HEPATOLOGY

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Letter to the Editor: Emergence of Zoonotic Rat Hepatitis E Virus Infection

TO THE EDITOR:

We noted the identification of further rat hepatitis E virus (HEV) cases in humans in Hong Kong.[1] This emergence, infection of a Canadian United Nations worker in Africa,[2] and serological evidence of exposure in German foresters[3] and hospitalized Vietnamese patients[4] raises the question of how widespread rat HEV infection is globally.

Rat HEV (*Orthohepevirus C* or HEV-C) is distinct from epidemic HEV strains circulating in Africa, Asia, and to zoonotic pig strains responsible for disease burden in industrialized countries (*Orthohepevirus A* or HEV-A). In Europe, hepatitis E is not notifiable, and, until recently, lack of awareness by physicians and poorly standardized diagnostics led to underreporting. In 2019, the European Centre for Disease Prevention and Control developed guidance on HEV surveillance (data collection/reporting, case definitions, and testing)[5]; this and other guidelines do not currently consider HEV-C. Despite similar clinical presentation of HEV-C and HEV-A (acute and chronic), molecular assays used clinically and for blood donor screening do not detect HEV-C. A commonly used anti-immunoglobulin M (IgM) assay can detect anti-HEV-C antibodies; however, HEV-A/HEV-C-discriminatory assays are unavailable. Of the 169 anti-IgM-positive patients, Sridhar et al. identified 5 HEV-C1 and 82 HEV-A RNA-positive patients.[1] Is more data on the remaining 82 HEV-IgM-positive/RNA-negative patients available? Could variability of HEV-C strains contribute to missed cases? There is a need to develop specific molecular and serological assays and associated control materials for HEV-C, to assess case frequency in previously healthy immunocompetent as well as immunosuppressed individuals and understand the risk to humans.

Zoonotic HEV-A is transmitted primarily through consumption of contaminated meat. While Sridhar et al. mentioned the proximity of the HEV-C-infected patients to rat infestations and droppings, there was no discussion about possible transmission of HEV-C by consumption of rat meat or contaminated meat products. More detailed epidemiological investigations (environmental risk factors, consumption habits, and water contamination) are needed to identify infection sources and transmission patterns.

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Potential conflict of interest: Nothing to report.

REPLY:

We thank Dr. C. Adlhoch and Dr. S.A. Baylis for their comments on our study describing the impact of rat hepatitis E virus (*Orthohepevirus C* genotype

1 or HEV-C1) on human health in Hong Kong.[1,2] We concur with them that HEV-C1 infection is currently a blind spot in hepatitis E diagnostic testing. As they point out, routinely used molecular assays for HEV diagnostics or blood donor screening would not be able to detect HEV-C1.[3] Although we demonstrated that the Wantai HEV immunoglobulin M (IgM) and immunoglobulin G kits (Wantai, Beijing, China) may cross-react with the sera of patients with HEV-C1,[1] HEV-A/HEV-C1 discriminatory assays would be a valuable asset to HEV diagnostics.

For 40 of the HEV IgM-positive/RNA-negative patients with sufficient sample volume, we also attempted conventional RT-PCR using universal consensus primers as described previously.[3] These primers would theoretically be able to detect highly divergent species within the family *Hepeviridae*, but all samples tested negative. As noted in our study, our real-time PCR primers and probes were specific for HEV-C genotype 1, which circulates in rats.[1] However, HEV-C is very diverse, with four putative genotypes circulating in a variety of rodents and ferrets.[4] Our HEV-C1 real-time PCR would not detect HEV-C genotypes 3 and 4, which circulate in field mice and voles.[4] However, we judge that urban dwellers in Hong Kong are less likely to be exposed to these genotypes.

The route of transmission of HEV-C1 between rats and humans is elusive. None of our patients had a history of rat meat consumption, and the practice is uncommon in Hong Kong. Indeed, almost all of them even denied rat infestation in their domestic premises. We considered adulteration of food products or natural HEV-C1 infection of pigs to be possibilities, so we tested for HEV-C1 in 212 pork products and samples. However, none of the samples tested positive.[1] We agree that extensive epidemiological investigations are required to identify the definitive source of HEV-C1 infection.

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Potential conflict of interest: Nothing to report.

Letter to the Editor: Does Augmenter of Liver Regeneration Deficiency Pave the Way for Nonalcoholic Steatohepatitis Progression?

