University of Groningen

CD4/CD8 ratio is a promising candidate for non-invasive measurement of liver fibrosis in chronic HCV-monoinfected patients

Feuth, Thijs; van Baarle, Debbie; van Erpecum, Karel J; Siersema, Peter D; Hoepelman, Andy I M; Arends, Joop E

Published in:
European Journal of Clinical Microbiology & Infectious Diseases

DOI:
[10.1007/s10096-014-2053-7](https://doi.org/10.1007/s10096-014-2053-7)

IMPORTANT NOTE: You are advised to consult the publisher's version (publisher's PDF) if you wish to cite from it. Please check the document version below.

Document Version
Publisher's PDF, also known as Version of record

Publication date:
2014

[Link to publication in University of Groningen/UMCG research database](#)

Citation for published version (APA):

Feuth, T., van Baarle, D., van Erpecum, K. J., Siersema, P. D., Hoepelman, A. I. M., & Arends, J. E. (2014). CD4/CD8 ratio is a promising candidate for non-invasive measurement of liver fibrosis in chronic HCV-monoinfected patients. *European Journal of Clinical Microbiology & Infectious Diseases*, 33(7), 1113-7. <https://doi.org/10.1007/s10096-014-2053-7>

Copyright

Other than for strictly personal use, it is not permitted to download or to forward/distribute the text or part of it without the consent of the author(s) and/or copyright holder(s), unless the work is under an open content license (like Creative Commons).

The publication may also be distributed here under the terms of Article 25fa of the Dutch Copyright Act, indicated by the "Taverne" license. More information can be found on the University of Groningen website: <https://www.rug.nl/library/open-access/self-archiving-pure/taverne-amendment>.

Take-down policy

If you believe that this document breaches copyright please contact us providing details, and we will remove access to the work immediately and investigate your claim.

Downloaded from the University of Groningen/UMCG research database (Pure): <http://www.rug.nl/research/portal>. For technical reasons the number of authors shown on this cover page is limited to 10 maximum.

CD4/CD8 ratio is a promising candidate for non-invasive measurement of liver fibrosis in chronic HCV-monoinfected patients

Thijs Feuth · Debbie van Baarle · Karel J. van Erpecum ·
Peter D. Siersema · Andy I. M. Hoepelman · Joop E. Arends

Received: 6 November 2013 / Accepted: 6 January 2014 / Published online: 22 January 2014
© Springer-Verlag Berlin Heidelberg 2014

Abstract The extent of liver fibrosis is an important factor in prognosis and clinical decision-making in chronic hepatitis C virus (HCV) infection. We investigated CD4/CD8 ratio in HCV-monoinfected and HIV/HCV-coinfected patients, in order to reveal its relation with liver fibrosis. CD4/CD8 ratio in the peripheral blood was assessed by flow cytometry in a cohort of 19 HCV-monoinfected, 14 HIV/HCV-coinfected, ten HIV-monoinfected patients and 15 healthy controls. Liver fibrosis was assessed by transient elastography ($n=25$) or by liver biopsy ($n=8$). Coinfection with HIV was associated with decreased CD4/CD8 ratios in chronic HCV-infected patients, despite adequate antiretroviral treatment. Furthermore, HCV-monoinfected patients with F3-F4 liver fibrosis demonstrated much lower CD4/CD8 ratios than patients with F0-F2 fibrosis (1.4 versus 2.5, $p=0.023$). Similarly, we observed a strong negative correlation between the CD4/CD8 ratio and liver stiffness measured by transient elastography ($R=-0.78$, $p=0.0006$). ROC analysis revealed that CD4/CD8 ratio as a non-invasive marker for fibrosis is very promising (area under the curve 0.8). Although our study was performed with a relatively small number of patients, our findings suggest that the

CD4/CD8 ratio is a promising candidate for non-invasive evaluation of liver fibrosis in HCV-monoinfected patients.

Introduction

Staging of liver fibrosis is essential in chronic HCV-infection since it has implications for treatment of HCV and screening for hepatocellular carcinoma [1]. Although various non-invasive methods have been developed, the gold standard for assessment of fibrosis still remains liver biopsy [2]. Because of shared routes of transmission, coinfection with HIV is relatively common in HCV-infected patients. Natural history of HIV/HCV-coinfection is characterised by accelerated HCV-disease progression, mainly when HIV is not well suppressed. Even when adequately treated, coinfection with HIV leads to altered immunity against HCV, leading to decreased treatment response to interferon [3]. Microbial translocation and increased immune activation are likely to contribute to this altered immunity against HCV in patients coinfecting with HIV on antiretroviral treatment (ART) [3, 4].

