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Combination therapy of sofosbuvir and ribavirin fails to clear chronic hepatitis E infection in a multivisceral transplanted patient

To the Editor:

Infections with zoonotic hepatitis E virus (HEV) have been increasingly reported in industrialized countries. Three months of detectable virus replication define chronic HEV infection,[1] which may rapidly progress to severe liver disease.[2] Patients under immunosuppression following solid organ transplantation represent the largest group at risk of chronic HEV infection. However, there have been anecdotal reports about chronic HEV infection in patients receiving immunosuppressive medication regimens including rituximab or inhibitors of TNF-α.[1,7] Chronic HEV infection has also been reported in patients under immunosuppression due to human immunodeficiency virus (HIV)[3] or following stem cell transplantation.[4]

Current treatment guidelines recommend the initiation of ribavirin (RBV) monotherapy for 3–6 months for patients failing to clear HEV after a reduction of immunosuppression.[1] Preclinical *in vitro* data by Dao *et al.* showed antiviral effects of sofosbuvir (SOF) in HEV infection and suggested additive antiviral effects when in combination with RBV.[5] However, there are only anecdotal and conflicting reports regarding the efficacy of SOF and RBV (SOF/RBV) in the treatment of patients chronically infected with HEV in the real world.[6,7,9,10]

Here we report the first case of a multivisceral transplanted patient with chronic HEV infection who failed to clear chronic HEV infection under combination therapy with SOF/RBV. In depth sequence analyses of virus isolates at 3 consecutive time points under SOF/RBV combination therapy showed a stepwise accumulation of RBV-associated mutations leading to treatment failure.

In 2011, a 30-year old male received transplantation of liver, pancreas, kidney, small bowel and right hemicolon for short bowel syndrome after multiple enteral resections due to Crohn's disease, total parenteral nutrition-related liver fibrosis and chronic renal insufficiency due to focal segmental glomerulosclerosis. His immunosuppressive medication comprised 5–8 mg tacrolimus/d and 1 mg sirolimus/d, aiming at steady-state trough plasma levels of 5–6 ng tacrolimus/ml and 3 ng sirolimus/ml.

At 6 months after transplantation, permanent elevation of aminotransferases was noted without an explanation of the underlying cause. In July 2014, infection with HEV genotype 3c was diagnosed and retained sample testing revealed persistent HEV infection at least since September 2013. As depicted in Fig. 1, RBV monotherapy was initiated in February 2015 at a dosage of 600–800 mg/d adapted to the impaired renal function and anemia of the patient. Seven weeks after the initiation of RBV treatment HEV viral load declined below the lower detection limit, but a viral rebound was observed after 20 weeks. After 21 months of viremia under continuous RBV treatment, 400 mg SOF/d was added. After 4 weeks of SOF/RBV combination therapy, HEV load dropped below the lower detection limit with detectable viral loads. To further support viral eradication, tacrolimus was reduced to 3 mg/d leading to steady-state trough plasma levels of 3 ng/ml. The dosage of sirolimus was not

