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Short Communication

ASSOCIATION OF NON-INVASIVE MARKERS OF LIVER FIBROSIS WITH HCV COINFECTION AND ANTIRETROVIRAL THERAPY IN PATIENTS WITH HIV

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The aim of this study was to assess the main effects and interaction between viral hepatitis C (HCV) coinfection and antiretroviral therapy (ART) by using a nonparametric ANOVA on direct and indirect markers of liver fibrosis in HIV-infected patients. The sample included 178 HIV patients aged from 23 to 65 (36% females). The following parameters were determined in blood of patients: hyaluronic acid, pro-matrix metalloproteinase-1, alanine aminotransferase, aspartate aminotransferase, and platelet count. The FIB-4 index was also calculated. The nonparametric ANOVA revealed no significant interaction between HCV coinfection and ART. This provides evidence for an independent contribution of each factor on promotion of the pathology. The results also demonstrated that the direct and indirect indicators of liver fibrosis are associated differently with the studied factors. Therefore, a combination of markers should be used for monitoring of liver fibrosis in HIV-infected patients.

Key words: *liver fibrosis, hyaluronic acid, pro-MMP-1, FIB-4, AST, ALT, nonparametric ANOVA.*

Liver diseases are becoming increasingly prominent in HIV-infected patients. These diseases are among the leading causes of non AIDS-associated deaths. The development of liver fibrosis represents the most clinically relevant and common pathway of hepatic injury, and early recognition of liver fibrosis is an essential component of its diagnostics and therapy (Kaspar and Sterling, 2017).

Liver biopsy remains the gold standard for the assessment of liver fibrosis. However, the use of biopsy is limited by possible complications associated with its invasive nature, heterogeneity of the liver, and some level of subjectivity in conclusions (Bravo *et al*., 2001). Therefore, this method is not available for massive screening of the disease. Non-invasive methods involve transient elastography (a method of measuring the mean stiffness of hepatic tissue) and investigation of blood serum. Taking into account a relatively high variability in the rate of progression of liver fibrosis (Konerman *et al*., 2014), biomarkers represent essential tools for the assessment of the disease by using a simple blood test (Neuman *et al*., 2016).

Biomarkers of liver fibrosis can be divided into two groups (Schmid *et al*., 2015). The first group includes direct markers of liver fibrosis and markers of hepatic metabolisms (e.g., hyaluronic acid, matrix metalloproteinases, and tissue inhibitor of matrix metalloproteinase). The second group consists of indirect biochemical markers of liver functioning (e.g., alanine aminotransferase, aspartate aminotransferase, and bilirubin). In addition, some indexes (e.g., APRI, FIB-4, Forns index) are used for a complex assessment of the fibrosis. All markers are suggested for the assessment of liver fibrosis in patients with HIV and viral hepatitis C (HCV) coinfection (Peters *et al*., 2011, Konerman *et al*., 2014; Schmid *et al*., 2015). However, the results of the previous studies opened inconsistent tendencies in the level of differentiation of patients with and without HCV coinfection (Larrousse *et al*., 2007; Schmid *et al*., 2015).

Observed inconsistency can be associated with the multifactorial determination of liver fibrosis in HIV patients. For example, absence or interruption of antiretroviral therapy (ART) is among the factors leading to progressing liver fi-

brosis in patients with HCV coinfection (Qurishi e*t al*., 2003; Peters *et al*., 2011, Kim *et al*., 2016). At the same time, multifactorial analysis of markers of liver fibrosis has been limited by less developed statistical tools for non-normally distributed variables. Recent developments in the field of nonparametric analysis opened an opportunity to assess the main effects and interactions of multiple factors through a nonparametric multiway ANOVA (Hettmansperger and McKean, 2011; Kloke and McKean, 2012). The aim of this study was to assess the main effects and interaction between HCV coinfection and ART by using a nonparametric ANOVA on direct and indirect markers of liver fibrosis in HIV-infected patients.