Peripheral CD4/CD8 T cell ratio is a well established marker of disease progression in untreated HIV, and has lately gained renewed attention as it may also reflect immune activation in treated HIV [5–7]. In addition, CD4/CD8 is associated with immune activation and poor outcome in a number of clinical conditions, either infectious (e.g. Dengue fever) or non-infectious (e.g. myocardial infarction, cervical carcinoma) [8–10].

T cells may be involved in development of liver fibrosis [11]. We previously showed a correlation between differentiated CD8 T cells and liver fibrosis, substantiating the role of CD8 T cells in liver fibrosis [7]. Therefore, we studied CD4/CD8 ratios in chronic HCV patients with or without treated

T. Feuth · A. I. M. Hoepelman · J. E. Arends (✉)
Department of Internal Medicine and Infectious Diseases, University
Medical Center Utrecht (UMCU), Huispostnummer F.02.126,
P.O. Box 85500, 3508, GA Utrecht, The Netherlands
e-mail: j.e.arends@umcutrecht.nl

D. van Baarle
Department of Immunology, University Medical Center Utrecht
(UMCU), Utrecht, The Netherlands

K. J. van Erpecum · P. D. Siersema
Department of Gastroenterology and Hepatology, University
Medical Center Utrecht (UMCU), Utrecht, The Netherlands

HIV-coinfection, in order to evaluate its possible use as a non-invasive marker for liver fibrosis.

Material and methods

Patients

Nineteen HCV-monoinfected patients, 14 HIV/HCV-coinfected patients and ten HIV-monoinfected patients were recruited from the Infectious Diseases outpatient clinic or from the Gastroenterology outpatient clinic of the University Medical Center Utrecht (UMCU), as previously described [7]. All HIV-infected patients were on effective antiretroviral therapy with undetectable HIV-RNA and none of them had another coinfection (TPHA, HBsAg, HEV-IgG negative). Blood samples from 15 anonymous healthy controls were requested from the bloodbank Mini Donor Dienst of the UMC Utrecht, and were tested negative for hepatitis B, hepatitis C and HIV.

Informed consent was obtained in writing from all patients in accordance with the WMA Declaration of Helsinki and in accordance with the ICH guideline for Good Clinical Practice (6th revision, 2008). The medical ethics committee for research in humans (METC) of the University Medical Center Utrecht, The Netherlands, approved the protocol of this study.

Assessment of liver fibrosis

Scoring of liver fibrosis was performed by liver biopsy using the METAVIR scale ($n=8$), or by using Fibroscan as previously described, with cut-off <9.5 kPa for F0-F2 fibrosis ($n=25$) [7].

Processing of blood for isolation of PBMCs and flowcytometric analysis

Peripheral blood mononuclear cells (PBMCs) were isolated by standard density centrifugation within 8 h from venipuncture and were directly incubated with antibodies to CD3 (Horizon™ V500, clone SP34, provided by BD Biosciences, San Diego, US), CD4 (APC-eF780, RPA-T4, eBioscience, San Diego, CA, USA) and CD8 (Pacific Blue™, RPA-T8, BioLegend, San Diego, CA, USA) for 20 min at 4°C. After washing, cells were directly analyzed by flow cytometry to determine percentages of CD4+ and CD8+ T cells.

Statistical analysis

Medians were compared with Mann–Whitney test. Kruskal–Wallis was used for testing differences of continuous data in multiple groups, followed by Mann–Whitney test. Chi-square test was used to test relation of categorical variables. Dependence of variables was tested using Spearman's correlation

coefficient. Diagnostic performance of the CD4/CD8 ratio in prediction of F3-F4 liver fibrosis was calculated by receiver-operating characteristic (ROC) curves and non-parametric area under the ROC curve. Statistical analysis was performed with IBM SPSS Statistics version 19.0 (SPSS Inc., IBM, USA) and GraphPad Prism 5 for Windows version 5.03 (GraphPad Software, Inc, USA).

