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Arginase 1: A potential marker of a common pattern of liver steatosis in HCV and NAFLD children

To the Editor,

We read with great interest a recent article published in this *Journal* by Moreau *et al.* [1] on the effect of hepatitis C virus (HCV) proteins in metabolic liver zonation and steatosis in previously established FL-N/35 transgenic mice [2]. In these mice with a quasi-pure C57BL/6 genetic background liver steatosis is mainly localized around the central vein of the hepatic lobule, while only a subset of hepatocytes seem to be involved in the activation of steatogenic enzymes with a prevalent periportal zonation, as well as the HCV proteins [3]. Interestingly, the authors found that fatty acid synthase, was redistributed from its normal periportal expression into the midzone of the lobule, matching with lipid accumulation. This lipogenic enzyme activation and redistribution in humans with HCV infection may precede the manifestation of a steatotic phenotype.

However, some markers of metabolic zonation, such as arginase 1, was not influenced by HCV protein expression. Arginase 1 is one of the two enzymes that competes with nitric oxide synthase for arginine, and it is mainly expressed in hepatocytes. The fact that arginases 1 and 2 are crucial to maintain liver lipid homeostasis has also emerged in a recent article by Navarro *et al.* which reported an increased expression of lipogenic genes and a consequent increased steatosis in Arg2$^{-/-}$ mice compared to wild-type, generating a model that resembled human NAFLD [4]. However, in both cases the role of arginase 1 is underestimated. In this regard, we have analyzed the expression of arginase 1 in liver tissue from four healthy children without any sign of steatosis, four children with HCV infection but no steatosis, four children with HCV and steatosis and seven children with NAFLD. As shown in Fig. 1A, the quantification of the immunohistochemistry by MetaMorph Software demonstrated that mean intensity of arginase 1 was increased in HCV patients with steatosis and in NAFLD subjects compared to healthy livers and HCV that displayed the same average intensity. Furthermore, as confirmed by Western blot analysis (Fig. 1B) arginase 1 protein expression was higher in HCV patients with steatosis than in NAFLD. Data presented by both Moreau *et al.* [1] and us highlight that arginase 1 increase may represent a marker of common patterns of steatosis in HCV and NAFLD when the derangement of lipid metabolism has already occurred.

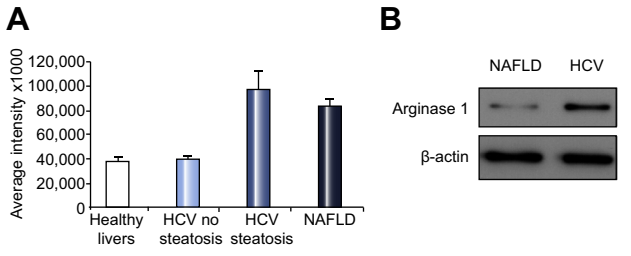

Fig. 1. HCV patients with steatosis, and NAFLD subjects show altered expression of arginase in liver. (A) MetaMorph quantification of arginase 1 immunohistochemical staining. Histograms reported mean ± standard deviation. (B) Western blot of pooled liver samples from HCV infected patients without steatosis, HCV infected patients with steatosis and NAFLD subjects.

The arginase 1 in inflammatory response is clearly demonstrated by the fact that the murine enzyme is inducible by lipopolysaccharide or oxidized and acetylated lipoproteins suggesting its role in diseases characterized by altered lipid metabolism and a pro-inflammatory pattern [5,6]. Further experimental data could be useful to confirm this hypothesis and to fully depict the molecular mechanisms that could link intra-hepatic lipid accumulation and consequent arginase 1 increase in HCV and NAFLD. Finally, these findings might open new therapeutic horizons for liver disease progression, especially as it has been reported that the inhibition of arginase 1 by silencing or by a specific Nω-hydroxy-nor-L-arginine could reduce growth rate of HCV-infected hepatocarcinoma cells and obesity-induced hepatic lipid abnormalities in mice with NAFLD, respectively [7,8].

Conflict of interest

The authors declared that they do not have anything to disclose regarding funding or conflict of interest with respect to this manuscript.