The study was conducted in the Rīga Eastern Clinical University Hospital from 2015 to 2018. The study was approved by the Ethics Committee of Rīga Stradiņš University and Ethics Committee of Rīga Eastern Clinical University Hospital. The inclusion criteria were: age ≥ 18, confirmed HIV monoinfection, and confirmed HIV infection with HCV coinfection. The exclusion criteria were: alcoholic liver diseases, opportunistic infection, viral hepatitis B, and liver cirrhosis or neoplasia.

The diagnosis of HIV was based on detection of positive HIV1/2 antibody by the 4th generation of *ELISA*, confirmed by Western Blot, and on detection of HIV RNS by real-time polymerase chain reaction (COBAS AmpliPrep/COBAS TaqMan HIV-1 Test 2.0, Roche, USA, the lower limit of detection was 20 copies/ml). The HCV coinfection was confirmed by detection of HCV antibody (ELISA, Bio-Rad, France or Ortho-Clinical Diagnostics Inc., USA) and by detection of HCV RNS (COBAS AmpliPrep/COBAS TaqMan HCV test, Roche, USA) or HCV Core Ag (ELISA Architec system HCV Ag, Abbot, USA) in plasma.

The study sample included 178 HIV patients aged from 23 to 65 (mean age was 38.48 ± 9.87, 36% females). The median level of CD4 cell count in the sample was 333 cells/μl, and interquartile range (IQR) was 193–467 cells/μl. The median viral load was 366 copies/ml (ranged 0–30300 copies/ml). The patients formed four groups according to HCV coinfection and ART status. The group with HCV coinfection involved 33 HIV patients without ART and 42 patients with ART. The group without HCV included 49 patients without ART and 54 patients with ART. Patients received ART according to the clinical guidelines for diagnosis, treatment, and prevention of HIV infection: two nucleoside reverse transcriptase inhibitors (NRTI) combined with one protease inhibitor (PI) or one non-nucleoside reverse transcriptase inhibitor (NNRTI).

The following parameters were determined in patient plasma: hyaluronic acid (HA), pro-matrix metalloproteinase-1 (pro-MMP-1), alanine aminotransferase (ALT), aspartate aminotransferase (AST), and platelet count by using validated methods. FIB-4 index, described by Sterling *et al*. (2006), was calculated as FIB-4 = (age × AST) / (platelets (10^9/L) × ALT$^{1/2}$).

Statistical analysis was performed with IBM SPSS for Windows 22.0 and R-package 'Rfit' 0.23.0, developed by Kloke and McKean (Kloke and McKean, 2012) as an implementation of the nonparametric approach to the multi-way analysis of variance with Type III sum of squares (Hettmansperger and McKean, 2011).

The Kolmogorov-Smirnov test (ranged from 0.19 to 0.35) and Shapiro-Wilk test (from 0.39 to 0.82) indicated non-normal distribution of selected markers. Therefore, a nonparametric approach to the between-subjects analysis of variance was applied. Table 1 presents descriptive statistics for the markers in HIV patients and the results of the two-way 2 (HCV) × 2 (ART) rank-based ANOVA.

Direct indicators of liver fibrosis demonstrated different trends. Pro-MMP-1 was significantly higher in HIV patients with ART without an association with HCV. The level of hyaluronic acid was higher in HIV patients without HCV independently of ART. Indirect indicators of fibrosis, ALT and AST, were higher in HIV patients with HCV than in those without HCV. ALT was also higher in HIV patients without ART. Similarly, the FIB-4 index of liver fibrosis was higher in HIV patients with HCV and in patients without ART. No one interaction of factors was statistically significant, which indicated an independent contribution of HCV coinfection and ART to liver fibrosis.

The results confirmed the negative effects of HCV coinfection and an absence of ART on liver fibrosis (Qurishi *et al*., 2003; Peters *et al*., 2011, Kim *et al*., 2016). The direct and indirect indicators of liver fibrosis related differently to factors promoting the pathology. Hyaluronic acid and AST were associated with HCV coinfection. Pro-MMP-1 was associated with ART only, while ALT and FIB-4 demonstrated an association with both factors under analysis. The last finding is in accordance with the suggestion of using liver fibrosis indexes for indirect assessment (Schmid *et al*., 2015).

