mean and median FORNS scores were higher in the coinfected group, ranging from 4.25 to 6.9. On average, patients with coinfection have a higher severity of liver fibrosis than those with monoinfection, but the distribution of fibrosis is different in the two groups.

Table XXVII. FORNS values * liver stage fibrosis *infection type

Infection type	FORNS	Fibrosis	Frequency	Percent
HIV monoinfection	<4.25	F0-F1	183	61,2
	>6.9	F2-F3-F4	24	8
	4.25-6.9	P=NS	92	30,8
HIV/hepatitis viruses coinfection	<4.25	F0-F1	18	48,6
	>6.9	F2-F3-F4	6	16,2
	4.25-6.9	P=NS	13	35,1

Table XXVIII. FORNS values * infection type

Infection type	Mean	Median	Min.	Max.
HIV monoinfection	3,773	3,560	0,060	15,070
HIV/hepatitis virus coinfection	4,920	4,500	0,300	11,410

Table XXIX. $CD4^+$ values * FORNS values * liver fibrosis stage * infection type

Infection type	FORNS	Fibrosis	N	Mean	Min.	Max.
HIV Monoinfection	<4.25	F0-F1	183	393,66	2	1176
	>6.9	F2-F3-F4	24	163,43	5	614
	4.25-6.9	P=NS	92	229,65	1	1423
		Total	299	324,47	1	1423
HIV/hepatitis virus coinfection	<4.25	F0-F1	18	241,39	8	629
	>6.9	F2-F3-F4	6	161,80	6	393
	4.25-6.9	P=NS	13	228,54	32	668
		Total	37	220,08	6	668

Similarly, on the FORNS score, monoinfected patients had higher $CD4^+$ counts on average than co-infected patients, with the stage of liver fibrosis being more advanced in immunosuppressed patients. In cases with significant fibrosis, F2F3F4, CD4+ cell counts were comparable between the two groups, suggesting that fibrosis progression has a similar impact on $CD4^+$ in both groups. The correlation was statistically significant in monoinfection ($p < 0.001$, $F = 13.131$) but not in HIV/hepatitis virus coinfection ($p = 0.532$, $F = 0.746$).

Table XXX. Mean, minimum, maximum RNA-HIV values * FORNS values *liver fibrosis stage * infection type

Infection type	FORNS	Fibrosis	N	Mean	Min.	Max.
HIV Monoinfection	<4.25	F0-F1	182	682782,91	40	29.000.000
	>6.9	F2-F3-F4	24	2479748,43	2430	16.420.000
	4.25-6.9	P=NS	92	1500016,43	157	17.985.698
		Total	298	1071957,10	40	29.000.000
HIV/hepatitis virus coinfection	<4.25	F0-F1	18	1059192,61	1998	10.000.000
	>6.9	F2-F3-F4	6	563961,40	100.807	1.370.000
	4.25-6.9	P=NS	13	571156,23	34.806	1.370.000
		Total	37	829197,43	1998	10000000

In the group of patients with HIV monoinfection and liver fibrosis F2F3F4, the mean value of HIV-HIV RNA was higher than in the co-infected group. F was 3.551, and p of 0.015 was statistically significant, which means that increased HIV-HIV RNA levels influence the degree of liver fibrosis. In the HIV/hepatitis virus coinfected group, p was 0.843, which was not statistically significant (F = 0.275).

Table XXXI. FORNS values * liver fibrosis stage * clinical stage of HIV * infection type

Infection type	FORNS	Fibrosis		Clinical stage of HIV infection A	B	C
HIV monoinfection	<4.25	F0-F1	Frequency	107	49	27
			%	58,5	26,8	14,8
	>6.9	F2-F3-F4	Frequency	6	8	10
			%	26,1	33,33	41,66
	4.25-6.9	P=NS	Frequency	35	21	36
			%	38,0	22,8	39,1
HIV/hepatitis virus coinfection	<4.25	F0-F1	Frequency	4	8	6
			%	22,	44,4	33,3
	>6.9	F2-F3-F4	Frequency	1	2	3
			%	16,66	33,33	50,0
	4.25-6.9	P=NS	Frequency	4	4	5
			%	30,8	30,8	38,5

Patients with HIV monoinfection and mild or no liver fibrosis (F0F1) were mostly in clinical stage A (58.5%) and those with significant fibrosis (F2F3F4) were in clinical stage C (41.66%). Cramer's V of 0.221 indicates a moderate association between the two variables, and p below 0.001 indicates that there are statistically significant differences between FORNS score values and the different clinical stages of HIV infection. Hepatic fibrosis was more common when co-infection was associated with clinical stage C (50%). Cramer's V of 0.217 indicates a weak association between these variables, and p of 0.746 indicates that the association is not statistically significant.

