reduces the sensitivity of the image but allows a real-time evaluation of the different vascular phases of the contrast medium through the lesion.[4,5]

The final aim of these efforts is to display, possibly in real-time and at acceptable costs, the microcirculatory kinetic characteristics of space-occupying liver lesions that are more informative about their nature and compete with the current available contrast-enhanced images of computed tomography and magnetic resonance.

In light of these considerations, the high diagnostic performance of the contrast-enhanced US method by Fracanzani et al. seems exceedingly optimistic and reflects, at least in part, both the high prevalence of the disease in the population studied (hepatocellular carcinomas represented half of the cases included in the study) and the clinical setting of the study (*i.e.*, a scientific work performed by highly motivated operators).

The clinical audience of HEPATOLOGY should be aware that the methods used by Fracanzani et al. represent the immediate past and not an updated work in progress. The new US equipment, scanning techniques, and contrast agents are on the way and the results of their clinical applications will supply a new body of information on noninvasive tissue characterization of liver lesions, especially when arising in a cirrhotic liver.

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Reply:

We thank Drs. Francica and Giorgio who have given us the opportunity to better explain a few points apparently not too clear in our manuscript. Contrary to what Drs. Francica and Giorgio imply, we did not analyze the tumor vascularity pattern after contrast enhanced power Doppler, but we performed Doppler spectral analysis on the intratumoral arterial vessel detected. Hepatocellular carcinoma is a highly vascular tumor and contrast-enhanced power Doppler is a sensitive method of detecting arterial flow signals in tumor nodules.[1,2] Further characterization of the nodules is possible using spectral analysis of the Doppler shift, which allows the calculation of the pulsatility and resistive indices of the arterial signals as indicated in our report. As for artifacts such as color "blooming" and saturation of the color box, they are of no clinical relevance if the appropriate concentration of contrast medium and correct gain settings are used as reported in the literature.[1] We stress the fact that our report is not a morphologic study of the tumor vascularity such as those using pulse inversion imaging with high mechanical index agents. The newer low mechanical index agents seem to be more promising in this respect. However it is important to realize that these new techniques only recently available to the clinician are still, in part, experimental and require dedicated equipment with the relative economical investments. Our study began 4 years ago when these new techniques were not available. The method we described is useful in the characterization of small lesions that occur during follow-up in cirrhotic patients. Finally we pointed out that this approach does not require specialized equipment but can be performed with currently available machines with power-Doppler capability. We obtained a sensitivity of contrast enhanced ultrasound of 95%, which is similar to that obtained by Tanaka et al.[3] with pulse inversion harmonic imaging and contrast agent (Levovist), and we found a similar percentage of HCC with no arterial vascularization (5% vs. 7%).

Regarding the point risen by Drs. Francica and Giorgio on the prevalence of HCC, it is worthwhile noting that the prevalence of HCC in our study does not seem to be higher than that reported in Italy (3.5% per year). In fact, from 500 cirrhotic patients in follow-up for 4 years we have found 20 monofocal HCC and 21 dysplastic or regenerative nodules. The remaining 12 lesions not included in the study were all HCC.

Thus, although we appreciate the interest of Drs. Francica and Giorgio in our work, we conclude that the setting they mention (equipment and series of patients) is not the one described in our report.

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## Hepatocyte Prostaglandin Synthesis and Mdr1 Expression

To the Editor:

Ziemann et al.[1] presented evidence that prostaglandins can induce increased expression of mdr1 messenger RNA and its transport function in primary rat hepatocyte cultures. Their evidence also suggested that prostaglandins produced in these cultures are partly responsible for the increased expression of mdr1 found over several days of culture. These results suggest that cyclooxygenase inhibitors could be useful in countering resistance to chemotherapy in patients with hepatocellular carcinoma.

These data are consistent with the literature. It is well known that primary rat hepatocyte cultures contain cyclooxygenase and make prostaglandins. However, primary hepatocyte cultures derived from rat, guinea pig, and human contain significant numbers of nonparenchymal cells, including approximately 5% Kupffer cells, a similar number of endothelial cells, and approximately 2% stellate cells.[2-4] These nonparenchymal cells can easily be detected by immunostaining or by uptake of fluorescent-labeled, acetylated, low-density lipoprotein. Physical methods for purifying hepatocytes, such as density gradient centrifugation or elutriation, are minimally effective, probably because nonparenchymal cells adhere to hepatocytes. Ziemann et al.[1] used arginine-free medium to try to select against nonparenchymal cells in their cultures, but this never has been shown to be effective.

Kupffer and endothelial cells can be completely eliminated from hepatocyte cultures by a brief incubation with the toxic A chain of ricin.[2] Ricin A chain is selectively absorbed by Kupffer and endothelial cells and inhibits their protein synthesis. Under appropriate conditions it does not affect hepatocytes. We found that ricin-purified hepatocytes produce insignificant amounts of cyclooxygenase products as compared with cultures without ricin purification.[5] Essentially, all the cyclooxygenase activity in freshly isolated hepatocytes can be accounted for by the contaminating Kupffer cells.[6] Kupffer cells are evidently the major source of cyclooxygenase products in adult liver. Hepatoma cell lines and fetal hepatocytes express cyclooxygenase, including cox-2.[7] However, the inducibility of cox-2 in rat hepatocytes rapidly disappears after birth under the influence of CCAAT/enhancer binding protein–$\alpha$.[7]

Ziemann et al.[1] were no doubt observing the effects of cyclooxygenase products made by Kupffer cells in their primary rat hepatocyte cultures. This in no way reduces the significance of their results. Cyclooxygenase products produced by Kupffer cells or by carcinoma cells could play a significant role in up-regulation of Mdr1 in liver or liver tumors *in vivo*. However, hepatocytes from adult liver are not an important source of cyclooxygenase products.