In summary, a nonparametric two-way ANOVA demonstrated usefulness for the assessment of the main effects and the interaction between HCV coinfection and ART in HIV patients. It can be concluded that there is no universal blood marker of liver fibrosis. A complex set of direct and indirect markers should be used in practice. As a result, indexes of liver fibrosis and particular markers can be analysed.

REFERENCES

Bravo, A. A., Sheth, S. G., Chopra, S. (2001). Liver biopsy. *New Engl. J. Med.*, **344**, 495–500.

Hettmansperger, T. P., McKean, J. W. (2011). *Robust Nonparametric Statistical Methods*. 2nd ed., New York, Chapman-Hall. 554 pp.

Kaspar, M. B., Sterling, R. K. (2017). Mechanisms of liver disease in patients infected with HIV. *BMJ Open Gastro*, **4**, e000166.

Kim H. N., Nance, R., Rompaey, S. V., Delaney, J. C., Crane, H. M., Cachay, E. R., Geng, E., Boswell, S. L., Rodriguez, B., Eron, J., Saag, M., Moore, R. D., Kitahata, M. M. (2016). Poorly controlled HIV infection: An independent risk factor for liver fibrosis. *J. Acquir. Immune Defic. Syndr.*, **72** (4), 437–443.

Table 1

DESCRIPTIVE STATISTICS AND 2 (HCV) × 2 (ART) RANKED-BASED ANOVA ON MARKERS OF LIVER FIBROSIS IN HIV PATIENTS

Group	pro-MMP-1 (ng/ml)	HA (μg/ml)	ALT (U/L)	AST (U/L)	FIB-4 Index
	Mdn (IQR)	Mdn (IQR)	Mdn (IQR)	Mdn (IQR)	Mdn (IQR)
HIV/HCV	4.58 (2.89–7.02)	26.04 (18.20–45.85)	49 (30–88)	40 (25–69)	1.02 (0.78–2.12)
With ART	5.18 (3.43–10.93)	29.61 (19.52–45.85)	60 (34–90)	44 (26–65)	0.92 (0.71–2.05)
Without ART	3.35 (2.26–5.72)	23.58 (16.72–45.66)	43 (26–76)	36 (25–76)	1.68 (0.88–2.29)
HIV	3.86 (2.41–7.28)	18.21 (14.63–27.73)	21 (15–34)	23 (17–30)	0.94 (0.70–1.34)
With ART	4.03 (3.36–8.33)	18.32 (15.16–24.13)	24 (16–35)	24 (17–32)	0.90 (0.69–1.19)
Without ART	3.67 (1.43–5.61)	17.77 (13.51–32.52)	20 (14–34)	22 (19–30)	1.09 (0.72–1.70)
Total	3.90 (2.60–7.02)	21.11 (15.33–36.41)	30 (17–57)	27 (20–45)	0.97 (0.73–1.68)
With ART	4.87 (3.38–8.97)	20.97 (15.67–32.18)	32 (19–63)	28 (19–48)	0.91 (0.70–1.25)
Without ART	3.43 (1.59–5.61)	21.94 (14.55–39.46)	27 (16–44)	27(19–48)	1.12 (0.78–1.91)
Source of variance	F	F	F	F	F
HCV	0.45	12.97***	48.70***	32.54***	5.21*
ART	8.96**	0.11	4.40*	0.20	6.78*
HCV × ART	0.29	0.62	1.29	0.26	0.79

HA, hyaluronic acid; pro-MMP-1, pro- matrix metalloproteinase-1; ALA, alanine aminotransferase; AST, aspartate aminotransferase; ART, antiretroviral therapy; HCV, viral hepatitis C; Mdn, median; IQR, interquartile range; ALT, alanine aminotransferase.

* $p < 0.05$. ** $p < 0.01$. *** $p < 0.001$.

Kloke, J. D., McKean, J. W. (2012). Rfit: Rank-based estimation for linear models rank-regression. *The R. Journal*, **4** (2), 57–64.