2.12 Cumulative effect of $CD4^+$ cell count, HIV-RNA and infection type on APRI, FIB 4 and FORNS liver fibrosis scores

We used stepwise multiple regression in SPSS to estimate the cumulative effect of $CD4^+$ cell counts, HIV plasma viremia and type of infection on liver fibrosis score values. The result was that the addition of each predictor factor increased the percentage change in liver fibrosis scores. For example, low $CD4^+$ cell counts combined with high HIV plasma viremia and advanced clinical stages of HIV infection explain some of the variation in these scores. However, other important factors also influence them.

2.13 Assessment of patients 1 year after HIV diagnosis and initiation of antiretroviral therapy

278 patients were evaluated 1 year after starting antiretroviral therapy, 29 of whom were HIV/hepatitis co-infected. We did not identify situations in which HIV monoinfected patients became co-infected with hepatitis viruses. Following the dynamics of the patients, we observed that 5.71% of the patients died, 5.08% were lost to the registry and 0.95% were transferred to other centres, leaving 305 patients (89.18%) in the registry.

2.13.1 CD4+ cell count assessment

We observed that compared to the $CD4^+$ cell count at the time of HIV diagnosis, the mean (500.61 cells/ mm^3), median (438 cells/ mm^3), minimum (38 cells/ mm^3) and maximum

(1691 cells/ mm^3) values were higher in the overall group after 1 year of ART. 44.53 % of patients had $CD4^+ > 500$ cells/ mm^3 and 41.02 % between 200 and 500 cells/ mm^3.

Table XXXII. Mean, median, minimum and maximum $CD4^+$ values * infection type

Infection type	N	Mean	Median	Min.	Max.
Monoinfection	231	512,58	455,00	38	1691
Coinfection	25	389,96	365,00	56	1007

The mean, median and maximum values were higher compared to the time of diagnosis in both groups, but lower in HIV/hepatitis virus co-infection.

Table XXXIII. $CD4^+$ values * infection type

Infection type	$CD4^+$	Frequency	Percent
HIV monoinfection	>500	107	46,32
	200-500	93	40,26
	<200	31	13,42
	Total	231	100,0
HIV/hepatitis virus coinfection	>500	7	28,0
	200-500	12	48,0
	<200	6	24,0
	Total	25	100,0

The percentage of patients with CD4+ less than 200 cells/ mm3 was lower in monoinfection, 46.3 % with values above 500 cells/ mm3.

2.13.2 Assessment of plasma HIV-RNA values

Plasma HIV viremia levels were assessed in 266 patients, of whom 242 were HIV monoinfected. Viral suppression was observed in 75.9 % of patients in the overall group. The median undetectable level indicates a lack of adherence to ARV medication in some patients. The median undetectable value suggests that at least half of the patients are virally suppressed. A higher percentage of coinfected patients had detectable HIV-RNA levels (37.5 %) compared to monoinfected patients (22.7 %).

Table XXXIV. RNA-HIV values * infection type

Infection type	N	Mean	Median	Min.	Max.
Monoinfection	242	5702,38	0,00	0	561000
Coinfection	24	150,63	0,00	0	1304

Table XXXV. detectability/ suppression of HIV-RNA* infection type

Infection type	RNA-HIV (copies/ml)	Frequency	Percent
HIV monoinfection	<40	187	77,3
	>40	55	22,7
	Total	242	100,0
HIV/hepatitis virus coinfection	<40	15	62,5
	>40	9	37,5
	Total	24	100,0

2.13.3 Assessment of transaminase levels

At the 1-year assessment, the majority of patients in the overall group had mean and median TGP and TGO levels within the normal range, with a decrease in the percentage of patients with elevated transaminase levels.

