

Low Transmission Rate of Hepatitis E Virus by Transfusion of Hepatitis E Virus RNA-Positive Blood Products

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Keywords

Hepatitis E virus · Transfusion transmitted infections · Blood donor screening · Infectious HEV RNA concentration

Abstract

Introduction: Hepatitis E virus (HEV) can cause transfusion-transmitted (TT) infections. Therefore, in many countries, blood donor screening for HEV RNA has been established. The amount of infectious HEV RNA concentration in blood donations and screening strategy (pool size, single-sample testing) are still debated. **Methods:** Blood donations, taken before the universal blood donor screening for HEV RNA, were investigated retrospectively for HEV RNA. Recipients of an HEV RNA-positive blood product were traced by look-back procedure. Archive samples of these recipients were investigated for TT-HEV infection by HEV RNA and anti-HEV IgG testing. HEV RNA concentration in the donor and in the transfused blood product was determined. **Results:** In 85/75,905 donations (0.1%), HEV RNA was detectable. A total of 28 recipients of 139 blood products from these donations could be further investigated. In 2/28 (7.1%) recipients, a possible TT-HEV infection occurred, but sequence analysis between donor and recipient could not be performed. HEV RNA concentration could be determined in 23 blood products and ranged from 10.9 IU/mL to 116,400 IU/mL donor plasma and absolute 120 IU–8,748,000 IU in the final blood product. In the cases of possible TT-HEV infection,

HEV RNA concentration was 62,880 IU/mL donor plasma, according to 691,680–943,200 in the red blood cell concentrate and 593 IU/mL donor plasma, according to 8,302–11,267 IU in the pooled platelet concentrate. **Conclusion:** Only a minority of HEV RNA-positive blood products caused a TT-HEV infection. With the limitation of the low number of investigated cases, even in blood products, which were contaminated with extremely high HEV RNA concentrations, there was no evidence for TT-HEV infection. Blood donor screening for HEV RNA by pooled samples should be favored over single-sample testing.

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Introduction

After the detection in the early 1980s [1], the hepatitis E virus (HEV) was considered as water-borne virus with spread in the developing countries and as an agent of a mostly mild and self-limiting hepatitis. In the following years, it became apparent that four genotypes of HEV are existent, which can infect humans. While HEV genotype (GT) 1 and 2 are prevalent in developing countries, causing mostly water-borne infections, HEV GT 3 (and 4) are present in developed countries. They cause autochthonous infections, which are often resolved spontaneously without symptoms in otherwise healthy

individuals like blood donors but can induce chronic infections in immunocompromised patients [2].

Humans are not the only reservoir of HEV GT 3 and 4; thus, the main transmission route is zoonotic and food borne. But besides that, they can also be transmitted by transfusion: after the first report of a transfusion-transmitted (TT)-HEV infection in Japan [3], TT-HEV infections were also reported from European countries [4, 5]. The topic of TT-HEV infection attracted more attention in the following years, and more data became available about the prevalence of HEV RNA in blood donors. Presence of HEV RNA in blood donations is not rare: it has been estimated between 0.01 and 0.1% [6–11] in several European countries and the USA. Therefore, in many countries, in blood donation services HEV RNA testing was established, but in other countries like Denmark and Australia, they decided against HEV RNA testing of blood donors [8, 12].

After cases of TT-HEV infection were reported in Germany [13, 14], also a general HEV RNA screening of blood donations was mandated by the German authorities [15]. Not every acute HEV infection in a blood donor causes TT-HEV infection in the recipient of the blood products [7]: the probability increases with the total HEV RNA concentration (the “viral load”) in a blood product and is dependent on the HEV RNA concentration in the donor’s plasma and the plasma content of the blood product.

Basing on a 95% limit of detection of approximately 20 IU/mL of the test used [16], detection of at least 2,000 IU HEV RNA/mL plasma in a single blood donation in pooled plasma should be sufficient to prevent most TT-HEV infections, according to the enactment of the German authorities [15]. It can therefore be assumed that some donations with only low HEV RNA concentrations are missed. This explains the finding of break-through TT-HEV infections despite HEV RNA testing by pooled plasma samples [17].