Konerman, M. A., Mehta, S. H., Sutcliffe, C. G., Vu, T., Higgins, Y., Torbenson, M. S., Moore, R. D., Thomas, D. L., Sulkowski, M. S. (2014). Fibrosis progression in human immunodeficiency virus/hepatitis C virus coinfected adults: Prospective analysis of 435 liver biopsy pairs. *Hepatol. Baltim. Md.*, **59**, 767–775.

Larrousse, M., Laguno, M., Segarra, M., De Lazzari, E., Martinez, E., Luis Blanco, J. L., León, A., Deulofeu, R., Miquel, R., Milinkovic, A., Lonca, M., Miró, J. M., Biglia, A., Murillas, J., Gatell, J. M., Mallolas, J. (2007). Noninvasive diagnosis of hepatic fibrosis in HIV/HCV-coinfected patients. *J. Acquir. Immune Defic. Syndr.*, **46**, 304–311.

Neuman, M. G., Cohen, L. B., Nanaua R. M. (2016). Hyaluronic acid as a non-invasive biomarker of liver fibrosis. *Clin. Biochem.*, **49**, 302–315.

Peters, L., Neuhaus, J., Mocroft, A., Soriano, V., Rockstroh, J., Dore, G., Puoti, M., Tedaldi, E., Clotet, B., Kupfer, B., Lundgren, J. D., Klein, M. B., for the INSIGHT SMART Study Group. (2011). Hyaluronic acid levels predict increased risk of non-AIDS death in hepatitis-coinfected persons interrupting antiretroviral therapy in the SMART Study. *Antivir. Ther.* **16** (5), 667–675.

Qurishi, N., Kreuzberg, C., Lüchters, G., Effenberger, W., Kupfer, B., Sauerbruch, T., Rockstroh, J. K., Spengler, U. (2003). Effect of antiretroviral therapy on liver-related mortality in patients with HIV and hepatitis C virus coinfection. *Lancet*, **362**, 1708–1713.

Schmid, P., Bregenzer, A., Huber, M., Rauch, A., Jochum, W., Müllhaupt, B., Vernazza, P., Opravil, M., Weber, R., Swiss HIV Cohort Study. (2015). Progression of liver fibrosis in HIV/HCV co-infection: A comparison between non-invasive assessment methods and liver biopsy. *PLoS ONE*, **10** (9), e0138838.

Sterling, R. K., Lissen, E., Clumeck, N., Sola, R., Cassia, M., Correa, M.C., Montaner, J., Sulkowski, M. S., Torriani, F. J., Dieterich, D. T., Thomas, D. L., Messinger, D., Nelson, M. for the APRICOT Clinical Investigators. (2006). Development of a simple noninvasive index to predict significant fibrosis in patients with HIV/HCV coinfection. *Hepatol. Baltim. Md*, **43**, 1317–1325.

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AKNU FIBROZES NEINVAZĪVO MARĶIERU SAISTĪBA AR HCV KOINFEKCIJU UN ANTIRETROVIRĀLO TERAPIJU PACIENTIEM AR HIV

Pētījuma mērķis bija novērtēt HCV koinfekcijas un antiretrovirālas terapijas (ART) mijiedarbību un galvenos efektus uz tiešajiem un netiešajiem aknu fibrozes asins marķieriem. Izlasi veidoja 178 pacienti ar HIV infekciju vecumā no 23 līdz 65 gadiem (36% bija sievietes). Pacientu asinīs bija noteikti sekojoši parametri: hialuronskābe, pro-matrices metālproteināze-1, alanīnaminotransferāze, aspartātaminotransferāze un trombocītu skaits. Papildus bija aprēķināts FIB-4 indekss. Neparametriskā dispersijas analīze neatklāja nozīmīgu HCV koinfekcijas un ART mijiedarbību. Tas apliecina katra faktora neatkarīgu pienesumu patoloģijas attīstībā. Rezultāti parādīja arī, ka aknu fibrozes tiešie un netiešie rādītāji ir dažādi saistīti ar faktoriem, kuri pastiprina patoloģiju. Tādējādi HIV pacientu aknu fibrozes monitoringam būtu jāizmanto marķieru kombinācija.