Figure 10. Percentage distribution of TGP (U/L) values (normal/increased)

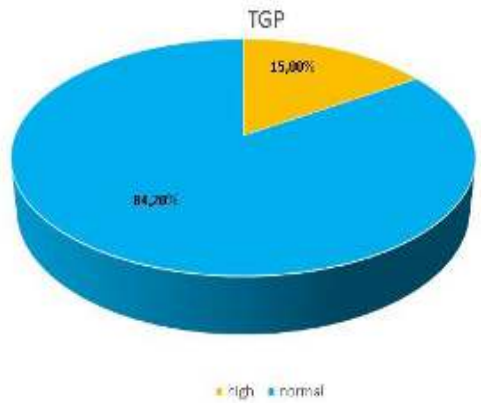
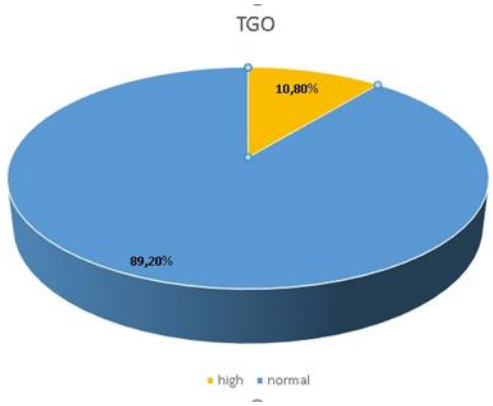


Figure 11. Percentage distribution of TGO (U/L) values (normal/increased)



Looking at transaminase levels by type of infection, we found that a higher percentage of patients with HIV/hepatitis co-infection had elevated TGP or TGO levels, but most of them had normal levels.

Table XXXVI. TGP values * infection type

Infection type	TGP	Frequency	Percent
HIV monoinfection	normal	213	85,5
	high	36	14,5
	Total	249	100,0
HIV/hepatitis virus coinfection	normal	21	72,4
	high	8	27,6
	Total	29	100,0

Table XXXVII. TGO values * infection type

Infection type	TGO	Frequency	Percent
HIV monoinfection	normal	226	90,8
	high	23	9,2
	Total	249	100,0
HIV/hepatitis virus coinfection	normal	22	75,9
	high	7	24,1
	Total	29	100,0

2.13.4 Assessment of liver fibrosis score values -APRI, FIB4 and FORNS

Table XXXVIII. APRI values * liver fibrosis stage

APRI	Fibrosis	Frequency	Percent
<0.5	F0F1	244	87,8
0.5-1.5	F2	29	10,4
>1.5	F3F4	5	1,8
	Total	278	100,0

In the overall group, the majority of patients (87.8 %) had APRI values < 0.5, corresponding to mild or absent liver involvement (F0F1). A small proportion (10.4 %) had moderate liver involvement (F2) and only 1.8 % of patients had severe liver involvement (F3F4).

Table XXXIX. APRI values * liver fibrosis stage * infection type

Infection type	APRI	Fibrosis	Frequency	Percent
HIV	<0.5	F0F1	223	89,6
monoinfection	0.5-1.5	F2	24	9,6
	>1.5	F3F4	2	0,8
		Total	249	100,0
HIV/hepatitis	<0.5	F0F1	21	72,4
virus coinfection	0.5-1.5	F2	5	17,2
	>1.5	F3F4	3	10,3
		Total	29	100,0

In the HIV monoinfected group, we identified 223 patients (89.6 %) with F0F1, 24 patients (9.6 %) with F2 and 2 patients (0.8 %) with severe fibrosis, F3F4. In the co-infected group, 21 patients (72.4 %) had F0F1 fibrosis, 5 patients (17.2 %) had moderate fibrosis, F2, and 3 patients (10.3 %) had severe liver fibrosis, F3F4.