TO THE EDITOR:

We read with interest the recent article by Kumar et al.[1] on the impact of augmenter of liver regeneration (ALR) deficiency on nonalcoholic fatty liver disease (NAFLD) progression from steatosis to nonalcoholic steatohepatitis (NASH). By using hepatocyte-specific ALR knockout/knockdown high-fat/high-

carbohydrate mouse models, they demonstrated an involvement of ALR in lipid metabolism, oxidative stress, and inflammatory response leading to fibrosis. However, ALR is expressed in three isoforms (15, 21, 23 kDa),[2] and their specific roles in this "loss-of-function" strategy is not addressed. In earlier publications, using an overexpression approach ("gain-of-function") of the mainly mitochondrial 23-kDa ALR, a reduction in hepatic fibrosis and ischemia reperfusion injury (IRI) in steatotic livers was shown.[2] Expression of cytosolic 15-kDa ALR reduced severity of liver injury in a methionine-choline–deficient NASH mouse model.[2] Furthermore, it was shown that cytosolic 15-kDa ALR, different from exogenously applied 15-kDa ALR, diminished triacylglycerol levels and lipoapotosis *in vitro* by attenuating endoplasmatic stress, increasing lipolytic and decreasing lipogenic gene expression.[3] Additionally, treatment with exogenous ALR reduced hepatic IRI in mice by reducing neutrophil infiltration through less hepatocytic chemokine expression.[4] More detailed knowledge of the function of individual ALR isoforms is necessary for future therapeutic interventions.

Kumar et al. found decreased ALR levels in human NASH patients (tissue and serum) and pointed to a lack of information about ALR regulation. This was addressed earlier, demonstrating reduced hepatic ALR mRNA levels in patients with steatosis and even more with NASH.[2,3] ALR is regulated, among others, by forkhead box A2 (FOXA2; hepatocyte nuclear factor 3β), which translocates from the nucleus to the cytosol upon treatment with free fatty acids, and whose expression correlates with ALR expression in NAFLD patients.[3] Nuclear factor erythroid 2–related factor 2 (Nrf2), another ALR-regulating transcription factor,[2] was shown to attenuate liver steatosis and therefore it is likely that FOXA2 and Nrf2 diminished endogenous ALR levels in hepatic steatosis/NASH. However, the conclusion that ALR deficiency may be a major determinant of accelerated progression of NASH to end-stage liver disease, that is, cirrhosis, is not supported by enhanced ALR levels in patients with cirrhosis[2] or by clinical characterization of patients with mutations in the growth factor erv1-like (GFER) gene (encoding for ALR) showing almost no distinctive feature regarding hepatic function[2] and therefore should to be taken with caution.

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Potential conflict of interest: Nothing to report.

REPLY:

We appreciate the important points raised by Weiss et al.[1] Our data indicate that the loss of 21/23 kDa Augmenter of liver regeneration (ALR), in mice and humans, renders the liver compromised to develop NASH.[2] Administration of 21/23 kDa ALR to hepatocyte-specific ALR-knockout (ALR-H-KO) mice between 1 and 2 weeks postpartum mitigated steatohepatitis.[3] The 15 kDa ALR is absent (Fig. 8)[2](Ref. 71 in [4]) or expressed at a very low level[5] in human liver and absent in mouse liver.[2,4,6] However, further investigation of ALR isoform(s) that can be of therapeutic intervention is important.

Hepatic ALR is down-regulated in steatosis regardless of its etiology in wild-type (WT) mice,

which do not develop inflammation or fibrosis.[2,3,5,6] Only a subset of humans with nonalcoholic fatty liver (NAFL) develop NASH, and some of these progress to cirrhosis, suggesting that inherent deficiency or anomaly in ALR could be an important predisposing factor. However, it will be of interest to examine forkhead box A2 or nuclear erythroid 2 p45-related factor 2 during NAFL disease progression caused by diet-induced or inherent ALR deficiency.

In the papers identified by Weiss et al.,[4] ALR is less stable (and probably also less efficient) because of the rare mutation(s) in the Growth factor, ERV1-like (*GFER*) gene in the patients who were affected. The mutations were associated with congenital cataract, muscular hypotonia, and developmental delay. Note that a liver biopsy of only one patient was performed, which showed mitochondrial damage and fibrosis. Although studying the effect of HF diet in mice with these mutation(s) in an organ-specific manner is interesting, they are unlikely to be present in NASH, a global epidemic. Other mutations identified in the *GFER* gene in the general population[6] are being investigated.