Only few data exist about the infection rate of the recipients of HEV RNA-positive blood products, as well as the concentration of HEV RNA in a blood product, which is presumably required to infect a recipient. The aim of our study was to assess retrospectively the rate of HEV transmission by transfusion of blood products from HEV RNA-positive blood donors, and to assess whether the minimum detection limit of HEV RNA in a single donation, required by the German authorities, is sufficient for the prevention of TT-HEV infections.

Material and Methods

Blood Donors

In the Institute of Transfusion Medicine of the University Hospital of Schleswig-Holstein, approximately 50,000 blood donations (whole blood, plasma and platelet

apheresis) are taken every year in the cities of Lübeck and Kiel in northern Germany. At each donation, according to the recommendations of the National Advisory Committee Blood [18], archive samples (EDTA) were also taken and stored for $\leq -30^{\circ}\text{C}$ for at least 3 years.

Blood donations, given before the mandatory HEV RNA screening in Germany [15], were retrospectively tested for HEV RNA by NAT (cobas HEV, Roche Diagnostics, Mannheim, Germany, 95% LOD: 18.6 IU HEV RNA/mL) in minipools up to 48 samples (corresponds to approximately a 95% LOD of 893 IU/mL in the single donation in the minipool) according the manufacturer’s recommendations. Reactive minipools were resolved by chessboard titration to identify the HEV RNA-positive donation. HEV RNA-positive donors were also investigated for IgG and IgM antibodies against HEV by ELISA (anti-HEV ELISA [IgG] and [IgM], Euroimmun, Lübeck, Germany) according to the manufacturer’s instruction. Sensitivity of the anti-HEV ELISA [IgG] is 95.5–100% and the specificity 100%. Sensitivity of the anti-HEV ELISA [IgM] is 100% and the specificity also 100%, both according to the manufacturer’s instruction. Results were given semiquantitative as “ratio” (extinction of the sample divided by the extinction of the calibrator). A ratio <0.8 was considered “negative,” a ratio ≥ 0.8 – <1.1 was considered “borderline,” and a ratio ≥ 1.1 was considered “positive.”

In every HEV RNA-positive donor, a look-back procedure was performed after identification of an HEV RNA-positive donation: recipients of the blood products, which were manufactured by the HEV RNA-positive donations, were identified by database analysis. Additionally, the archive sample of the preceding as well as the subsequent donation was tested for HEV RNA by single-sample testing.

Blood Products

Red blood cell (RBC) concentrates, buffy coat, and fresh frozen plasma (FFP) were manufactured from leukocyte-reduced whole blood after centrifugation by standard procedures. Buffy coat-derived platelets from four different donors were resuspended in additive solution to obtain a pooled platelet concentrate (PC). The plasma remnant in RBC was 11–15 mL and 200–280 mL in plasma from whole blood. After deduction of additive solution, in PC the plasma remnant from altogether four donors was 55–75 mL, according to approximately 14–19 mL plasma from each of the four donors in a PC. Platelets were also obtained by standard apheresis procedure: the plasma remnant of apheresis platelet concentrate (APC) was 65–146 mL, after deduction of additive solution.

Recipients

Blood products are provided to the clinics of the University Hospital of Schleswig-Holstein by the blood banks of the Institute of Transfusion that are situated in

Lübeck and Kiel. From EDTA-blood samples of patients, which were sent to our blood bank in Lübeck from the treating physicians, for pre-transfusion testing (e.g., RBC crossmatch), an archive sample was stored at $\leq -30^{\circ}\text{C}$, if feasible.

After identification of a recipient by look-back procedure, an archive sample, taken before transfusion of an HEV RNA-positive blood product, if available, was investigated for the presence of anti-HEV IgG (Euroimmun). Further archive samples, taken thereafter, served as follow-up samples and were investigated for anti-HEV IgG by undiluted sample and for HEV RNA. As only sparse amounts of each patient plasma (maximum: 650 μL) were stored, the samples had been diluted two- to threefold with HEV RNA-negative plasma before insertion in the NAT. To assess the absolute amount of HEV RNA, which has been given by transfusion, HEV RNA in the donor sample was determined quantitative and multiplied with the plasma remnant of the blood product. Samples for quantitative HEV RNA determination were sent frozen to another laboratory (Institute for Laboratory and Transfusion Medicine, Heart and Diabetes Center North Rhine-Westphalia). Quantitative HEV RNA determination was performed by the AltoStar HEV RT-PCR test (Altona Diagnostic Technologies, Hamburg, Deutschland) as described elsewhere [19].