Tracing the distribution of FIB4 score values in the total group and then according to the type of infection, we observed that 247 patients (88.8 %) (227 with monoinfection and 20 with coinfection) had FIB4 values below 1. 45 (Ishak 0-1), 25 patients (9 %) (18 with monoinfection and 7 with coinfection) had FIB4 values between 1.45 and 3.25 (Ishak 2-3) and 6 patients (2.2%) (4 with monoinfection and 2 with coinfection) had FIB4 values above 3.25 (Ishak 4-6).

Table XL. FIB4 values * liver fibrosis stage

FIB4	Fibrosis	Frequency	Percent
<1.45	Ishak 0-1	247	88,8
1.45-3.25	Ishak 2-3	25	9,0
>3.25	Ishak 4-6	6	2,2
	Total	278	100,0

Table XLI. FIB4 values* liver fibrosis stage* infection type

Infection type	FIB4	Fibrosis	Frequency	Percent
HIV	<1.45	Ishak 0-1	227	91,2
monoinfection	1.45-3.25	Ishak 2-3	18	7,2
	>3.25	Ishak 4-6	4	1,6
		Total	249	100,0
HIV/hepatitis	<1.45	Ishak 0-1	20	69,0
virus coinfection	1.45-3.25	Ishak 2-3	7	24,1
	>3.25	Ishak 4-6	2	6,9
		Total	29	100,0

Tabelul XLII. FORNS values on total and individual batches

Infection type	FORNS	Fibrosis	Frequency	Percent
HIV	<4.25	F0-F1	194	77,9
monoinfection	>6.9	F2-F3-F4	3	1,2
	4.25-6.9	P=NS	52	20,9
		Total	249	100,0
HIV/hepatitis	<4.25	F0-F1	16	55,2
virus coinfection	>6.9	F2-F3-F4	5	17,2
	4.25-6.9	P=NS	8	27,6
		Total	29	100,0
Total	<4.25	F0-F1	210	75,5
	>6.9	F2-F3-F4	8	2,9
	4.25-6.9	P=NS	60	21,6
		Total	278	100,0

The FORNS score was below 4.25 (F0F1) in 210 patients (75.5%) (194 with monoinfection and 16 with co-infection) (F0F1), above 6.9 (F2F3F4) in 8 patients (3 with monoinfection and 5 with co-infection), and between 4.25 and 6.9 in 60 cases (21.6%) (52 monoinfected, 8 co-infected), without differentiating between the stages of liver fibrosis.

3. Discuss

For the period studied, we observed a general downward trend in the annual number of new cases diagnosed with HIV infection, except for 2019 and 2023. HIV monoinfection was more common, with annual percentage variations, with all patients diagnosed with HIV infection being monoinfected in 2020.

In the study group, about 60 % of newly diagnosed HIV-infected patients had HIV- RNA levels above 105 copies/ml, with coinfected patients more likely to have higher HIV plasma loads at diagnosis.

We observed that in HIV monoinfected patients, higher plasma viremia levels correlated with late diagnosis.

In terms of immune status, the majority of patients had $CD4^+$ counts below 500/mm^3, with more than a third in immunological stage 3. Two thirds of patients diagnosed in clinical stage A had $CD4^+$ cell counts below 500/mm^3. In the presence of HIV co-infection with hepatitis viruses, a higher percentage of patients tended to have $CD4^+$ cell counts below 500/mm^3.

Patients with HIV/hepatitis co-infection had a higher frequency of elevated transaminase levels at the time of HIV diagnosis. In monoinfection, we found a statistically significant association between TGP and TGO levels and $CD4^+$ cell count, which increased with decreasing $CD4^+$ cell count. In coinfection, the association was significant for TGP levels, which were higher when $CD4^+$ cell counts were below 200 cells/mm^3.

Irrespective of the type of infection, we observed an increase in transaminase levels with increasing plasma HIV viral load. The correlation was statistically significant in both groups, but stronger in the co-infected group.

For the groups studied, there were significant differences between the mean values of the APRI, FIB4 and FORNS liver fibrosis scores, with co-infected patients having higher scores corresponding to moderate/severe liver fibrosis. The values of these scores increased with decreasing $CD4^+$ cell count and increasing HIV-RNA, and the association was statistically significant in HIV monoinfection. Stepwise analysis of the cumulative effect of $CD4^+$ cell count, HIV-RNA and infection type on liver fibrosis score showed that the more factors cumulated, the higher the liver fibrosis score. Taking each predictor separately, the $CD4^+$ cell count had the greatest effect, followed by the presence of hepatitis C virus co-infection, and then the plasma HIV-RNA level. The greatest cumulative effect of the predictors was on the FORNS score, with the combination of the three parameters explaining 19 % of the variance.