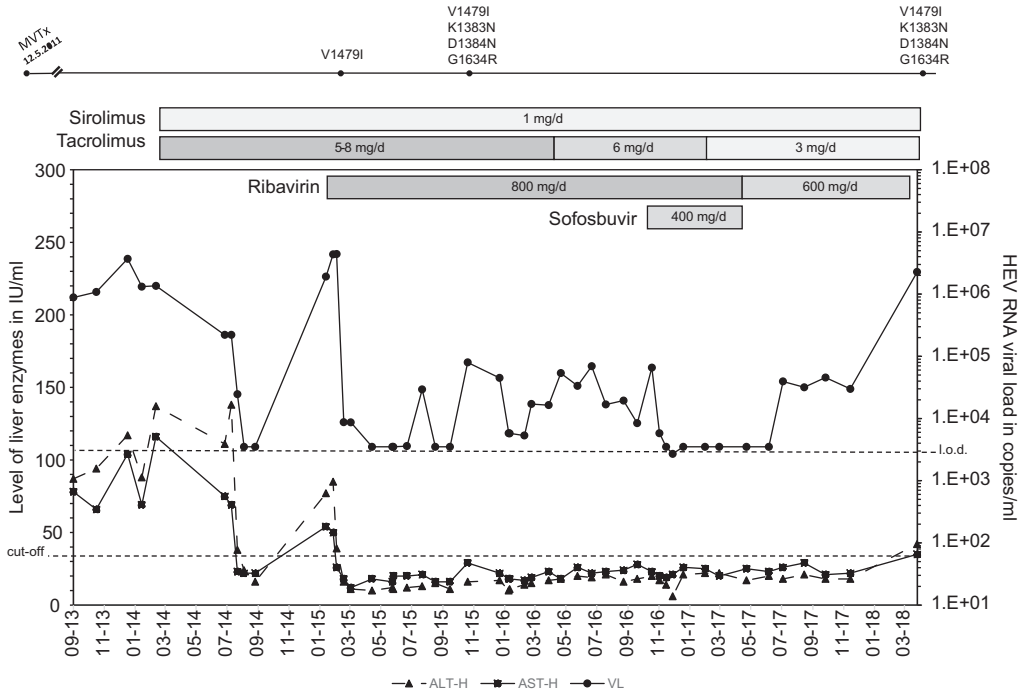

Fig. 1. Treatment course in patient with chronic HEV infection.

Table 1. Single nucleotide polymorphisms found in 3 samples during the treatment interval. (Note: the samples from 2015 and 2017 were analyzed by ultra-deep sequencing approach while the sample from April 2018 was analyzed with the Sanger method, see text).

	Position of		03–2015	10–2017	04–2018
	nt exchange	aa exchange	Proportion in %		Detectable y/n
Resistance–associated mutations	G4490A	V1479I	77.8	80	y
	G4955A	G1634R	–	66.7	y
	A4204T	K1383N	–	80	y
	G4205A	D1384N	–	70	y
Single nucleotide polymorphisms (no association with RBV resistance)	T3684C	V1210A	–	14.0	n
	C3723T	P1223L	11.2	–	n
	A3459T	Y1435F	–	–	y
	C3741T	T1229I	–	12.3	n
	G3739A	A1228T	–	44.4	n
	G3968A	V1305I	–	87.2	n
	A4004T	T1317S	–	–	y
	G4019A	A1322T	–	33.3	n
	G4049A	A1332T	–	–	y
	G4053A	R1333K	–	33.3	n
	C4065T	T1337I	–	44.4	n
	A4359T	Y1435F	–	–	y
	G4076A	V1478I	–	33.3	n
	C4083T	A1343V	–	–	y
	G4126T	E1357D	30.2	27.3	n
	C4151T	L1366F	–	30.4	n
	C4157T	L1368F	–	–	y
	G4178A	V1375I	–	10.0	y
	T4207C	D1384N	–	30.0	n
	C4157T	L1368F	–	–	y
	G4334A	A1427T	–	30.0	n
	C4340T	L1429F	–	–	y
	T4375G	D1440E	–	70.0	n
	G4409A	G1452S	–	23.4	n
	T4439A	S1462T	–	30.0	n
	G4475A	G1474S	71.5	–	y
	T4488C	V1478A	–	30.0	n
	C4551T	A1499V	–	16.5	n
	G4649A	A1532T	70.8		n
	T1653C	I1533T	–	10.0	n
	T4807G	I1584M	–	18.8	n
	C4908T	P1618L	–	13.0	n
	G4916A	A1621T	46.7	–	n
	G5087A	V1678I	–	–	y
	G5096A	V1681I	–	15.5	n
	A5124G	Q1690R	–	12.9	n
	G5150A	V1699M	–	–	y
	T5156C	C1701R	55.3	48.5	n

Table 2. Comparison of SOF/RBV treatment regimens in patients with chronic HEV.