In the case of HEV RNA detection in a recipient after transfusion of an HEV RNA-positive blood product, recipients as well as donors HEV RNA sequences were analyzed as reported elsewhere [20, 21]. If HEV RNA became detectable in a recipient of an HEV RNA-positive blood product, medical reports were reviewed retrospectively, to get information about the underlying disease, and whether clinical signs of hepatitis were present. Also, the liver enzymes (GOT, GPT, γ -GT) were reviewed as surrogate markers for hepatitis.

Results

All donations that had been donated between May 26, 2017, and December 31, 2018, were tested for HEV RNA retrospectively. A total of 75,905 donations (whole blood, platelet and plasmapheresis) were considered. These donations had been donated by 23,703 donors (6,413 first-time donors and 17,290 regular donors). Here, 77 donations from 76 donors (six first-time donors and 70 regular donors) tested positive for HEV RNA, by pooled samples, according to 0.32% of all donors and 0.1% of all donations.

Look-back procedures revealed eight further HEV RNA-positive donations from these 76 donors. In one out of these 85 HEV RNA-positive samples, only anti-HEV IgM was detectable, anti-HEV IgM and IgG were detectable in three samples, and only IgG was detectable

in five samples. In 76 samples, neither anti-HEV IgG nor IgM was detectable.

From those 85 blood donations, 194 single blood products were produced (68 RBC, 79 FFP), and 47 PC or APC. Fifty-five FFPs were supplied as plasma for fractionation. Sixty-eight RBCs, 24 FFPs, and all 47 PCs or APCs were transfused to 139 different patients. In 109/139 (78.4%) patients, no archive samples for further investigation were available, but in 30 (21.6%) patients, an archive sample was available (Fig. 1). Two patients, in whom an archive sample was available only at day 1 and day 4 after transfusion, were excluded from the analysis. In six further recipients, archive samples taken only between day 6 and day 14 after transfusion were available. In the other recipients, archive samples taken between day 19 and day 967 after transfusion could be investigated. Overall, in 19/28 (67.9%) recipients, there was no evidence for HEV infection before transfusion or thereafter. In 9/28 (32.1%) recipients, there was evidence for HEV infection: anti-HEV IgG was detectable already before transfusion in 3/28 (10.7%) recipients (recipient 1–3; Table 1) of an HEV RNA-positive blood product (Fig. 1). No HEV RNA occurred thereafter. In 4/28 (14.3%, Fig. 1) recipients (recipients 4–7; Table 2), which were anti-HEV IgG-negative at time of transfusion, transient anti-HEV-IgG antibodies close above the cut-off of the test appeared, but no HEV RNA became detectable. No additional anti-HEV IgG was detectable in the HEV RNA-positive blood products.

In 2/28 recipients (recipients 8 and 9, 7.1%; Fig. 1), which were also anti-HEV IgG-negative at time of transfusion or in the first sample thereafter, HEV RNA became detectable after transfusion. The course of infection is displayed in Table 3.

Unfortunately, sequence homology between HEV RNA, detected in the donor and HEV RNA, and detected in the recipient was not possible, as HEV RNA concentration was too low for sequencing. Recipient 8 (Table 3) was a female at an age of 69 years. She suffered from squamous cell carcinoma of the mouth. The treatment was surgical as well as by radiochemotherapy. She received an HEV RNA RBC, and HEV RNA concentration in the donor sample was 62,880 IU HEV RNA/mL plasma, according to an overall HEV RNA concentration of 691,680–943,200 IU in the RBC. HEV RNA had disappeared at day 84 spontaneously after transfusion. No therapy against HEV had been administered.