Results

None of the patients received treatment for HCV at the time of inclusion or within the preceding year. All HIV-infected patients (both monoinfected and HCV-coinfected) were on stable cART at the time of this study, resulting in CD4⁺ T-cell counts well above 200 cells/mm³ (median 540/mm³ in HIV/HCV-coinfected and 661 in HIV-monoinfected patients; $p=0.07$) and HIV-RNA <50 copies/mL. Patient groups were comparable in terms of age, gender, HCV-RNA level, and duration of antiretroviral treatment (Table 1). Absolute numbers of lymphocytes were comparable between HCV monoinfected and HIV-HCV coinfected patients (median 2.0 and 2.3; $p=0.58$).

Both HIV-monoinfected and HIV/HCV-coinfected patients depicted significantly lower CD4/CD8 ratios (median 1.6, $p=0.035$ and 0.9, $p=0.001$) than healthy controls (2.0). CD4/CD8 ratios of HIV/HCV-coinfected patients were also lower than those of HCV-monoinfected patients (1.9, $p=0.001$) while the difference with HIV-monoinfected patients was borderline significant (1.6, $p=0.08$) (Fig. 1a).

Interestingly, when we compared CD4/CD8 ratios between HCV-monoinfected patients with severe (F3) fibrosis or cirrhosis (F4) (median 1.4) versus those with moderate or absent (F0-F2) fibrosis (median 2.5; $p=0.03$), a low CD4/CD8 ratio was associated with enhanced liver fibrosis. Furthermore, there was a strong negative correlation between CD4/CD8 ratio and liver stiffness as measured by Fibroscan (Spearman $R=-0.78$, $p=0.0006$). This was not the case for HIV/HCV-coinfected patients, as all those patients showed low CD4/CD8 counts (median 0.9), independent of the presence of liver fibrosis (Spearman $R=-0.12$, $p=0.76$) (Fig. 1b–c). No correlation existed between CD4/CD8 ratio and HCV viraemia in either HCV monoinfection (Spearman $R=-0.08$, $p=0.75$) or HIV/HCV coinfection (Spearman $R=0.04$, $p=0.89$) (data not shown). Correlation between CD4/CD8 ratio and routinely clinically measured CD4-counts were borderline significant in HIV-monoinfected (Spearman $R=0.63$, $p=0.05$) and HIV/HCV-coinfected patients ($R=0.51$, $p=0.06$) (Fig. 1e–f).

A receiver operating curve (ROC)-curve was created to evaluate whether the CD4/CD8 ratio would be valuable as a diagnostic test for detection of liver fibrosis in HCV-monoinfected patients. Area under the receiver operating curve (AUROC) was 0.80 (confidence interval 0.58–1.0,

Table 1 Patient characteristics

Characteristics	HCV <i>n</i> =19	HCV/HIV <i>n</i> =14	HIV <i>n</i> =10	P-value
General characteristics				
Age, median (IQR), years	55 (7)	48 (14)	46 (6)	0.12
Gender, % male	74 %	86 %	90 %	0.27
HCV disease characteristics				
HCV-RNA, median (IQR), IU/mL	4.5e5 (1.1e6)	1.9e6 (4.8e6)		0.07
HCV genotype, % genotype 1	79 %	75 %		1.00
ALT, median (IQR), IU/L	69 (85)	65 (80)		0.92
Liver fibrosis/cirrhosis				
F0-F2, number (%)	9 (47 %)	8 (57 %)		0.73
F3-F4, number (%)	10 (53 %)	6 (43 %)		
HIV disease characteristics				
Viral load, copies/mL		All <50	All <50	–
CD4-count, median (IQR)/mm ³		540 (330)	661 (247)	0.11

Fig. 1 CD4/CD8 ratios in patients and controls. **a** CD4/CD8 ratios in healthy controls, HCV-monoinfected, HIV/HCV-coinfected and HIV-monoinfected patients measured by flow cytometry; *lines* depict median values. **b** CD4/CD8 ratios in HCV-monoinfected (*left*) and HIV/HCV-coinfected patients (*right*) with no or minimal fibrosis (F0-F2, *open dots*) or severe fibrosis or cirrhosis (F3-F4, *full dots*). **c** Correlation and linear regression line of liver stiffness measured by FibroScan and CD4/CD8 ratio in HCV-monoinfected patients (*black dots*) and HIV/HCV-coinfected patients (*grey dots*). **d** ROC-curve of CD4/CD8 ratio as a diagnostic test for liver fibrosis (F3-F4). **e-f** Correlations and linear regression analysis of CD4 counts (X-axis) and CD4/CD8 ratios (Y-axis) in HIV-HCV coinfecting (**e**) and HIV-monoinfected (**f**) patients

$p=0.027$), confirming that CD4/CD8 ratio in HCV-monoinfected patients is a potential candidate for non-invasive discrimination between F0-F2 and F3-F4 liver fibrosis (Fig. 1d).

