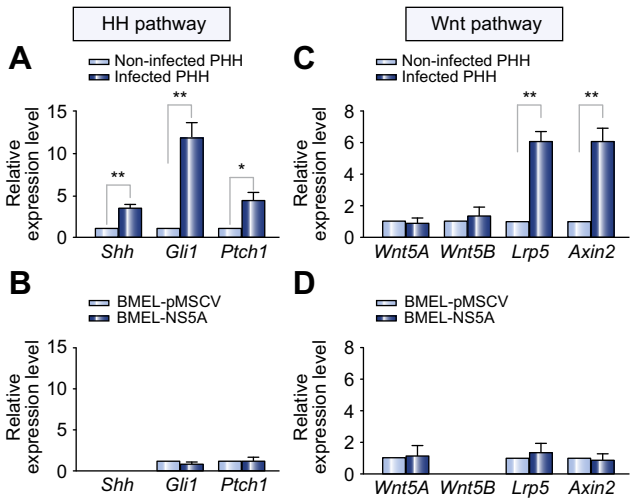

Fig. 1. Activation of HH and Wnt pathways by HCV does not account for NS5A-induced EMT. All experimental conditions were described in [3]. (A) Control and HCV-infected PHH (see Fig. 3D in Akkari *et al.* [3]) were collected three weeks after the infection and analysed by RT-qPCR to evaluate mRNA levels of components of HH and Wnt pathways as well as *Axin2*, a Wnt signalling target gene. Means ± SEM of three samples from one of three independent experiments are shown. (B) Expression of HH and Wnt pathways components and a Wnt target (*Axin2*) was analysed in BMEL-pMSCV and BMEL-NS5A cell lines. RT-qPCR quantification of mRNA was normalised to *HPRT* level and was arbitrarily set as 1 in control cells. *Shh* and *Wnt5b* mRNAs were below the level of detection in BMEL cells. Means and values from two independent experiments quantified in triplicate are shown. Statistical analysis was performed by unpaired Student's *t* test. $^{*}p$ <0.05, $^{**}p$ <0.01.

activated up to at least three weeks following HCV infection of PHH (Fig. 1C). However, although we cannot exclude an early transient activation of Wnt/β-catenin signalling in BMEL expressing NS5A, we found no evidence either of increased expression of components of this pathway or, more significantly, of sustained transcriptional activation of axin2 in BMEL-NS5A cells undergoing EMT (Fig. 1D). Thus, our results suggest that neither Hedgehog nor β-catenin signalling is required for NS5A-mediated EMT. It remains an open question whether these pathways participate in EMT induction orchestrated by other HCV proteins.

Conflict of interest

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

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Effect of albumin on survival in septic cirrhotic patients other than spontaneous bacterial peritonitis. The question remains open

To the Editor:
We read the manuscript by Guevara *et al.* with interest [1]. The authors evaluated the effect of albumin administration on the 3-month survival in cirrhotic patients hospitalized for infections other than spontaneous bacterial peritonitis (SBP). After randomization, 54 and 56 patients received respectively antibiotics alone, and both antibiotics plus intravenous albumin (1.5 g/kg the first day and 1 g/kg at day 3). The authors concluded that albumin administration with antibiotics showed a potential survival benefit in per-protocol analysis as compared with the control group, and that such a beneficial effect was probably due to the improvement in effective arterial blood volume reflected by the improvement in renal function. However, we feel that their conclusions may require closer examination.

First, this hopeful conclusion contrasts with the absence of a 3-month survival benefit when the analysis was performed on

an intention-to-treat basis. The reason for the lack of significance in the intention-to-treat analysis could be related to the patients withdrawn from the per-protocol analysis. Indeed, the overall prognosis of these patients excluded from analysis was worse (these patients had septic shock or unrecognized cardiomyopathy) than those who remained in the analysis, and this may overestimate the albumin efficacy. Thus, the per-protocol analysis represents a "best-case scenario" to demonstrate the treatment effect [2]. The patient selection could also explain this lack of significance since a progressive form of renal failure has been observed only in sub-diaphragmatic infections [3]. Moreover, the author's assumption that albumin could improve the survival rate by 30% (i.e., 3-month mortality rates of 55% in the control group and 25% in the albumin group) was an over-optimistic projection that is not supported by the literature [4,5]. This also explains the absence of any beneficial effect of albumin in their intention-to-treat analysis. Even in the sickest cirrhotic patients with SBP, Sort *et al.* observed a 3-month mortality rate of 59% in the group treated with antibiotics alone (control group), and the difference in the survival rate between this control group and the group of patients treated with both albumin and antibiotics was only 19% [4].

Second, the multivariate analysis performed to identify potential predictors of death included more variables than allowed by the low number of events (18 deaths) observed. Consequently, this may fail to provide valid estimates applicable to other sets of patients. To avoid such overfitting, a predefined ratio of candidate prognostic variables to the number of deaths should have been set at 1:10 [6].

Third, the authors observed a significant decrease in plasma renin activity, aldosterone and norepinephrine concentration, along with the stability of arterial pressure in the albumin group, contrary to that observed in the control group. These data probably support the improvement in renal function during the first week following albumin administration. However, we should interpret this supposed improvement in renal function with caution, because plasma renin activity, aldosterone and norepinephrine concentration were measured in only 48 of the 97 patients enrolled in the analysis, which precludes the extrapolation of measured values to the true mean values for the whole population. From a clinical point of view, the incidence of renal failure (serum creatinine >133 μmol/L) was low and not different between the two groups (27.4% in the control group and 23.9% in the albumin group). As stated honestly by Guevara *et al.*, the low incidence of renal insufficiency suggested that their study population was not so sick. The absolute difference of only 2.2% in the 3-month survival rate between the two groups (80.4% in the control group *vs.* 82.6% in the albumin group) also precludes any assumptions regarding the putative survival benefit achieved by improving renal function with albumin infusion.

Finally, this randomized study allows us to draw some conclusions and assumptions for future trials. As reported previously, albumin administration has demonstrated its beneficial effects on systemic hemodynamic and renal function in cirrhotic patients with SBP, in terms of both an improvement in cardiac function and a decrease in arterial vasodilatation [7]. This effect may be unchanged in infections other than SBP. In Guevara's study [1], the beneficial effect of albumin on survival is more controversial, and the high number of patients (approximately 5000 per group) that would have been required to detect a 2.2% difference in survival rate, between those receiving albumin infusion or not, will preclude further trials with such a "hard" end point. Assessment of renal function is surely a valid surrogate, because (1) creatinine level can be measured simply in the post-treatment period, (2) renal insufficiency is strongly associated with death in cirrhotic patients, and (3) renal function improves after albumin treatment. As clearly stated by Guevara *et al.*, the key issue to highlight a beneficial effect of albumin on survival would be to investigate more fragile cirrhotic patients, particularly those with ascites (but without SBP) associated or not with renal failure. Large randomized studies are needed to confirm the results of Guevara's study. We will soon report first results from our ongoing French "ALBCIRINF" study evaluating the effect of albumin on renal function in severe cirrhotic patients, suffering from bacterial infection other than SBP.

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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