	Patient characteristics	HEV GT	RBV first line therapy	SOF/RBV treatment regimen	Viral response to SOF/RBV
Todesco *et al.* 2017[3]	HIV/HBV coinfection	3i	1 course: 3 months of pegylated-interferon alfa 2a + 1,000 mg RBV/d	3 months of 400 mg SOF/d + 1,000 mg RBV/d	Initial decrease of VL without viral clearance, viral rebound after treatment cessation
van der Valk *et al.* 2017[6]	Chronic lymphatic leukemia	3	2 courses: – 2 months of 600 mg RBV/d – 4 months of 600 mg RBV/d + not stated period of 1,200 mg RBV/d and 800 mg RBV/d	2 months of 400 mg SOF/d + 400 mg RBV/d	Initial decrease of VL below detection limit, viral rebound under treatment
Todesco *et al.* 2018[7]	Heart transplantation	3c	2 courses: – 3 months of 800 mg RBV/d – 9 months of 1,000 mg RBV/d, 8 months of 1,200 mg RBV/d	6 months of 400 mg SOF/d + 9 months of 1,200 mg RBV/d	Initial decrease of VL, viral rebound under treatment
Drinane *et al.* 2018[9]	Kidney/pancreas transplantation	n.d.	2-courses: 4 months of 600 mg RBV/d – 9 months of 600 mg RBV/d	3 months of 400 mg SOF/d + 4 months of 1,200 mg RBV/d; RBV decreased to 800 mg RBV/d after 4 weeks	Sustained virologic response

HEV, hepatitis E virus; GT, genotype; n.d., not determined; SOF, sofosbuvir; RBV, ribavirin; VL, viral load.

changed. However, HEV replication remained detectable at very low levels and never became negative throughout a total of 6 months of SOF/RBV combination therapy. Six weeks after SOF was stopped and RBV monotherapy was continued at a dosage of 600 mg per day, HEV load relapsed again. Transient elastography (FibroScan®; Echosens, France) showed no signs of liver fibrosis (stage F0-1) before or after treatment initiation.

As depicted in Table 1, sequence analysis performed at 3 consecutive time points under therapy confirmed the development of all 4 well-characterized RBV-associated resistance mutations (K1383N, D1384N, V1479I, and G1634R).[8] In contrast, out of 39 additional single nucleotide polymorphisms in the HEV RNA dependent RNA polymerase, 35 were detected only in 1 sample, indicating that they were induced by regular mutation processes in RNA viruses and not selected through drug-driven pressure.

In summary, this is the first report of a chronically HEV-infected multivisceral transplanted patient failing to clear HEV under SOF/RBV combination therapy. It might be of interest to note that although the patient was visceral transplanted, steady-state trough plasma levels of tacrolimus did not show any signs of malabsorption, arguing against a potential malabsorption of SOF or RBV. Our observation is consistent with previous reports suggesting insufficient efficacy of SOF/RBV combination therapy in patients with chronic HEV infection, including a patient with stem cell transplantation[6] and immunosuppression due to chronic HIV infection.[3] A recent report of a sustained virologic response after treatment with SOF/RBV[9] has been discussed.[10] As depicted in Table 2, our patient and other case reports comprising patients with different subtypes of HEV genotype 3 treated with varying SOF/RBV treatment regimens showed comparable response patterns, exhibiting an initial decrease of viral load followed by a viral rebound either under treatment or after cessation of treatment. This indicates that the combination of SOF/RBV has an effect on chronic HEV that does not respond to RBV monotherapy, but is unable to eradicate the virus in these patients. Of note, extending SOF/RBV combination therapy for 24 weeks in a heart-transplanted patient with chronic HEV infection also failed to induce a sustained virologic response.[7]

Taken together, the addition of SOF appears to be inefficient in patients with chronic HEV infection failing RBV monotherapy, highlighting the unmet need for novel innovative treatment regimens for these patients at high risk of rapid disease progression.

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Conflict of interest

The authors declare no conflicts of interest that pertain to this work.

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Authors' contributions

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Supplementary data

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