Discussion

Since liver fibrosis is an important factor in prognosis and clinical decision-making in HCV-infection, we investigated whether the CD4/CD8 T cell ratio reflects degree of liver fibrosis in HCV-monoinfected and HIV/HCV-coinfected patients. The clinical significance of the peripheral CD4/CD8 T cell ratio is well established in both HIV-infection as well as other clinical conditions [5, 6, 8–10]. Furthermore, CD8 T cells may be involved in liver fibrosis [7, 11].

Interestingly, CD4/CD8 ratio was associated with HIV-coinfection and with liver fibrosis in HCV-monoinfected patients. Furthermore, our data demonstrate that CD4/CD8 ratio is a promising candidate for non-invasive evaluation of liver fibrosis.

Decreased CD4/CD8 ratio may reflect either low CD4 counts, high CD8 counts or combined changes in both T-cell compartments. Low peripheral CD4/CD8 ratios in untreated HIV-infection may not only reflect HIV-induced loss of infected CD4 T cells, but may also be the result of migration of T cells or altered T cell differentiation [12]. Indeed, a recent study demonstrated that coinfection with HCV was associated with decreased CD4 counts in HIV-1 natural suppressors [13]. This is in line with our results in adequately treated HIV-patients, suggesting that either T cell differentiation or migration is involved. Since the HCV-infected liver is enriched for CD8 T cells, CD4 T cell migration to the liver seems less likely to be responsible for low peripheral CD4/CD8 counts [11]. Furthermore, HIV/HCV-coinfected patients depict similar intrahepatic CD4 and CD8 T-cell responses compared to those of HCV-monoinfected individuals [14], suggesting that decreased CD4/CD8 ratio in HIV/HCV-coinfected patients is not the result of migration. Therefore, we hypothesize that low CD4/CD8 ratio in coinfecting patients is caused by altered differentiation, which may be the result of increased immune activation in HIV/HCV-coinfected patients [15], since low CD4/CD8 ratio is associated with increased T cell activation in HIV-monoinfected patients with long-term viral suppression [6].

However, in the case of HCV-monoinfection it is less obvious which factors contribute more to the low CD4/CD8 ratio. Our recent finding that liver fibrosis is associated with increased percentages of effector CD8 T cells in the same patient group is suggestive for changes in the CD8 rather than in the CD4 T cell compartment [7]. Even so, livers of HCV-monoinfected with cirrhosis depict relatively more hepatic CD8 T cells than those without cirrhosis [11], suggesting that our findings in the peripheral blood may mirror changes in hepatic T-cell populations. No correlation was found between

CD4/CD8 ratio and liver stiffness in HIV/HCV-coinfected patients, possibly because HIV-coinfection itself already induces low CD4/CD8 ratios.

Although CD4/CD8 ratio is highly influenced by absolute CD4-counts, the correlation between these routinely clinically measured CD4-counts and CD4/CD8 ratio was only borderline significant in this study. This can be explained by previous findings by others, demonstrating discordance between CD4 T-cell counts and CD4/CD8 ratios in patients with liver disease [16].

In the present study, liver fibrosis was assessed by transient elastography in the majority of patients. In general, measurement of liver fibrosis by transient elastography corresponds highly with liver biopsies in severe fibrosis and cirrhosis (F3 and F4), but it is less accurate in differentiating F3 from F2 fibrosis, which has major implications in clinical decision-making for initiation of HCV-treatment [17]. To investigate whether CD4/CD8 ratio could bridge this gap in noninvasive measurement of liver fibrosis it would be preferable to measure liver fibrosis by biopsy as the golden standard in order to establish cut-off value for CD4/CD8 T cell ratio.

Since venous blood samples are easily obtained and can be sent for analysis of CD4/CD8 ratio to a central laboratory, assessment of CD4/CD8 ratio could be easily implemented even in HCV-endemic countries where resources are often limited [18]. Considering the AUROC of 0.80, CD4/CD8 ratio is a promising candidate for non-invasive measurement of liver fibrosis in HCV-monoinfected patients.

However, the number of HCV-monoinfected patients in the present study is small. Our findings need to be confirmed in a population-based study and an optimal cut-off value needs to be determined in order to further assess the value of CD4/CD8 T cell ratio alone as a marker for liver fibrosis, or in combination with other variables such as APRI-score or Fibro-test [19].

