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Title: CD4+ T cells and NK cells: Biomarkers for hepatic fibrosis in HIV/HCV-coinfected patients

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1 What did this study explore?

The identification of noninvasive liver fibrosis biomarkers is still an open research area. In this context, we reasoned that the study of the phenotype of peripheral blood cells may unravel interesting clues towards the identification of such biomarkers. Some evidence indicates that the characteristics of the immune cells, including NK cells, observed in peripheral blood are similar to those seen in liver with relatively lower levels of magnitude. Accordingly, the aim of this study was to characterize peripheral blood NK cell phenotypes by flow cytometry as potential biomarker for liver fibrosis in patients chronically coinfected with hepatitis C and HIV.

2 How did the authors perform all experiments?

Twenty-nine subjects were included in the study. Cryopreserved PBMC from 24 HIV/HCV-coinfected individuals and 5 HIV/HCV-seronegative individuals (healthy controls, HC) were used in this study. PBMC were thawed and stained with fluorochrome-conjugated antibodies distributed in five different panels (depending on PBMC availability) to evaluate expression of different markers on NK cells.

3 How did the authors process all experimental data?

Data were analyzed using the FlowJo software (TreeStar, Ashland, Oregon, USA). NK cell populations were defined according to the corresponding isotype control.
Plasma viral load levels (Abbott RealTime HIV-1 RNA version 3; Abbott Molecular, Inc., Des Plaines, IL, USA) were assessed in HIV-infected subjects and CD4+T-cell counts (flow cytometry double platform, BDFACSCanto; BD Biosciences, San Diego/California, USA) were assessed in HIV and HIV-negative individuals. For categorical variables, both chi-square and Fisher's exact test were

applied. For continuous variables, the nonparametric Kruskal-Wallis and Mann-Whitney test were used. Area under the receiving operating curve (ROC) was used to calculate the cut-off point in NK cell percentage with the best sensitivity of high liver fibrosis. Statistical analyses were performed using the Statistical Package for the Social Sciences software version 19.0 (SPSS Inc., Chicago, IL, USA).

4 How did the authors deal with the pre-study hypothesis?

Regarding HIV/HCV-coinfected individuals, no differences were observed in NK cell phenotypes according to the different degrees of liver fibrosis. Nevertheless, we could observe a statistically significant difference in the percentage of peripheral blood NK cells in patients with high scores compared to patients with low liver fibrosis. Patients with advanced fibrosis have lower percentage of NK cells than those with low fibrosis scores. Moreover, we observed that a percentage of NK cells lower than 6.6% had 90% sensitivity and 77% specificity to predict the presence of advance fibrosis (METAVIR F3-F4). This observation could indicate, for the first time, that the evaluation of the NK cells compartment is a potential biomarker for fibrosis staging in HIV/HCV-coinfected patients.

5 What are the novel findings of this study?

It was observed that HIV/HCV coinfected patients with higher levels of liver fibrosis are those with lower percentage of NK cells and with lower LTCD4+ count. These are two simple parameters, that might be perform in a routine laboratory test and that may serve as noninvasive biomarkers of liver fibrosis, identifying patients in need for HCV therapy in the short term.

Thank you again for publishing our manuscript in the World Journal of Hepatology.

Sincerely yours,

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