In this study, we found a clear association between HIV viral load and the degree of liver fibrosis as measured by APRI, FIB4 and FORNS scores, with patients with a higher viral load

(>105 copies/ml) having advanced liver fibrosis (F3F4) and those with a lower viral load (<10^4 copies/mL) generally having mild or no liver fibrosis (F0F1).

Following ART administration, we observed an improvement in the immune status of the patients at the 1-year evaluation, with an increase in the mean and median $CD4^+$ cell counts in each group. 85.55 % of patients had $CD4^+$ cell counts above 200/mm^3 and 44.53 % had $CD4^+$ cell counts above 500/mm^3. Although higher than at the time of HIV diagnosis, these levels were lower in the HIV/hepatitis co-infected group. $CD4^+$ cell recovery was better in patients with monoinfection. The majority of patients had undetectable plasma HIV levels, with viral suppression being more common in monoinfected patients. There were also cases with detectable viremia with significantly elevated values, most likely due to lack of adherence to treatment. Regardless of the type of infection, the majority of patients had mean and median transaminases in the normal range. However, there were a few patients with elevated values, more frequently in the co-infected group, requiring further investigations to determine the etiology. The liver fibrosis scores were generally below the detection limit of moderate/severe liver fibrosis, with mild or absent liver involvement predominating. Increased values of these scores, respectively advanced liver fibrosis, were more common in coinfection.

4. Conclusions

1. Liver involvement in HIV infection is complex, with multifactorial causality: infection "per se", co-infection with viruses with liver tropism, opportunistic infections, toxicity of drugs used to treat patients, metabolism.
2. A comprehensive and complete assessment of patients at the time of initial diagnosis allows the establishment of monitoring and control of all systems and organs affected by HIV infection, including the liver and its function.
3. The essence of effective medical intervention in the comprehensive care of HIV-infected patients is regular and permanent monitoring of their viro-immunological status.
4. In the group studied, patients with HIV/hepatitis virus co-infection had higher HIV plasma viremia levels and consequently lower $CD4^+$ cell counts.
5. The advanced clinical stage of HIV monoinfection correlates with high plasma HIV loads and with male sex, which is also likely to have lower $CD4^+$ cell counts, regardless of infection type.
6. Patients with HIV monoinfection had a more severe clinical stage of infection at diagnosis.
7. Patients co-infected with HIV/ hepatitis viruses and those with advanced stages of HIV infection are more likely to have elevated transaminase levels.

8. In HIV monoinfection, increased transaminase values correlate with $CD4^+$ cell counts below 200/ mm^3 and high plasma viral loads.

9. Regardless of the type of infection, TGO values are more elevated compared to TGP.

10. There is a strong correlation between TGO and HIV-HIV RNA values for both types of infection, the correlation being stronger in HIV/ hepatitis virus coinfection.

11. Coinfection is associated with higher APRI, FIB4 and FORNS scores.

12. In HIV monoinfection, the cumulative effect of advanced clinical stage, low $CD4^+$ cell counts ($< 200/mm^3$) and elevated HIV-RNA levels ($> 10^5$ copies/ml) will result in higher APRI, FIB4 and FORNS liver fibrosis scores, thus more advanced stages of liver fibrosis.

13. In HIV infection, the viro-immunologic status of the patient influences the stage of liver fibrosis. When FibroScan is not available, the calculation of liver fibrosis score values, particularly APRI and FIB4, may be useful in assessing liver fibrosis.

14.Liver involvement in HIV infection is decisively influenced by the viro-immunologic status of the patient, but especially by the association with hepatotropic viruses.

15. After the administration of antiretroviral drugs, in most cases there is a progressive improvement in the patient's viro-immunological status, with an increase in the $CD4^+$ cell count and viral suppression, as well as an improvement in the degree of liver damage, reflected by a decrease to normalisation of transaminase values and liver fibrosis scores APRI, FIB4 and FORNS.