Altogether, our data reveal that a low CD4/CD8 ratio is associated with HIV-coinfection and with liver fibrosis in HCV-infected patients, making it a promising candidate for non-invasive evaluation of liver fibrosis in HCV-monoinfection. Implementation of CD4/CD8 ratio for estimation of liver fibrosis could especially be useful in resource-limited settings.

Conflict of interest The authors declare that they have no conflict of interest.

References

1. Massard J, Ratziu V, Thabut D et al (2006) Natural history and predictors of disease severity in chronic hepatitis C. *J Hepatol* 44: S19–S24

2. Ahmad W, Ijaz B, Gull S et al (2011) A brief review on molecular, genetic and imaging techniques for HCV fibrosis evaluation. *Virol J* 8:53
3. Operskalski EA, Kovacs A (2011) HIV/HCV co-infection: pathogenesis, clinical complications, treatment, and new therapeutic technologies. *Curr HIV/AIDS Rep* 8:12–22
4. Kim AY, Chung RT (2009) Coinfection with HIV-1 and HCV—a one-two punch. *Gastroenterology* 137:795–814
5. Taylor JM, Fahey JL, Detels R, Giorgi JV (1989) CD4 percentage, CD4 number, and CD4:CD8 ratio in HIV infection: which to choose and how to use. *J Acquir Immune Defic Syndr* 2:114–124
6. Serrano-Villar S, Gutierrez C, Vallejo A et al (2013) The CD4/CD8 ratio in HIV-infected subjects is independently associated with T-cell activation despite long-term viral suppression. *J Infect* 66:57–66
7. Feuth T, Arends JE, Fransen JH et al (2013) Complementary role of HCV and HIV in T-cell activation and exhaustion in HIV/HCV coinfection. *PLoS One* 8:e59302
8. Liu CC, Huang KJ, Lin YS, Yeh TM, Liu HS, Lei HY (2002) Transient CD4/CD8 ratio inversion and aberrant immune activation during dengue virus infection. *J Med Virol* 68:241–252
9. Syrjala H, Surcel HM, Ilonen J (1991) Low CD4/CD8 T lymphocyte ratio in acute myocardial infarction. *Clin Exp Immunol* 83:326–328
10. Sheu BC, Hsu SM, Ho HN, Lin RH, Torng PL, Huang SC (1999) Reversed CD4/CD8 ratios of tumor-infiltrating lymphocytes are correlated with the progression of human cervical carcinoma. *Cancer* 86:1537–1543
11. Bonacini M, Govindarajan S, Kohla M, Lai MM, Lindsay KL (2007) Intrahepatic lymphocyte phenotypes in hepatitis C virus infection: a comparison between cirrhotic and non-cirrhotic livers. *Minerva Gastroenterol Dietol* 53:1–7
12. Rosenberg YJ, Anderson AO, Pabst R (1998) HIV-induced decline in blood CD4/CD8 ratios: viral killing or altered lymphocyte trafficking? *Immunol Today* 19:10–17
13. Sajadi MM, Pulijala R, Redfield RR, Talwani R (2012) Chronic immune activation and decreased CD4 cell counts associated with hepatitis C infection in HIV-1 natural viral suppressors. *AIDS* 26:1879–1884
14. Alatrakchi N, Graham CS, He Q, Sherman KE, Koziel MJ (2005) CD8+ cell responses to hepatitis C virus (HCV) in the liver of persons with HCV-HIV coinfection versus HCV mono-infection. *J Infect Dis* 191:702–709
15. Page EE, Nelson M, Kelleher P (2011) HIV and hepatitis C coinfection: pathogenesis and microbial translocation. *Curr Opin HIV AIDS* 6:472–477
16. Hull MW, Rollet K, Oduyungbo A et al (2012) Factors associated with discordance between absolute CD4 cell count and CD4 cell percentage in patients coinfecting with HIV and hepatitis C virus. *Clin Infect Dis* 54:1798–1805
17. Feuth T, Arends JE, Lieveld FI et al (2013) Impact of transient elastography on clinical decision-making in patients with chronic viral hepatitis. *Scand J Gastroenterol* 48:1074–1081
18. Lavanchy D (2011) Evolving epidemiology of hepatitis C virus. *Clin Microbiol Infect* 17:107–115
19. Castera L (2011) Invasive and non-invasive methods for the assessment of fibrosis and disease progression in chronic liver disease. *Best Pract Res Clin Gastroenterol* 25:291–303