Recipient 9 (Table 3) was a female at an age of 40 years with multiple myeloma. During aplasia due to an autologous stem cell transplantation, a PC was transfused. An archive sample, drawn at the day of transfusion or before, was not available, as no cross-matching was performed before transfusion of the PC. HEV RNA concentration in the donor's archive sample was 593 IU/

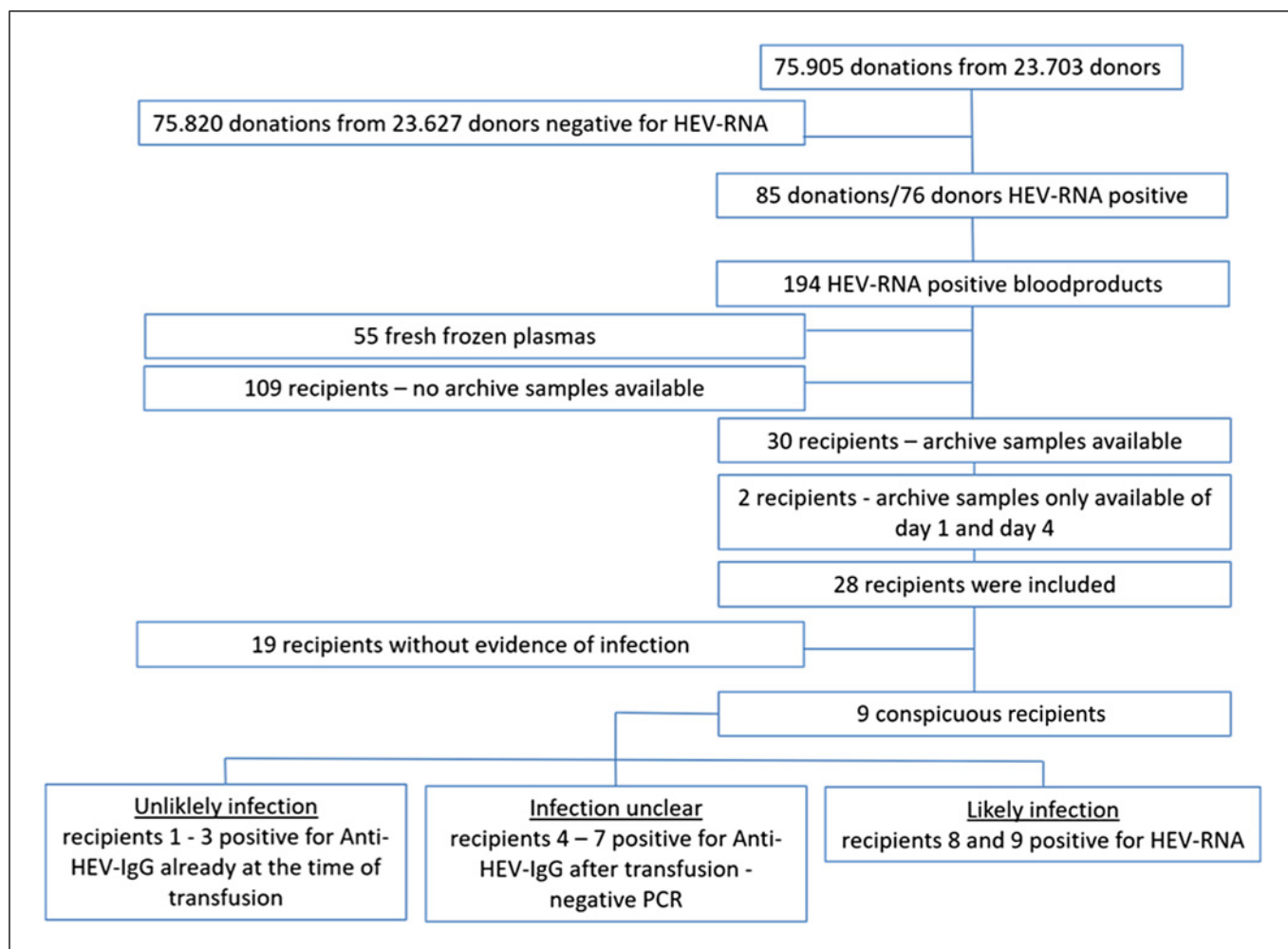


Fig. 1. Results of HEV RNA blood donor screening and look-back procedure.