Our conclusion that inherent ALR deficiency or anomaly could be critical in NASH progression to cirrhosis was based on (1) HF diet-induced excessive steatosis, inflammation, and fibrosis in otherwise normal ALR-hepatocyte-heterozygous mice and augmented inflammation and cirrhosis in ALR-H-KO mice with pre-existing underlying pathology; and (2) lower ALR levels in human NASH and NASH cirrhosis.[2,5,6] However, the high ALR expression observed in some NASH-cirrhotic livers (Supporting Fig. S10; Supporting Table S4)[2] indicates heterogeneity in human NASH. Patients expressing higher hepatic ALR had non-NASH cirrhosis,[Ref. 71 in (4)] which may be attributed to etiological differences from NASH cirrhosis.

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Potential conflict of interest: Nothing to report.

Letter to the Editor: Thromboelastography-Guided Blood Product Transfusion in Cirrhosis With Coagulopathy: Real Saving or Just Less Waste?

TO THE EDITOR:

We read with interest the article by Kumar et al.[1] and wish to make comments. A thromboelastography (TEG)–guided transfusion policy (with arbitrary thresholds of $r > 10$ minutes for fresh frozen plasma [FFP] and maximal amplitude [MA] <55 mm for platelet transfusion) led to sparing blood products

compared to standard of care (SOC) in patients with advanced cirrhosis, nonvariceal bleeding, and coagulopathy, defined by an international normalized ratio (INR) >1.8 and/or a platelet count <50 × 10^9/L.

Presently, all acknowledge that the INR is inappropriate to investigate the coagulopathy of patients with cirrhosis, partly due to the absence of thrombomodulin in its formulation. Instead, thrombin generation (TG) assays performed with thrombomodulin, mimicking what occurs *in vivo*, indicate that TG is normal/increased in cirrhosis because of the deficiency of both procoagulants and anticoagulants.[2] Furthermore, infusion of FFP in patients with cirrhosis prior to invasive procedures does not modify TG, despite INR shortening.[3] Accordingly, although INR-based guidelines are still pervasively applied worldwide, they should be reconsidered.

Kumar et al.[1] arbitrarily chose a TEG reaction time of >10 minutes as an indication for FFP infusion. Such a policy allowed sparing 50% of FFP units but does not answer the question of whether FFP is really required. We surmise that if the clotting system is rebalanced, FFP infusion lacks biological plausibility.

What about platelets? Kumar et al.[1] chose an MA <55 mm to guide platelet transfusion and spared 50% platelet units. Again, such a threshold might be questionable as elevated von Willebrand factor levels in cirrhosis support *in vitro* platelet adhesion, despite reduced platelet count.[2] Besides primary hemostasis, platelets support TG by assembling activated coagulation factors on their surface. The platelet count still able to secure *in vitro* TG corresponding to the lower reference limit is around 60 × 10^9/L.[2] Thus, and at variance with FFP infusion, a rationale for platelet infusion in patients with thrombocytopenic cirrhosis exists, though the effect *ex vivo* of a single adult platelet unit in such patients barely affects TG or thromboelastometry.[4]

In conclusion, although TEG-guided policy allows sparing FFP or platelet transfusion compared to SOC, it is not biologically plausible. Whether such sparing of resources is a real benefit rather than a lesser waste should be ascertained by studies comparing TEG-guided transfusion versus restrictive protocols (i.e., no transfusion, unless severe coagulation derangement), which have not been considered in most studies dealing with thromboelastometry-guided transfusion policy.

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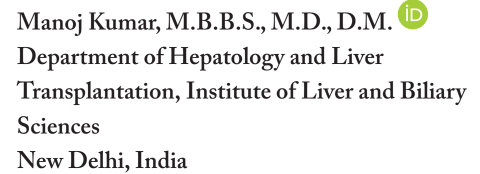
Potential conflict of interest: Nothing to report.

REPLY:

We thank Primignani et al. for taking interest in our article. We agree with their comments that the cutoffs of conventional tests and thromboelastography used in our study for transfusion of blood products is arbitrary. There are also no studies, to our knowledge, assessing whether blood products are actually needed in patients with cirrhosis in various clinical settings and with different degrees of coagulopathy. Appropriately designed studies are needed in the future to answer the following questions:

1. What are the cutoffs for coagulation tests and viscoelastic hemostatic assays at which blood products should be transfused in patients with cirrhosis in different clinical settings (for patients undergoing invasive procedures, variceal and nonvariceal bleeding, and so on)?
2. And if blood products are needed, can the currently used arbitrary cutoffs be relaxed?



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