16. HIV-induced liver damage can be effectively controlled by optimised HIV therapy without long-term adverse effects on the clinical course of patients.

B. FibroScan assessment of liver fibrosis in HIV-infected patients on antiretroviral therapy

1. Material and method

Some of the patients evaluated at the time of HIV diagnosis and then after one year of ART were evaluated during 2024 with FibroScan (Fibroscan 530 Compact device). To determine the stage of liver fibrosis and the degree of hepatic steatosis, the value obtained consecutively to the measurements performed was interpreted using myFibroscan app https://www.echosens.com/products/my-fibroscan/. We entered into the database: patient's gender, HBsAg, HCVsAg, HDVsAg (positive/negative), TGP (U/L), TGO (U/L), GGT (U/L), total cholesterol (mg/dl), platelets/mm3, CD4+(cells/mm3) and HIV-RNA (copies/ml) values, time since diagnosis of HIV infection (years/months), ART administered, APRI, FIB4 and FORNS liver fibrosis scores, liver fibrosis stage, and steatosis grade determined by FibroScan.

2. Results

The group consisted of 45 patients, 15 female (33.3 %) and 30 male (66.7 %). The age distribution was as follows: 1 patient < 20 years, 6 patients (20-29 years), 9 patients (30-39 years), 13 patients (40-49 years), 11 patients (50-59 years) and 5 patients (> 60 years). 39 patients were HIV monoinfected and 6 patients were HIV/ hepatitis virus coinfected (2 HIV/HBV coinfected patients and 4 patients HIV/HCV coinfected).

Patients were exposed to ART for varying lengths of time (from a few months to 9 years). More than half of the patients (53.3 %) had a $CD4^+$ cell count above 500 $CD4^+$ cells/mm^3, with a significant proportion (40.0 %) having a $CD4^+$ cell count between 200 and 500 cells/mm^3.

The mean $CD4^+$ count was 576.33 cells/mm^3, the median 522 cells/mm^3, the minimum 120 cells/mm^3 and the maximum 1583 cells/mm^3. Most patients had an undetectable viral load 84.4 %. The mean HIV-RNA level was 47.71 copies/ml and the median was less than 40 copies/ml. Patients with detectable HIV-RNA with a peak value of 736 copies/ml had been diagnosed with HIV infection in 2023 and 2024, respectively, and had been on antiretroviral treatment for several months. TGP levels were normal in 33 patients and elevated in 12. TGO levels were normal in 35 patients and elevated in 10 patients. The mean and median values of TGP and TGO were within the normal range.

Table XLIII. Distribution of liver fibrosis stages according to APRI, FIB 4 and FORNS values

Liver fibrosis score	Stage of liver fibrosis	Distribution of cases	
APRI	F0F1	Frequency	35
		%	77.8
	F2	Frequency	10
		%	22.2
FIB4	Ishak 0-1	Frequency	35
		%	77.8
	Ishak 2-3	Frequency	10
		%	22.2
FORNS	F0F1	Frequency	28
		%	62.22
	F2F3F4	Frequency	1
		%	2.22
	F=NS	Frequency	16
		%	35.56

We calculated liver fibrosis scores and classified patients according to the stage of liver fibrosis identified. APRI and FIB4 scores similarly identified liver fibrosis stages. In 16 cases (35.56 %), the FORNS score could not differentiate between mild/absent and severe fibrosis stages.

By performing the FibroScan for the patients in the study, we identified the stages of fibrosis and hepatic steatosis, respectively. From the data obtained we observed that patients with mild or absent fibrosis (F0F1) mostly (57.1 %) had no hepatic steatosis or mild steatosis (22.9 %), rarely with moderate or advanced steatosis. Patients with moderate, severe fibrosis or cirrhosis of the liver (F2, F3, F4) also had varying degrees of hepatic steatosis (S2S3, S3).