Table 1. Results recipient 1–3 – infection unlikely

Recipient 1							
Day	–3	+7	+13	+19	+26	+36	+62
HEV RNA	Not tested	Not tested	Negative	Negative	Negative	Negative	Not tested
Anti-HEV IgG	Borderline	Borderline	Borderline	Borderline	Positive	Positive	Positive
Recipient 2							
Day	+1	+9	+25	+35	+54	+57	
HEV RNA	Not tested	Not tested	Negative	Invalid	Invalid	Negative	
Anti-HEV IgG	Positive	Positive	Positive	Positive	Positive	Borderline	
Recipient 3							
Day	–1	+8	+14	+21	+31	+40	
HEV-NAT	Not tested	Negative	Negative	Negative	Negative	Not tested	
Anti-HEV IgG	Positive	Positive	Positive	Positive	Positive	Negative	

Table 2. Results recipient 4–7 – infection unclear

Recipient 4							
Day	–3	+15	+17	+20	+39	+62	+104
HEV RNA	Not tested	Negative	Negative	Negative	Negative	Negative	Not tested
Anti-HEV IgG	Negative	Negative	Positive	Negative	Negative	Negative	Negative
Recipient 5							
Day	–1	+9	+18	+27	+36	+44	+63
HEV RNA	Not tested	Negative	Negative	Negative	Negative	Negative	Not tested
Anti-HEV IgG	Negative	Positive	Borderline	Negative	Negative	Negative	Negative
Recipient 6							
Day	0		+34		+77		+91
HEV-NAT	Not tested		Not tested		Negative		Negative
Anti-HEV IgG	Negative		Positive		Positive		Borderline
Recipient 7							
Day	0			+4			+6
HEV-NAT	Not tested			Not tested			Negative
Anti-HEV IgG	Negative			Positive			Negative

Table 3. Results recipient 8–9 – infection possible

Recipient 8				
Day	–4	+55	+84	+559
HEV RNA	Negative	Positive	Negative	Not tested
Anti-HEV IgG	Negative	Negative	Positive	Positive
Anti-HEV IgG ratio	0.2	0.6	4.4	1.5
GOT, U/L	40 (<35)	20	27	24
GPT, U/L	31 (<35)	11	18	18
γ-GT, U/L	22 (<40)	<7	Not tested	Not tested
Recipient 9				
Day	0		+45	+52
HEV RNA	Not tested		Positive	Positive
Anti-HEV IgG	Not tested		Negative	Negative
Anti-HEV IgG ratio	Not tested		0.2	0.1
GOT, U/L	18 (<35)		26 (<35)	21
GPT, U/L	33 (<35)		38 (<35)	31
γ-GT, U/L	43 (<40)		46 (<40)	190

mL plasma, according to absolute 8,302–11,267 IU HEV RNA in the PC. No antibodies against HEV were formed during the observation period, HEV RNA was detectable in two archive samples, and the last one was taken

52 days after transfusion. Overt clinical signs of hepatitis did not occur.

Quantitative HEV RNA determination was possible in 21 donor archive samples of whom 23 blood

Table 4. HEV RNA concentration in blood donors and blood products with traceable recipients and their characteristics