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Reply to: "Arginase 1: a potential marker of a common pattern of liver steatosis in HCV and NAFLD children"

Arginase, NASH and HCC: What role for macrophages?

To the Editor,

We thank Alisi and colleagues for their comments [1] on our recent report characterizing the effects of the hepatitis C virus (HCV) proteins on metabolic liver zonation and steatosis [2]. In a transgenic mouse model with hepato-specific expression of the full complement of HCV proteins we found that fatty acid synthase, a major lipogenic enzyme, was redistributed to the midzone of the lobule, coinciding with zonated accumulation of lipids. Based on these and additional results, we hypothesized that low levels of viral proteins are sufficient to drive striking alterations of hepatic metabolic zonation. However, not all genes that display zonated expression in the liver were altered in the animal model studied. Notably, we found no change in the pattern of expression of E-cadherin or arginase 1. In contrast to the results reported by Alisi *et al.* we did not study the global level of expression of the latter.

Alisi *et al.* compared arginase 1 expression in liver tissues from children with no hepatic pathologies or suffering from non-alcoholic fatty liver disease (NAFLD) or hepatitis C [1]. The quantification of the immunhistochemical staining showed identical mean intensity of arginase 1 in healthy livers and in HCV-positive patients without steatosis. This is consistent with our results showing no difference of arginase 1 expression in hepatocytes from control and HCV-transgenic mice [2]. In our work, appearance of steatosis did not alter arginase distribution. In contrast, Alisi *et al.* observed increased arginase 1 expression in HCV patients with steatosis and in NAFLD. Their results highlight the link between lipid accumulation and arginase expression, as well as the effect of HCV infection on arginase 1 accumulation. Noteworthy, Alisi *et al.* give no information on the HCV genotype, while our study focused solely on genotype 1. It would be of interest to determine whether the reported observations are genotype specific or more generalizable.

As Alisi and colleagues correctly pointed out, the role of arginase 1 in steatosis is not well understood and possibly underestimated. Interestingly, its increased expression has been described in HCV infection and it was suggested that it might participate in promoting cell growth and proliferation [3]. Indeed, arginase 1 converts arginine to ornithine, which is further metabolized to polyamines that promote cellular proliferation. Thus, elevated arginase 1 expression may be hepatoprotective during chronic infection by promoting survival and proliferation of HCV-infected hepatocytes. In consequence, the inflammatory immune response that contributes to liver damage would be inefficient in eliminating virus-infected cells.

Elevated arginase 1 expression is associated with many tumor types [4] and therapeutic strategies aiming at inhibiting this metabolic pathway are being tested in the clinic. Interestingly, in HCC, the predominant peri-tumoral distribution of arginase 1 expression is in agreement with proteomic analyses of samples from HCV infected HCC and non-tumoral liver [3,5].

Recent evidence indicates that interactions between tumor cells and the host microenvironment have a major role in driving cancer progression and metastasis [6,7]. The abundance of macrophages, pivotal members of tumor stroma, strongly correlates with poor prognosis in different types of solid tumors, including HCC [8]. It has been proposed that tumor-associated macrophages (M2-polarized) play an important role in generating overall immunosuppressive milieu within the tumor microenvironment that suppresses anti-tumor immunity and promotes tumor progression [6]. Interestingly, the differential metabolism of L-arginine provides a means of distinguishing the two macrophage activation states. M1, or classically activated macrophages, upregulate iNOS to catabolize L-arginine to nitric oxide and citrulline, while M2, or alternatively activated macrophages, induce arginase 1, the enzyme upstream of polyamines production, and thus increase collagen synthesis and cellular proliferation [9].

In HCC, high numbers of peri-tumoral M2-polarized TAMs are associated with poor patient prognosis, and while no analyses of arginase 1 expression have yet been conducted in this context, it is likely that they express high levels of this enzyme along with the other M2 genes described. In this context, TAM-derived arginase 1 might promote tumorigenesis in a non-cell autonomous manner. Furthermore, several TAM-derived factors, such as