Table XLIV. Distribution of liver fibrosis/steatosis stages identified by FibroScan

Hepatic steatosis		Liver fibrosis				Total
		F0F1	F2	F3	F4	
S0	Frequency	20	0	0	0	20
	%	100	0	0	0	100
S1	Frequency	8	0	1	0	9
	%	88.9	0	11.1	0	100
S2S3	Frequency	7	4	1	1	13
	%	53.8	30.8	7.7	7.7	100
S3	Frequency	0	1	1	1	3
	%	0	33.3	33.3	33.3	100
Total	Frequency	35	5	3	2	45
	%	77.8	11.1	6.7	4.4	100

Table XLV. Characteristics of patients with advanced liver fibrosis stages (F3, F4)

	F3	F3	F3	F4	F4
Infection type	HIV monoinfection	HIV monoinfection	HIV monoinfection	HIV monoinfection	HIV monoinfection
CDC stage of HIV infection	A2	C3	B1	B3	A2
Year of HIV diagnosis	2021	2023	2023	2022	2023
RNA-HIV (copies/ml)	<40	89	<40	181	<40
Hepatic steatosis	S1	S2S3	S3	S3	S2S3

We analyzed the relationship between $CD4^+$ cell counts and the stage of liver fibrosis identified by FibroScan.

Table XLVI. Distribution of liver fibrosis stage by $CD4^+$ cell count

			Liver steatosis				
			F0F1	F2	F3	F4	Total
$CD4^+$ (cells/mm^3)	>500	Frequency	22	1	2	0	25
		%	88,0	4,0	8,0	0,0	100,0
	200-500	Frequency	12	3	1	1	17
		%	70,6	17,6	5,9	5,9	100,0
	<200	Frequency	1	1	0	1	3
		%	33,3	33,3	0,0	33,3	100,0
Total		Frequency	35	5	3	2	45
		%	77,8	11,1	6,7	4,4	100,0

Patients with high $CD4^+$ values (>500 cells/ mm^3) were mostly at stage F0F1. Those with low $CD4^+$ values (< 200 cells/ mm^3) had a more varied distribution of liver fibrosis stages. For this association the p value was 0.086. The Cramer's V of 0.355 suggests a moderate association between the two variables that is, however, not strong enough to be considered statistically significant.

Table XLVII. Distribution of liver fibrosis stages according to plasma HIV-HIV-RNA detectability/ undetectability

			Liver steatosis				
			F0F1	F2	F3	F4	Total
RNA-HIV (copies/ml)	< 40	Frequency	31	4	2	1	38
		%	81,6	10,5	5,3	2,6	100,0
	>40	Frequency	4	1	1	1	7
		%	57,1	14,3	14,3	14,3	100,0
Total		Frequency	35	5	3	2	45
		%	77,8	11,1	6,7	4,4	100,0

The stages of liver fibrosis also varied according to HIV plasma viremia. Patients with HIV-RNA < 40 copies/ml had mild or absent liver fibrosis (F0F1), whereas patients with detectable HIV-RNA (> 40 copies/ml) had various stages of fibrosis, including more advanced stages (F3 and F4). The p-value of 0.383 indicates that the observed differences in the distribution of liver fibrosis stages between the HIV-RNA < 40 copies/ml and > 40 copies/ml groups are not statistically significant. Cramer's V 0.261 indicates a weak association between HIV-RNA and liver fibrosis stage.

Table XLVIII. Distribution of liver fibrosis stages according to APRI and FibroScan scores, respectively

			F0F1	F2	F3	F4	Total
APRI	F0F1	Frequency	30	3	1	1	35
		%	85.7	8.6	2.85	2.85	100.0
	F2	Frequency	5	2	2	1	10
		%	50	20	20	10	100.0
Total		Frequency	35	5	3	2	45
		%	77.8	11.1	6.7	4.4	100.0

A total of 35 patients (85.7 %) were identified as having the same stage of liver fibrosis, namely F0F1. The p value was 1, below the statistical significance threshold, meaning that this association was not statistically significant.

Table XLIX. Distribution of liver fibrosis stages according to FIB4 and FibroScan score, respectively

			F0F1	F2	F3	F4	Total
FIB4	Ishak 0-1	Frequency	30	3	1	1	35
		%	85.7	8.6	2.85	2.85	100.0
	Ishak 2-3	Frequency	5	2	2	1	10
		%	50	20	20	10	100
Total		Frequency	35	5	3	2	45
		%	77.8	11.1	6.7	4.4	100.0

35 patients were identified with mild or absent fibrosis (F0F1) by both criteria. The p-value corresponding to this association was 0.329 meaning that this correlation was not statistically significant.