Donor					Recipient		
donor No.	HEV RNA/ IU/mL plasma	blood product	RNA total in the blood product/IU (min-max)	archive samples (days before transfusion)/ first/last after transfusion	sex	age	underlying disease
1	2,154.9	RBC	23,704–32,324	(0)/11/240	m	41	Polytrauma
2	10.9	RBC PC	120–164 153–207	(0)/5/8 (–1)/8/40	f f	35 66	Acute myeloid leukemia Hip arthroplasty
3 ^a	593.0	PC	8,302–11,267	(not available)/45/52	f	40	Multiple myeloma (recipient 9)
4	4,698.4	PC	65,778–89,270	(1)/9/57	m	12	Acute lymphoblastic leukemia
5	116,640.0	PC	1,632,960–8,748,000	(–3)/3/29	m	55	Dilatative cardiomyopathy
6	1256.3	RBC	13,819–18,845	(0)/14/130	f	54	Unknown
7	850.9	RBC FFP	9,360–12,764 170,180–238,252	(–1)/9/14 (0)/9	m m	57 81	Myocardial infarction Major burn
8	163.0	PC	2,282–3,097	(0)/4/6	f	75	Rectal cancer
9	1,367.2	RBC	15,039–20,508	(–2)/3/361	f	32	Systemic lupus erythematosus
10 ^a	62,880.0	RBC	691,680–943,200	(–4)/55/559	f	62	Oral squamous cell carcinoma (recipient 8)
11	10,889.6	RBC	119,786–163,344	(–1)/14/383	f	51	Unknown
12	428.2	RBC	4,710–6,423	(0)/7/49	f	70	Acute myeloid leukemia
13	65,456.0	PC	916,384–1,243,664	38	f	52	Non-small cell lung cancer
14	1,017.3	RBC	11,190–15,260	(0)/28/967	m	77	Unknown
15	1,574.1	FFP	314,820–440,748	(0)/11/19	m	66	Adenocarcinoma of the bile duct, post-pancreatectomy severe bleeding
16	331.8	RBC	3,650–4,977	(0)/41/125	m	70	Unknown
17	189.4	RBC	2,083–2,841	(–2)/1/14	f	71	Mitral valve repair
18	31.2	APC	2,028–4,555	(–3)/15/104	f	11	Intracranial nongerminomatous germ cell tumor
19	3,321.6	APC	215,904–484,954	(–4)/16/43	f	11	Intracranial nongerminomatous germ cell tumor
20	1,688.8	RBC	18,577–25,332	(–1)/9/63	m	61	Myocardial infarction
21	1,737.3	RBC	19,110–26,060	(–3)/6/51	f	74	Thyroidectomy, miscellaneous complications
22	Not tested	RBC	Not tested	(0)/34/91	m	64	Unknown
23	Not tested	RBC PC	Not tested Not tested	(–1)/7/21 (–3)/7/62	m m	71 89	Polytrauma Sepsis/abscess
24	Not tested	RBC PC	Not tested Not tested	(–3)/10/14 (–5)/61	m f	86 82	Myocardial infarction Mitral valve repair

RBC, red blood cell concentrate; PC, pooled platelet concentrate; APC, apheresis platelet concentrate; FFP, fresh frozen plasma. ^aProbably TT-HEV infection.

products were administered to a patient with available archive sample. HEV RNA concentration ranged between 10.9 IU/mL plasma and 116,400 IU/mL plasma, according to an absolute HEV RNA con-

centration between 120 IU and 8,748,000 IU in the administered blood product. An overview about HEV RNA concentration in the archive sample, in the blood product and point in time when the first sample

and the last sample after transfusion were taken as well as clinical characteristics of all 28 recipients, is given in Table 4.

Discussion

While seroprevalence of HEV already has been estimated in the past to range between 6.8% and 19% in our blood donor population [22, 23], HEV RNA was actually found in 0.1% of the donations, taken in our institute. This rate of donations with detectable HEV RNA is at the upper limit of HEV RNA-positive donations, which have been reported from other institutions (0.01–0.1% of all donations) [6–11]. Reasons might be a different sensitivity of the NAT assays used, different eating habits of the donor population, and also the size of the pools in whom HEV RNA was investigated: the look-back procedure revealed additional eight HEV RNA-positive donations only by single-sample testing. The burden of HEV RNA is therefore high in our donor population, and thus, TT-HEV infections are to be expected: overall 194 blood products were derived from HEV RNA-positive donations in the observation period, and we were able to investigate 28 recipients for a TT-HEV infection. However, probable HEV infection occurred in only two of the recipients. Unfortunately, it was not possible to better prove the blood product as the source of infection as sequencing failed due to a low HEV RNA concentration, in either the blood donor or the recipient. However, TT-HEV infection seemed most likely in recipient 8: the timing of the disappearance of HEV RNA and the appearance of antibodies fits well to a time of infection, when the RBC was administered. In the immunosuppressed recipient 9, we cannot completely exclude a preexisting, chronic HEV infection without antibody formation, as we had no archive samples, drawn at the day of transfusion of the HEV RNA-positive PC.