Table L. Distribution of liver fibrosis stages according to FORNS and FibroScan scores respectively

			F0F1	F2	F3	F4	Total
FORNS	F0-F1	Frequency	23	4	1	0	28
		%	82.1	14.3	3.6	0	100.0
	P=NS	Frequency	10	2	2	2	16
		%	62.5	12.5	12.5	12.5	100.0
	F2F3F4	Frequency	1	0	0	0	1
		%	100	0	0	0	100
Total		Frequency	35	5	3	2	45
		%	73.3	11.1	6.7	4.4	100.0

28 patients had liver fibrosis stage F0F1 according to the FORNS scores, and 23 patients had the same fibrosis stage according to FibroScan. However, in 16 patients the values of this score could not distinguish between mild and significant fibrosis. Using the Chi-square test, the p-value was < 0.0001, which means that for the group studied there was a statistically significant correlation between the liver fibrosis stages identified by the two methods.

3. Discuss

In the study group, the liver fibrosis stages identified by FibroScan were 80% of those determined by calculating the APRI and FIB4 liver fibrosis scores. These 3 methods identified the same number of cases with liver fibrosis stage F0F1. The difference was in the distribution of cases with moderate/severe liver fibrosis. The FORNS score has the disadvantage that it cannot distinguish between mild and advanced fibrosis for a given range of values. However, in 66 % of cases it identified an F0F1 stage similar to that identified by FibroScan, and this association was statistically significant for the group studied.

The stages of liver fibrosis determined by FibroScan varied according to the patient's virological status. For example, in cases of severe immunological suppression - $CD4^+$ cell counts < 200/mm^3 or detectable plasma HIV RNA levels - the stages of liver fibrosis are more advanced.

In our study, we did not observe any differences between the stages of liver fibrosis and the duration of exposure to antiretroviral drugs, which means that drugs with a very good liver safety profile are currently used.

4. Conclusions

1. The patient's viro-immunologic status, in case of HIV infection, influences the stage of liver fibrosis.

2. When FibroScan is not available, calculation of liver fibrosis score values may be useful for initial assessment and monitoring of liver fibrosis over time.

3. After initiation of antiretroviral therapy, not only the patient's viro-immunologic status, but also the degree of liver damage is improved.

IV. Conclusions (summary)

1. HIV infection causes multi-system damage, including liver damage, depending on the degree of immunosuppression of the patient.
2. Chronic inflammation of the liver, secondary to HIV itself or in association with other hepatotoxic factors - co-infection with hepatotropic viruses, opportunistic infections, metabolic, drug-induced - leads to liver fibrosis which, over time, leads to progression of liver disease.
3. The viro-immunological status of the patient, the stage of HIV infection at the time of diagnosis and co-infection with other hepatotropic viruses influence the degree of liver involvement.
4. Liver fibrosis may be present at the time of HIV diagnosis or may develop, persist and even progress in the absence of adherence to antiretroviral therapy.
5. FibroScan may be used routinely to determine the stage of liver fibrosis at the time of initial diagnosis of HIV infection and then repeated dynamically to monitor its progression.
6. In the absence of the FibroScan, the liver fibrosis stage may be assessed using the APRI, FIB4 and FORNS liver fibrosis scores.
7. Complete and comprehensive assessment of the patient from the moment of diagnosis of HIV infection, immediate initiation of individualized antiretroviral treatment and regular monitoring of the patient will ensure not only the improvement of the patient's viro-immunological status, but also the control of all systems and organs affected by HIV infection and increase the quality of life of patients.

V. Originality and innovative contributions of the thesis

1. Introduction of comprehensive and complex assessment of liver involvement in the newly diagnosed patient with HIV infection.
2. Determination of initial and subsequent liver fibrosis stage in dynamics using APRI, FIB4 and FORNS liver fibrosis scores.
3. Use of routine FibroScan in HIV-infected, naïve patients.

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