In a previous study [7], 42% (18 of 43) of the recipients of an HEV RNA-positive blood product suffered from TT-HEV infection. This is a considerable higher rate of TT-HEV infections than in our study (7.1%). Several reasons are possible for this difference: in the study of Hewitt et al. [7], of the 18 recipients with evidence of a TT-HEV infection, in six recipients only antibodies against HEV but no HEV RNA were detectable. It is unclear by the provided data, whether these patients were negative for anti-HEV already at baseline/at the point in time of transfusion or shortly after. Therefore, it cannot be excluded that these patients were positive for anti-HEV IgG already before transfusion. Alternatively, anamnestic immune response to HEV is also think-

able as a reason for high-titer anti-HEV IgG. But if only recipients, in whom HEV RNA was detectable in the study from the UK, are considered, rate of TT-HEV infection is still higher than in our study. Point in time of the sampling in our study is not optimal in some cases due its retrospective character: in six recipients, only an archive sample, drawn before day 15 after transfusion, was available and we cannot exclude a later seroconversion. HEV RNA is approximately at least 14 days after infection detectable in the plasma [2], and also detection of HEV RNA later in the course cannot be excluded in these six recipients. Therefore, a TT-HEV infection might be missed in these cases and rate of TT-HEV infections might even be higher in our study. Also, seroconversion might be missed in some of the investigated recipients, as we had no archive samples available, which had been drawn more than 6 weeks after transfusion. However, as HEV RNA should be detectable 14 days after infection and persists for a mean time of 68 days (at least in blood donors) [24], thus, at least HEV RNA should become detectable in these recipients.

Four recipients had detectable anti-HEV IgG after transfusion of an HEV RNA-positive blood product. These antibodies were transient in recipients 4, 5, and 7 and became weaker in recipient 6. No HEV RNA was detectable in all four recipients. Thus, other explanations than TT-HEV infection for this phenomenon are more likely, like unspecific reactions or passive antibody transfer by other, only anti-HEV IgG antibody-positive blood products.

Whether a TT-HEV infection might occur depends, besides other host-related factors, on the absolute HEV RNA concentration in the blood product, frequently referred as “viral load.” As higher the HEV RNA concentration in the blood stream of the donor and as higher the plasma content of the blood product, as higher is the absolute HEV RNA concentration of the blood product. Already, Hewitt and colleagues [7] observed a connection between plasma content of a blood product and infectivity, with highest rates of TT-HEV infections by FFP [7]. 2×10^4 HEV RNA IU in a blood product is considered to induce a TT-HEV infection in 55% of the recipients [25], and blood products with $>5 \times 10^4$ IU HEV RNA are considered to be infectious in almost any cases [26]. We cannot confirm this observation: seven of the administered blood products in our study (that of donor 4, 5, 7, 11, 13, 15, and 19; Table 4) had HEV RNA concentration far above 5×10^4 IU, but no evidence for TT-HEV infection was observed. One of these blood products (donor 4) was even transfused to a presumably highly immunosuppressed patient. While the observation period was short in the recipients of the blood products

of donor seven, in the recipients of the blood products from other donors, either detection of HEV RNA and/or anti-HEV IgG had to be expected in case of infection. We can only speculate about the reasons for this observation: a reason might be that in the blood stream, HEV is present in association with lipids [27]. The lipid surface might protect HEV for neutralizing antibodies, but also infectivity is reduced: at least in a cell culture systems, lipidated HEV was less infective compared to the “naked” HEV, which is present in feces [28, 29]. However, whether these in vitro observations, made by cell culture systems, can explain the low rate of TT-HEV infection in our study remains unclear and has to be elucidated by further studies. One explanation for the lacking infectivity of HEV RNA-positive blood products can be excluded surely: the simultaneous presence of potentially neutralizing antibodies in the blood products. As already reported elsewhere [19], only in the minority of the HEV RNA-positive blood donors, also antibodies against HEV were detectable.

Host-related factors are responsible for the course of acute HEV infection. Many HEV infections – at least with GT 3– are resolved spontaneously in immunocompetent individuals without clinical or laboratory signs, like GPT elevation [19]. This was also the case in one out of the two recipients of an HEV RNA-positive blood product (recipient 8). Spontaneous resolution of the infection occurred despite administration of radiochemotherapy, in contrast to recipient 9: HEV RNA was detectable in the last archive sample, drawn 52 days after transfusion, with a lacking seroconversion at this point in time. Seroconversion is known to be delayed in immunosuppressed patients [7], and we cannot exclude that HEV infection became chronic in this hematologic patient. However, an archive sample which has been drawn >6 months after transfusion was unfortunately not available to prove this. Overall, the number of TT-HEV infections in our study is too low to better characterize the clinical course of TT-HEV infection, but many reports have demonstrated a poor outcome in patients, who suffer from TT-HEV infection [7, 13].

To avoid TT-HEV infections, HEV RNA testing by NAT of blood donations has become a cornerstone in many countries and is mandated in Germany since 2020. HEV NAT testing is as cost-intensive as NAT testing for other viruses [30], and to lower costs, testing of pooled samples is preferred [31]. A large pool size (e.g., pools of 96 samples) enables reliably detection of a blood donation with a high HEV RNA concentration and prevents transfusion of blood components with high HEV RNA concentrations. Minimum absolute HEV RNA concentration, the “infectious dose,” for TT-HEV infection has been estimated to range between 3.16×10^3

IU and 7.05×10^3 IU [32]. The limit of detection for HEV RNA in the single sample in the pool is 2,000 IU HEV RNA/mL plasma stipulated by the German authorities. Fourteen of our donations might have been overseen by this limit of detection, and eight out of these 14 donations were below the 95% limit of detection of our test, using a pool of 48 donations (Table 4). More safety concerning TT-HEV infections is yielded by smaller pools [10] with a maximum of safety, if single-sample testing is performed [33]. In most of these 14 donations, only the recipient of the RBC could be traced and we cannot exclude TT-HEV infection in recipients of the PC or FFP. But the number of recipients with TT-HEV infection in our study is overall low, even if blood products with high HEV RNA concentrations were transfused. It is therefore not to be expected, that other blood products from these 14 donations pose a higher risk for TT-HEV infections. As even donations with higher HEV RNA concentration do not result in TT-HEV infection, of course donations with low or very low HEV RNA concentrations do rarely induce TT-HEV infection. This was in our study likely the case only in one PC, donated by donor 3 (Table 4), with the limitation, that we cannot exclude a preexisting HEV infection in recipient 9.

Safety of blood components also should be weighed against costs: to avoid any TT-HEV infection, single-sample NAT testing for HEV RNA of all blood donations can be performed, but costs of this measure are certainly outside of what the blood donation services or the healthcare system can pay for. Therefore, blood donation screening for HEV RNA in minipools with the required detection limit of 2,000 IU HEV RNA/mL plasma for the single donation in the pools seems to be a reasonable compromise and is preferable to single-sample testing (which is not discussed for other viruses like HBV, HCV, or HIV). Clinicians should also be aware about the low probability of TT-HEV infection, despite HEV RNA testing of blood donations, and should be encouraged to additionally monitor susceptible patients also for HEV RNA.

In conclusion, the rate of TT-HEV infections in our study was lower than reported in previous studies. With the limitation of the low number of investigated cases in our study, our data suggest that routine NAT testing for HEV RNA of blood donors may have limited clinical benefit. Even blood products with high HEV RNA concentration above the to-date considered absolute infectious HEV RNA concentration in a blood product of 5×10^4 IU did not result in TT-HEV infections in many cases from yet unknown reasons. If testing for HEV RNA in blood donors should be continued, selection of the pool size for HEV RNA testing should be weighed against costs and a larger pool size can be accepted.

Statement of Ethics

This study protocol was reviewed and approved by the local ethical review board (AZ 22-134) of the University of Lübeck. Written informed consent from participants was not required in accordance with local/national guidelines.

Conflict of Interest Statement

D.J. was a member of the journal's Editorial Board at the time of submission. The other authors have no conflicts of interest to declare.

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